

## Preliminary Study using Visible and SW-NIR Analysis for Evaluating the Loss of Freshness in Commercially Packaged Cooked Ham and Turkey Ham

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### Abstract

GIRÓN J., IVORRA E., SÁNCHEZ A.J., FERNÁNDEZ-SEGOVIA I., BARAT J.M., GRAU R. (2014): **Preliminary study using visible and SW-NIR analysis for evaluating the loss of freshness in commercially packaged cooked ham and Turkey ham.** Czech J. Food Sci., 32: 376–383.

A non-destructive Vis-NIR spectroscopy (400–1000 nm) method was developed to evaluate the loss of freshness of sliced and commercially packaged cooked ham and turkey ham without any sample manipulation. The spectra were recorded at 0, 30, 40, and 60 days using a camera, spectral filter (400–1000 nm) and a halogen floodlighting system which had been developed and calibrated for the purpose. Physico-chemical, biochemical, and microbiological properties such as pH, total volatile basic nitrogen (TVB-N), ATP breakdown compounds, and colony-forming units were determined to predict the degradation of freshness. The image spectra obtained from visible and SW-NIR spectroscopy were related to the storage time of the samples. A PLS-DA model was developed independently for packaged or unpackaged samples using the second derivative of the spectra. Mean  $R^2$  prediction obtained for cooked ham was 0.915 and 0.949 for Turkey ham. The technique developed could be applied to monitoring the freshness of commercial packed cooked ham and turkey ham as a non-destructive technique. Further studies will be needed to check the spectra obtained from samples of different commercial brands in order to evaluate more precisely the efficiency of the method.

**Keywords:** spectroscopy; hyperspectral camera; storage time; cold meat

The meat processing industry and food retail stores are constantly looking for new solutions that improve the production or sales (more automation, productivity, control, traceability, safety, easier sanitation, and maintenance, etc). In particular, the meat sector for cooked whole muscle or mixed meat products is seeking ways to automating and achieving maximum reduction in time and costs in different phases of the process, with the goal of maximum increase in productivity and profitability. For this reason spectroscopic methods have gained importance in the evaluation of food quality attributes (NÁDAI 1983; NÁDAI & MIHÁLYI-KENGYEL 1984). The fact that NIR spectra reflect several parameters

of the material makes the method ideal for evaluating complex quality (WILLIAMS & NORRIS 2001). The near-infrared (NIR) spectroscopy technique is promising for fast, non-destructive analysis of biological materials (WU *et al.* 2008) as is able to evaluate complex parameters of the samples. The short-wave NIR region (700–1000 nm) allows NIR energy to penetrate more deeply into the sample with a much lower heating effect than the long-wave NIR region (1100–2500 nm). Also, short-wave NIR spectra can be used with inexpensive light sources (tungsten halogen lamps) and detectors (silicon diode arrays) (MAYES & CALLIS 1989). Various near infrared spectroscopic methods have also been published dealing with the

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prediction of the sensory quality criteria (WARM *et al.* 2001), evaluation of freshness (NILSEN *et al.* 2002), or detection fish spoilage (LIN *et al.* 2006). Monitoring the shelf-life of dairy products by diffuse reflectance spectroscopy has also been described (SINELLI *et al.* 2005; HORVÁTH *et al.* 2008). Also, spectroscopy has been evaluated as a powerful tool for the quality evaluation of meat products (OZAKI *et al.* 2007; PRIETO *et al.* 2009; WEERANANTANAPHAN *et al.* 2011). At present, there is little research into this technique to evaluate packaged and sliced meat products, and in most of these studies, the samples were manipulated (minced, placed in Petri dishes, etc.) prior to the measurement, changing their structure and the format in which the meat products are marketed. Therefore the aim of this study was to evaluate the feasibility of the image acquisition equipment developed, based on visible and short-wavelength near-infrared (SW-NIR) diffuse reflectance spectroscopy as a non-destructive method for evaluating the loss of freshness of sliced and commercially packaged cooked ham and turkey ham. Although the image acquisition equipment can work with moving samples, in this preliminary study the images were acquired without movement as it was first necessary to develop the image processing protocol.

