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Detection of expired vacuum-packed smoked salmon based on PLS-DA method using hyperspectral images.

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Abstract

Consumers want fresh food with a long shelf-life, which in 2010, resulted in an important increase in packaged and processed food. This is especially important for fishery products due to their highly perishable nature. One problem is that it is not possible to measure freshness in packaged food only using the visible spectrum. Moreover, the detection of freshness is a complex problem as fish has different tissues with different biodegradation processes. Therefore, it would be especially interesting to have a non-destructive method to evaluate the shelf-life of packed processed fish. This paper proposes a method for detecting expired packaged salmon. Firstly, this method uses hyperspectral imaging spectroscopy (HIS) using visible and SW-NIR wavelengths. Secondly, a classification of different salmon tissues is carried out by image segmentation. Finally, classifications of expired or non expired salmon are realized with the PLS-DA statistical multivariate method due to the large amount of captured data. In a similar way, spectral data and the physicochemical, biochemical and microbiological properties of salmon are correlated using partial least squares (PLS). The result obtained has a classification success rate of 82.7% in cross-validation from real commercial samples of salmon. Therefore, this is a promising technique for the non-destructive detection of expired packaged smoked salmon.

Keywords: Hyperspectral imaging; Colour model segmentation; SW-NIR; Fish shelf-life;

1. Introduction

The global retail value of packaged food sales was estimated to be around US$1.95 trillion in 2010 at present exchange rates. Since 2006, the real growth was 7% and forecasts predict an increase of nearly 10% in 2015[1]. Moreover, due to the high perishability of fishery products, 90 percent of trade in fish and fishery products consists of processed packaged products, salmon being the fourth group in terms of volume of sales according to the United Nations Food and Agriculture Organization (FAO) in 2006 [2]. Therefore, there is a great need for a method to measure the shelf-life of processed packed salmon.

Nevertheless, the shelf life of fishery products is very difficult to measure despite its great importance in the sector. The European Commission (Council Directive 95/149/EEC, March 1995) established a sensory assessment method to evaluate this quality i.e. Quality Index Method (QIM). In the case of any doubt arising with this method, a technique such as Total Volatile Basic Nitrogen (TVB-N) could be used. However, these two methods are expensive, time consuming and require highly qualified personal. In addition, sensorial methods are subjective and TVB-N is destructive, so there is a need for a new standard based on current technologies able to overcome these problems, with the added possibility of in-line use [3]. Several attempts have been made to use potentiometric sensors [4], classical destructive physical and chemical measures [5–7], ion mobility spectrometry [8], electronic noses [9], fluorescence spectroscopy[10], or near-infrared spectroscopy [11]. Nevertheless, all of them have disadvantages such as being destructive, requiring sample preparation, high expense or being too slow.

Given the large number of publications dealing with hyperspectral imaging spectroscopy and its promising results [12], [13] in the field of food quality, this seems to be a good way to attain a reliable low cost system to measure the freshness of fish, addressing industrial requirements [14]. Furthermore, spectroscopy in the range of visible and short wave infrared (SW-NIR) is specially indicated to evaluate chemical composition and quality in fish [15–18].

New works apply hyperspectral imaging spectroscopy in the SW-NIR range [19], [20] for evaluating freshness in fish. Nevertheless, in most of these studies samples were manipulated (minced, placed in Petri dishes, etc.), to be measured, changing their structure and the format in which the fish products are marketed. Moreover, fish have different kinds of tissues with different biodegradation rates which should be taken into account when assessing freshness [19]. This is especially important in the case of salmon [21], which has two different types of tissue. This classification is carried out by image segmentation from a Red-Green-Blue (RGB) image obtained applying a colour model which transforms a hyperspectral image (HI) to a visual image. Using this RGB image each tissue can be easily recognized.

As a result, this study evaluates the use of a non-destructive method using hyperspectral imaging SW-NIR spectroscopy with different data filters (random pixel selection, mean and median and image segmentation) to analyze the shelf-life of vacuum-packed smoked salmon fillets.