## MATERIAL AND METHODS

The study was carried out on 60 samples of sliced turkey ham (a ready-to-eat product made from cured turkey thigh meat; USDA-FSIS 2003) and the same number of samples of cooked ham obtained from a commercial brand. At each sampling time, 10 samples of each were used. In order to establish the initial freshness parameter, products from different batches, but with the same expiration period (40 days), were chosen. The sampling was performed at 0, 30, 40 (expiration date given by the producer), and 60 days (in order to ensure shelf-life). For nucleotide analysis performed in triplicate, samples were taken on days 0 and 40, and also on days 15 and 25. During the study the samples were stored at 4°C.

At each sampling time, images were captured before opening the product packaging and just after taking them out of the fridge. After opening the packages aseptically, a representative sample of each was taken for microbiological analysis, according to the microbiological procedure. Then, with the opened samples, images were captured again and physicochemical analyses were carried out (after grinding and homogenising).

## Destructive analyses

*Analytical determinations.* For pH determination, mixtures of 10 g of the homogenised sample and 90 ml of distilled water were measured with a portable pH meter MM40 (Crison Instruments S.A, Alella, Barcelona, Spain) following the procedure proposed by FUENTES *et al.* (2008). Total Volatile Basic Nitrogen (TVB-N) was determined according to the procedures described by MALLE and TAO (1987).

*Nucleotides analysis.* The ATP-related compounds, consisting of inosine-5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), were assayed by HPLC according to the method described by BARAT *et al.* (2008), with some minor modifications. The extraction of nucleotides was performed following the method described by BURNS and KEE (1985). A total of 2 extracts were obtained per sample.

The compounds were identified using the retention times comparison of unknowns with those of standards, and by standard addition or "spiking" (JOHNSON & STEVENSON 1978). IMP, Ino, and Hx standards were obtained from Sigma-Aldrich (St. Louis, USA). Standards solutions were prepared in 0.6M HClO<sub>4</sub> neutralised with solid potassium carbonate.

*Microbiological analysis.* The aerobic plate counts (expressed as CFU/g) were estimated following the method proposed by the ISO 4833:2003. 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°C for 48 hours.

All destructive analyses were done in triplicate after the samples grinding and homogenising.

## Non-destructive analysis

*Spectra collection.* The images were taken using a CCD 102f camera (Basler Vision Technologies, Ahrensburg, Germany) and Specim ImSpector V10 1/2 filter (Specim Spectral Imaging, Ltd., Oulu, Finland), which works as a linear hyperspectral camera. The illuminants were two tungsten halogen lamps 50 W 230 V HI-SPOT (Havells Sylvania, Gennevilliers, France) producing indirect light to reduce reflections. The positions of the illuminant and camera relative to the sample were always constant to control the lighting conditions and to obtain a constant image size. The distance between the illuminant and the sample was

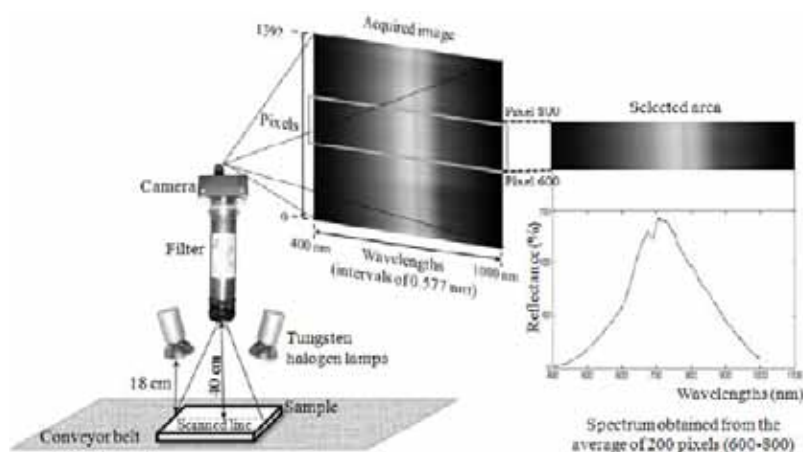


Figure 1. Spectral acquisition setup

18 cm, with a distance of 40 cm between the camera and the sample. The diffuse reflectance spectrum was collected using 1040 different wavelengths (each wavelength is digitalised by 8 bits) from the spatial averaging spectrum obtained from the scanned line composed of 1392 points (spectra), after removing those with scattering effects. The wavelengths were distributed in the range from 400 to 1000 nm with a resolution of 11.2 nm along 1040 pixels (Figure 1).