2. Materials and methods

2.1 Sample preparation

The study was carried out on 30 samples of commercial smoked salmon. Samples were from different batches but with the same expiry period (in order to establish an initial freshness parameter). Each sample was divided into 3 and repackaged in aseptic conditions under vacuum, obtaining 90 samples. Sampling was performed at 0, 10, 20, 40 (expiry date given by the producer), 60 and 70 days (in order to ensure shelf life), although destructive analyses were only carried out at 0, 20, 40 and 60 days.

At each sampling time 15 samples were used. Images were captured before opening the product packaging. After opening the packages aseptically, a representative sample was taken for microbiological analysis according to the microbiological procedure. Then the remaining samples of each unpacked product were used for the subsequent analysis after grinding and homogenizing. During the study samples were stored at 4 ºC.

2.2. Destructive analyses

Moisture (xW) was measured by oven drying to constant weight at 100ºC [22]. Water activity (aw) was analyzed with Aqualab GB-X Fast-Lab (GBX, Romans-sur-Isère, France) equipment, working at a temperature of 25ºC. For the determination of pH, solutions of 10g of homogenized salmon and 90ml of distilled water were measured with a portable pH meter MM40 (Crison Instruments S. A, Alella, Barcelona, España) following the procedure proposed by Fuentes [23]. Total Volatile Basic Nitrogen (TVB-N) was determined according to the procedures described by Malle and Tao [24]. The aerobic plate counts (expressed as cfu g^{-1}) were estimated following the method proposed

by the UNE [25]. 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 per 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
Fig.1 is a schematic diagram of the technique employed to detect expired salmon from acquisition to obtaining the result. Each step is explained in detail in the following subsections.

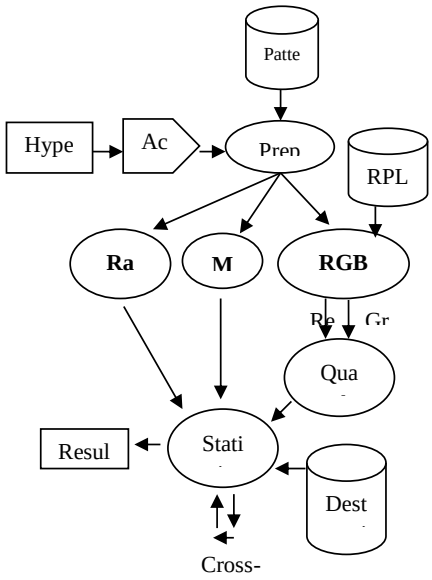

Figure 1. Schematic diagram of the technique employed to detect expired salmon from acquisition to obtaining the result.

2.3.1 Image acquisition and preprocessing
Images were taken using a CCD 102f camera (Basler Vision Technologies., Ahrensburg, Germany) and SpecimImSpector V10 1/2″ filter (Specim Spectral Imaging, LTD., Oulu, Finland), which works as a linear hyperspectral camera. The illuminants were two halogen lamps 50 W 230 V HI-SPOT (Havells Sylvania, Gennevilliers, France) producing indirect light to reduce reflections (Fig. 2).
The position of the illuminant and camera relative to the sample was always constant to control the lighting conditions and to obtain a constant image size. The distance between the illuminant and the sample was 18 cm, with a distance of 40 cm between the camera and the sample. The image (scanned line) obtained was composed of 256 grey levels (8 bits). The diffuse reflectance spectrum was collected using 53 different wavelengths (each wavelength was digitalized by 8 bits). These wavelengths were distributed at intervals of 11.2 nm in the range from 400 to 1000 nm. The scanned line was composed of 1392 points, so that an image was recorded with a resolution of 1392 × 1040 pixels.
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 adjustable speed drive controller Altivar31 (Schneider Electric Rueil-Malmaison, France).
The camera was operated by the software pylon Viewer 2.1.0.1664 (Basler Vision Technologies, Ahrensburg, Germany).

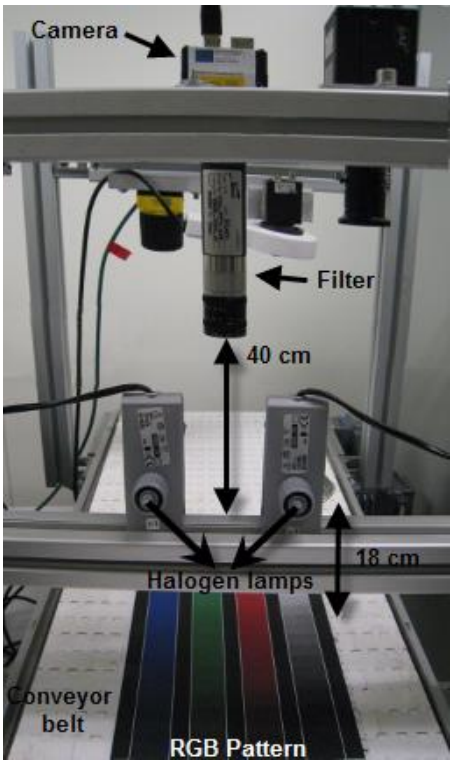

Figure 2. Spectral acquisition setup.

Reflectance calibration was performed to normalize the non-linear light source reflectance. This was done by applying equation 1 where r_W is the reflectance value of a white pattern reflectance acquired under the same conditions, r_D is the dark measure covering the camera's objective and r_s is the sample reflectance.

$$R(\lambda) = \frac{(r_S - r_D)}{(r_W - r_D)} \qquad (1)$$

Other operations carried out for further statistical processing were mean-centring and unit variance normalization.
Image reflectance calibration and preprocessing were performed by a code developed on Matlab R2008a (The Mathworks, Natick, Massachussets, USA).

2.3.2: Colour model

In order to segment the different salmon tissues, an RGB colour model was developed. There are several ways of building a RGB image from an HI one. One of them uses standard colour matching functions established by the International Commission on Illumination such as CIE 1931 or CIE 1964 [26]. However this method requires good spectral calibration to apply each colour function in the correct way. Another method uses the specific sensor response of the camera to each colour but it also needs a spectral calibration to evaluate the influence of the filter. The last method, which was used in this paper, uses a "black box" approach [27] where the RGB colour model is calculated in a robust statistical way without spectral calibration.
Empirical characterization was performed acquiring 215 HI from a printed RGB pattern (Fig. 3) in a continuous way. A program was developed to assign a known RGB value extracted from the digital pattern image for each HI. After preprocessing the HI (eq.1), a spatial mean was carried out on each image for each colour row including the white-dark one to correlate the 860 (215x4) spectral responses with their RGB colour level. In order to obtain the mathematical model for building a RGB image, a robust partial least square (RPLS) [28] was used. For the purpose of testing, another printed colour pattern was scanned (Fig. 4a) with a colour not used in the RGB model developed. Fig. 4b shows the enhanced colour pattern reconstructed employing the model obtained (R^2=0.912) which was obtained using 4 LV (latent variables).

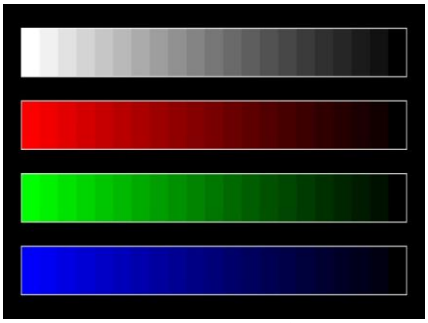

Figure 3. RGB pattern used for training the colour model

a

b

Figure 4.a: Original colour pattern used for testing. b: Enhanced colour pattern reconstructed**2.3.3: Segmentation**

Once the HI is transformed into an RGB image using the previous colour model, segmentation is carried out following two steps. Firstly, background and glare are removed from the image and secondly the image is split into four quartiles (Q), once for the red channel and again for the green channel.

The objective of segmentation is to detect pixels with similar intensities in the Red and Green channels which relate to the same tissue [29]. The Blue channel was not studied as it provides the same information as the Green channel for this application. In addition, it was decided to split channel intensities into four quartiles as it

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was proved experimentally that up to 25% of pixels could be dark tissues (Q1) and up to another 25% could be bright tissues (Q4).

The first step is carried out as the samples are packed with a transparent film which can reflect light (glare). Apart from this noise, it is demonstrated that the film does not affect the spectral response [30]. This step is performed using a filter based on a fixed colour threshold to remove background and glare.