Image acquisition was performed using the following procedure: A line of light reflected from the sample enters the camera lens and is dispersed simultaneously into different wavelengths by an optical device. Then a two dimensional image, with spatial wavelength resolution, is formed and saved on the computer.

Reflectance calibration was performed in order to normalise the non-linear light source reflectance. This was done by dividing the reflectance value by the white pattern reflectance measured under the same conditions. The dark measurement was obtained by switching off the light source and adjusting the gain and offset settings.

Image preprocessing was required to remove the surface reflection effects and other non-chemical biases from the spectral information. After image preprocessing, a the the segmentation process was performed to select the sample information. The samples spectra were calculated as the means of their selected pixels on the spatial dimension. Other operations carried out for further statistical processing were mean-centring and unit variance normalisation. In addition the second derivative of the entire spectrum was calculated employing the method based on Savitzky-Golay convolution functions (BOUZIDI *et al.* 2005). The second derivative technique is often used to process the NIR data. It helps to separate the overlapping absorption bands, remove baseline shifts, and increase apparent spectral resolution (LIN *et al.* 2004).

Image acquisition was operated by the software pylon Viewer 2.1.0.1664 (Basler Vision Technologies, Ahrensburg, Germany), and image reflectance calibration and preprocessing were performed by a code developed on Matlab (The Mathworks, Natick, USA) (SÁNCHEZ *et al.* 2008).

Although the image acquisition equipment can work at 15 frames per second, which permits working with moving samples, in this preliminary study the images were acquired without motion as it is necessary first to develop the image processing protocol.

### Statistical analysis.

The effect of the storage time on the variables (TVB-N,  $K_1$ , and  $\log(\text{CFU/g})$ ) was subjected to a variance study (ANOVA). In those cases where the effect was significant, the means were compared using Fisher's least significant difference (*LSD*) procedure.

The data spectra classification based on the storage time was carried out employing Partial Least Squares Discriminant Analysis (PLS-DA) (WALLAYS *et al.* 2009). PLS-DA is very similar to Linear Discriminant Analysis (LDA). In fact, BARKER and RAYENS (2003) have shown that PLS-DA is essentially the inverse least squares approach to LDA and produces essentially the same results but with the noise reduction and variable selection advantages of Partial Least Squares (PLS). In PLS-DA,  $\text{PLS}_s$  is used to develop a model that predicts the class number for each sample.  $\text{PLS}_s$  is a powerful multivariate calibration method used to correlate NIR spectra with other variables.

To estimate the average deviation of the model from the data, the Root-Mean-Square Error of Calibration (RMSEC) was used. It is defined as (Eq. 1):

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

where:  $\hat{y}_i$  – value of the predicted variable when all samples are included in the model formation;  $y_i$  – known value;  $n$  – total number of objects in the data set

Cross-validation was used to evaluate the performance of the developed models. The Cross-validation method employed was Random Subsets: different test sets were determined through random selection of  $n/8$  samples, so that no single sample was in more than one test set. This procedure was repeated three times. An upper limit of 20 PLS factor ( $LV_s$ ) was set for PLS-DA models. The Root-Mean-Square Error of Cross-Validation (RMSECV), which is the measure of the model ability to predict the samples that were not used to build the model, was used to evaluate and compare the accuracy of the different PLS-DA models developed. Equation (1) was used, but in this case the parameters  $\hat{y}_p$ ,  $y$ , and  $n$  were defined as:  $\hat{y}_i$  – reference values of the variable that are estimated by cross-validation (where the value for each object  $i$  is estimated using a model that was built with a set of objects that does not include object  $i$ );  $y$  – values of the variable;  $n$  – total number of objects in the data set.

The relationship between the spectra and the values obtained from the destructive analyses was obtained by partial least square regression (PLS) setting an upper limit of 20 PLS factor ( $LV_s$ ) (BHARATI & CHAMPAGNE 2004).

For PLS-DA and PLS statistical procedure 2/3 of the samples were used for the calibration and cross-validation and the remaining 1/3 for testing the model.

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

## RESULTS AND DISCUSSION

Table 1 shows the destructive analyses (pH, TVB-N and microbiological counts) and the standard deviations (SD) for sliced, packed, cooked ham and turkey ham on days 1, 30, 40, and 60 at 4°C. With both products, all parameters presented significant differences ( $P < 0.05$ ) during the storage time, therefore the influence of the sampling time was evaluated.

The pH values, initially higher than 6, as a consequence of the brining (SAMELISA *et al.* 2000), decreased significantly ( $P < 0.05$ ) with time. The decrease in pH may be due to the lactic acid bacteria that prevail in the cooked end product (BORCH *et al.* 1988; BLIXT & BORCH 2002; PING *et al.* 2009), reaching values close to 5.3 for the spoiled product (SAMELISA *et al.* 2000; VASILOPOULOS *et al.* 2008; PING *et al.* 2009).

In both products, TVB-N (mg/100 g) levels increased significantly with time ( $P < 0.05$ ) (Table 1), although at the end of the study (60 days) the values were lower than the limit set for fish (30 mg/100 g) (EEC 2005). This analysis is only legislated for some types of fish, although it can give information on the biochemical changes occurring during the storage period of some products such as dry-cured ham (LORENZO *et al.* 2008) and camel meat (AL-BACHIR & ZEINO 2009) but not to express the expiry date of the product.

Microbiological analysis showed statistical differences ( $P < 0.05$ ), for both products, during storage. Aerobic plate count (PCA) values increased gradually, reaching values close to 7 log (CFU/g) at the expiration date (40 days). A combination of micro-aerophilic conditions, the presence of NaCl and NaNO<sub>2</sub> and a reduced  $a_w$  inhibits the growth of Gram-negative spoilage flora, favouring the growth of psychrotrophic

Table 1. pH, total volatile basic nitrogen (TVB-N), aerobic plate counts (log (CFU/g)), and the standard deviation (SD) of packaged sliced cooked ham and turkey ham during storage time (0, 30, 40, and 60 days)

Sample	Time	pH	TVB-N (mg/100 g)	log (CFU/g)
Cooked ham	0	6.13 ± 0.05 <sup>a</sup>	18.2 ± 0.2 <sup>a</sup>	3.91 ± 0.08 <sup>a</sup>
	30	5.86 ± 0.08 <sup>b</sup>	21.0 ± 0.3 <sup>b</sup>	5.82 ± 0.01 <sup>b</sup>
	40	5.68 ± 0.01 <sup>c</sup>	23.9 ± 0.2 <sup>c</sup>	6.91 ± 0.01 <sup>c</sup>
	60	5.47 ± 0.06 <sup>d</sup>	25.3 ± 0.2 <sup>d</sup>	8.71 ± 0.06 <sup>d</sup>
Turkey ham	0	6.01 ± 0.04 <sup>a</sup>	16.3 ± 0.2 <sup>a</sup>	1.51 ± 0.01 <sup>a</sup>
	30	5.99 ± 0.04 <sup>b</sup>	24.0 ± 0.3 <sup>b</sup>	4.83 ± 0.80 <sup>b</sup>
	40	5.97 ± 0.02 <sup>c</sup>	26.2 ± 0.1 <sup>c</sup>	7.73 ± 0.01 <sup>c</sup>
	60	5.25 ± 0.01 <sup>d</sup>	27.6 ± 0.4 <sup>d</sup>	7.81 ± 0.04 <sup>c</sup>

Means in a column with different letters (for each product) are significantly different ( $P < 0.05$ )



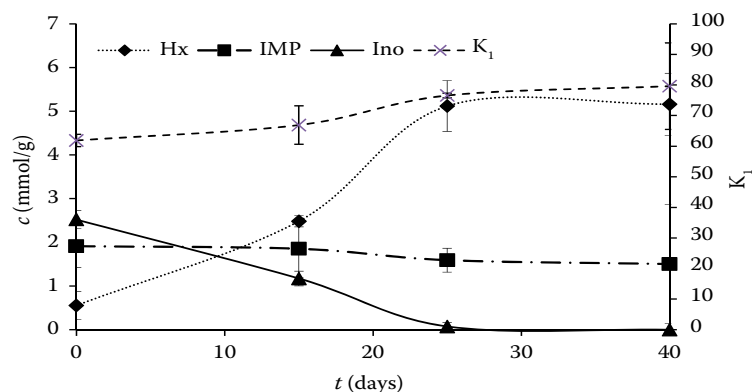


Figure 2. Changes in the concentration of nucleotide compounds

IMP – inosine 5'-monophosphate; Ino – inosine; Hx – hypoxanthine) for cooked ham; values shown are the means of measurements in triplicate and the standard deviation (bars)