For the second step, the quartiles were calculated using three adaptive thresholds. Figure 5 shows a histogram where these thresholds (T1, T2 and T3) can be observed together with the four quartiles (Q1, Q2, Q3 and Q4) into which the intensities are divided. T1 is calculated as the median of the intensity values in the Red channel of the remaining pixels after removing background and glare. The second and third thresholds are also the median of the red values but only for the pixels under T1 for the second and above T1 for the third. Thus an image is divided into four quartiles using these thresholds. This second step is repeated for the Green channel obtaining another four quartiles.

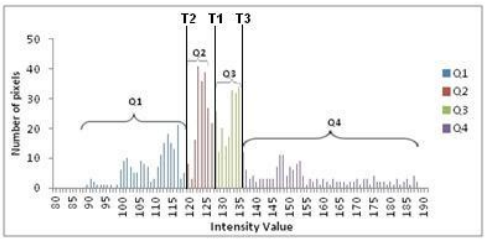

Figure 5: Histogram with the four quartiles division for one channel.

2.4 Statistical analysis

The effect of storage time on the variables (TVB-N, x^w, a_w, pH and log (cfu g−1)) was subjected to a variance study (ANOVA). In those cases where the effect was significant (p-value < 0.05) the means were compared using Fisher's least significant difference (LSD) procedure.

Partial least squares discriminant analysis (PLSDA) [31] was employed to obtain predictive methods of calibration and storage time. PLS-DA is very similar to Linear Discriminant Analysis (LDA). In fact, Barker and Rayens [32] showed that PLS-DA is essentially the inverse-least squares approach to LDA and produces essentially the same result but with the noise reduction and variable selection advantages of Partial Least Squares (PLS). In PLS-DA, PLS is used to develop a model that predicts the class number for each sample. PLS is a powerful multivariate calibration method used to correlate NIR spectra and the component of interest.

The relationship between the spectra and the values obtained from the destructive analyses was obtained by partial least square regression (PLS) [33].

To estimate how well developed models fit the calibration data, the Root-Mean-Square Error of Calibration (RMSEC) was used. It is defined as:

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

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.

A random cross-validation method was employed to evaluate the developed models. In this method, subsets of n/9 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 Root-Mean-Square Error of Cross-Validation (RMSECV), which is a measure of a model's ability to predict samples that were not used to build the model, was used to evaluate and compare the accuracy of the different PLS-DA models developed using the random cross-validation method described previously. RMSECV is based on equation 2, but in this case the parameter $\widehat{y_i}$, n and y were defined as:

- $\widehat{y_i}$ = values of the variable that are estimated by cross-validation (where the value for each object is estimated using a model that was built using a set of objects that does not include object i);
- y = values of the variable;
- n = total number of objects in the dataset.

All statistical procedures were performed on PLS Toolbox 6.3 (Eigenvector Research Inc., Wenatchee, Washington, USA), a toolbox extension within the Matlab 7.6 computational environment (The Mathworks, Natick, Massachusetts, USA).

3. Results and discussion

3.1 Destructive analysis

Table 1 shows the destructive analyses (TVB-N, x^w, a_w, pH, and microbiological counts) and the standard deviations (S.D.) for packaged sliced salmon on days 0, 20, 40 and 60 at 4º C. All parameters, except pH, presented significant differences (P-value <0.05) during storage time,

therefore the influence of sampling time was evaluated.

TVB-N (mg/100g) levels increased significantly with time (P-value<0.05), exceeding the limit value (30 mg/100 g) set for some kinds of fish (EEC, 2005) at 40 days (expiry date given by the producer). This analysis is only legislated for some types of fish, although it can give information about the biochemical changes occurring during the storage period of some products such as dry-cured ham [34] and camel meat [35] but not to express the expiry date of these products. Product moisture (x^w) increased initially (during the first 20 days), then values were almost constant (x^w=0.62). Water activity (a_w) decreased during the storage from values of 0.980 to 0.953. The development of compounds such as TVB-N, by autolytic deamination of free amino acids produced by proteolysis and degradation of nucleotides [36], could be responsible for this decrease. Aerobic plate counts increased from 3.22 (log cfu g -1) to 7.23 (log cfu g -1), although at day 40 values (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,[36 - 37]). The increase was mainly produced by lactic acid bacteria (LAB) which are the dominant flora in vacuum-packed smoked salmon at the end of the storage period [39]. Acetic acid is a common metabolite for a number of LAB which should have decreased pH values, though they were found to be constant (Table 1). Leroi [39] and Montiel [40] observed similar values, so pH could be neutralized by the bases (nitrogen compounds) formed as mentioned previously [36].

Table 1. TVB-N, Water (x^w), water activity (a_w), aerobic plate counts (log cfu g^{-1}) and pH, and their standard deviations (SD) of the vacuum-packed smoked salmon during storage (0, 20, 40 and 60 days).

Time (days)	TVB-N (mg/100g) ± SD	x^w ± SD	a_w ± SD	log cfu g^{-1} ± SD	pH ± SD
0	23.46 ± 0.23^a	59.44± 0.52^a	0.980 ± 0.001^a	3.22 ± 0.06^a	6.23 ± 0.06^a
20	27.29 ± 0.59^b	63.79± 0.93^b	0.961 ± 0.090^b	4.85 ± 0.10^b	6.27 ± 0.04^a
40	33.05 ± 0.38^c	61.68± 0.12^c	0.963 ± 0.001^b	5.50 ± 0.03^c	6.25 ± 0.03^a
60	44.18 ± 2.73^d	62.96± 0.14^{bc}	0.953 ± 0.001^c	7.23 ± 0.03^d	6.27 ± 0.08^a

Means in a column with different letters are significantly different (P≤0.05)

3.2 Image analysis

Image analysis of the data was carried out using three approaches to deal with the great amount of data from HI. They were random pixel selection (without filter), spatial mean (basic filter) and pixel selection based on colour (advance filter). Samples were divided into two groups according to the expired time: non-expired (days 0, 10, 20) and expired (days 40, 60, 70).

In the first approach, the PLS-DA model was developed without any filters, using 62 random pixels (px) for each sample. It was decided to use only 62 px for memory issues due to the large amount of data. The low cross validation results obtained using this technique (R^2C=0.37; R^2CV= 0.3) can be seen in Fig. 6. In order to increase the cross-validation results it was necessary to apply some kind of filter (data treatment).

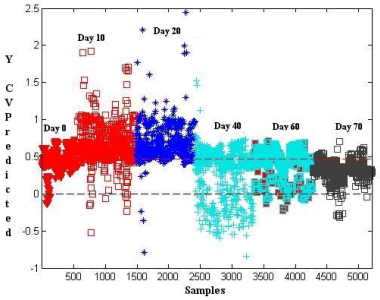

Figure 6: PLS-DA CV prediction model with random px.

The second approach used a basic, fast filter for the data before the PLS-DA model. In this case filters were the median and the mean of all the pixels for each sample. Table 2 shows the R^2C and R^2CV values as well as their RMSE for median and mean data treatment. There was a great improvement in comparison with the first approach (unfiltered data processing), but values of R^2CV suggested that noise could be present in the model building. Therefore, a third approach was used.

Table 2: Statistical results of vacuum-packed smoked salmon's PLS-DA models using the mean and the median px values.

	R^2Cal	R^2CV	RMSEC	RMSECV	Nº LV
Mean	0.926	0.797	0.136	0.253	12
Median	0.973	0.769	0.081	0.244	12

In the last approach pixels were chosen based on the visual colour from the reconstructed RGB image. To check the good performance of the RGB image reconstruction with the real samples, an entire sample of salmon (Fig 6a) was scanned with a resolution of 216 hyperspectral images. Using the RPLS model developed, as explained in the section materials and methods *2.3.1 Colour model*, an RGB image reconstruction was obtained. To reduce noise and increase the contrast between different tissues, a segmentation method was employed [41–43], in particular, a colour enhancement using multiscale fundamental forms (MFF) [44] based on a Mallat dyadic

wavelet [45]. MFF was carried out with a detail scale of 4, a compromise between increased detail and reduced noise. The improved RGB reconstruction after the dyadic wavelets is show in Fig 6b.

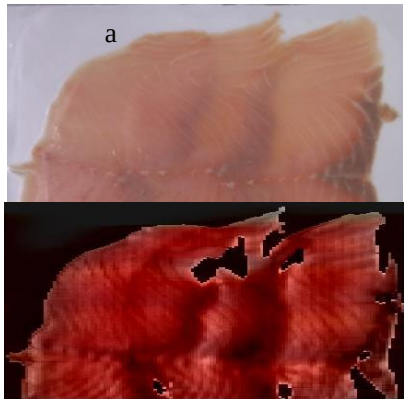

Figure 6a) Photograph of a vacuum-packed smoked salmon sample. b): Enhanced reconstructed image of salmon using the developed colour model.

Results of the statistical analysis for the four quartiles in the red and green channels for all the samples are shown in table 3. The best results were obtained employing the green channel. This result could be due to the fact that different salmon tissues were better segmented in this channel [46] while in the red channel several kinds of tissues were mixed in each quartile. The best quartile for detection of expired and non-expired samples was Q2 (R^2C= 0.907; R^2CV = 0.80). This quartile is composed of mainly lean tissue.

Table 3: Statistical results of the PLS-DA models for vacuum-packed smoked salmon based on red and green channel values

	Red channel				Green channel			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
R^2C	0.726	0.632	0.757	0.746	0.814	0.907	0.736	0.725
R^2CV	0.506	0.464	0.675	0.300	0.726	0.800	0.537	0.288
RMESC	0.260	0.302	0.246	0.251	0.215	0.152	0.256	0.261
RMSECV	0.396	0.377	0.289	0.572	0.284	0.228	0.367	0.537
Nº LV	7	6	6	9	9	7	10	9

Combinations of several quartiles in the green channel were also studied (Fig. 7) and it was found that using Q2 and Q3 the results were enhanced (R^2C= 0.97; R^2CV = 0.83, RMSEC: 0.08, RMSECV: 0.21). This could prove that the assumption that at least 50% of the pixel values are free of noise is correct.

Furthermore, the better prediction results that this last proposed data filter offers, also gives more guarantees that the data employed to perform the PLS-DA model are precise and there is no noise modelled, which could be important in a product like salmon, in which there are different tissues.

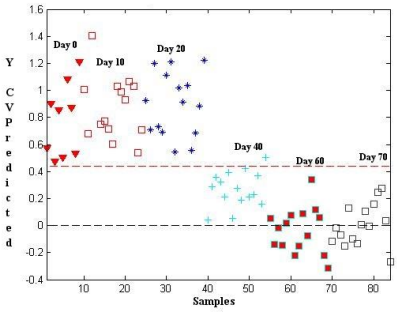

Figure7: PLS-DA CV prediction model using Q2 and Q3 pixel values.

4. Conclusions

The research was focused on resolving the important problem of detecting expired vacuum-packed smoked salmon in a non-destructive way. Hyperspectral imaging spectroscopy in the SW-NIR was proved to have enough information and accomplish the requirements for this application. However, hyperspectral images have noise that should be removed, and different salmon tissues are present inside the information. Thus, it is necessary to use an adequate data filter in order to achieve a reliable system. Experimental results comparing three filters (random pixel selection, mean and median and colour model segmentation) using PLS-DA models showed that the proposed colour model segmentation explained in this paper has the better R^2 in cross-validation (82.7%). The result achieved makes this technique very promising to resolve the problem set out.

As future work, it is planned to work on segmenting different salmon tissues with more accuracy using texture segmentations with edge detection [47] and neural networks [48]. Moreover, selection of wavelengths used could help not only to reduce the quantity of data but also enhance the results [49]. The possibility of extending this study to other food products with different tissues is also under consideration.

Acknowledgements

Vitae

Eugenio Ivorra received his degree in technical engineering in computer systems and master's degree in automatic and industrial computer science from *Universidad Politécnica de Valencia*, Spain, in 2008 and 2011. He is currently a Ph.D. student at the *Instituto Automática e Informática Industrial*, Spain. His research focus is hyperspectral imaging, and 3D applied to the food industry.

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