lactic acid bacteria (BORCH *et al.* 1996; KORKEALA & BJORKROTH 1997; HU *et al.* 2009). Hence, the obtained results are within the growing models proposed for lactic acid bacteria in cooked ham (LEROY *et al.* 2009).

The evolution of the concentrations of the three ATP derivatives (inosine 5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx)) during storage is shown in Figure 2. The values of hypoxanthine increased until day 25, then remaining constant until the end of the study. IMP showed no variation during the storage time, so the Hx evolution could be related to the Ino decreases and maybe to the action of the nucleoside phosphorylase (NP) enzyme produced by the same spoilage bacteria as reported in some studies on fish muscle (HERNÁNDEZ-CÁZARES *et al.* 2011). So Hx could express the loss in freshness during the first 25 days. A similar behaviour was observed for turkey ham.

### Non-destructive analysis (Image analysis)

The extracted mean spectrum of turkey ham after image preprocessing, mean-centering, unit variance normalisation, and smoothing is shown in Figure 3. Smoothing the spectrum was only carried out for a better visualisation of the information. Visual inspection of the spectra can easily divide the means of the samples from day 0 to day 60 and days 30–40 but the dispersion of each sample made multivariate statistical analysis necessary to achieve reliable results.

The results of the calibration, cross-validation, and storage time prediction employing Partial Least Squares Discriminate Analysis (PLS-DA) for both products, employing the second derivative of the spectra, are shown in Table 2. When non derivative procedure of the spectra was performed, 8 PLS factors (latent values  $LV_s$ ) were obtained for cooked ham with a mean  $R^2$  prediction ( $R^2 P$ ) of 0.787. When the second derivative was employed, only 3  $LV_s$  were

selected with a mean  $R^2 P$  of 0.915. The same behaviour was observed with the samples of Turkey ham.

The low values of the RMSE and the high ones of the coefficients of determination ( $R^2$ ) for prediction in both products showed how this imaging technique could be used to define the storage time of the samples. Moreover, the use of small numbers of PLS factor, 3 for turkey ham and 5 for cooked ham (Table 2), makes the models calculations faster and easier to implement for a possible future in-line device.

Figure 4 shows the three firsts  $LV_s$  obtained from the PLS-DA study of the second derivative of the spectra of the turkey ham samples. As can be observed the clusters formed by the PLS-DA could be differentiated very well according to the storage time. In addition, it was used packed and unpacked samples with no effects on the discrimination of the model. In fact, the samples with the presence or not of the plastic film did not form any subcluster inside the time clusters. These observations were consistent with our previous results (GRAU *et al.* 2010) where it was found that the addition of film to chicken meat samples caused only

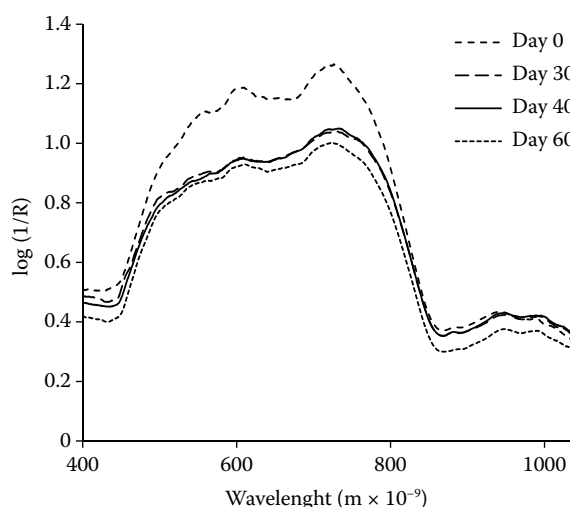


Figure 3. Mean spectra of turkey ham after mean-centering, unit variance normalisation, and smoothing

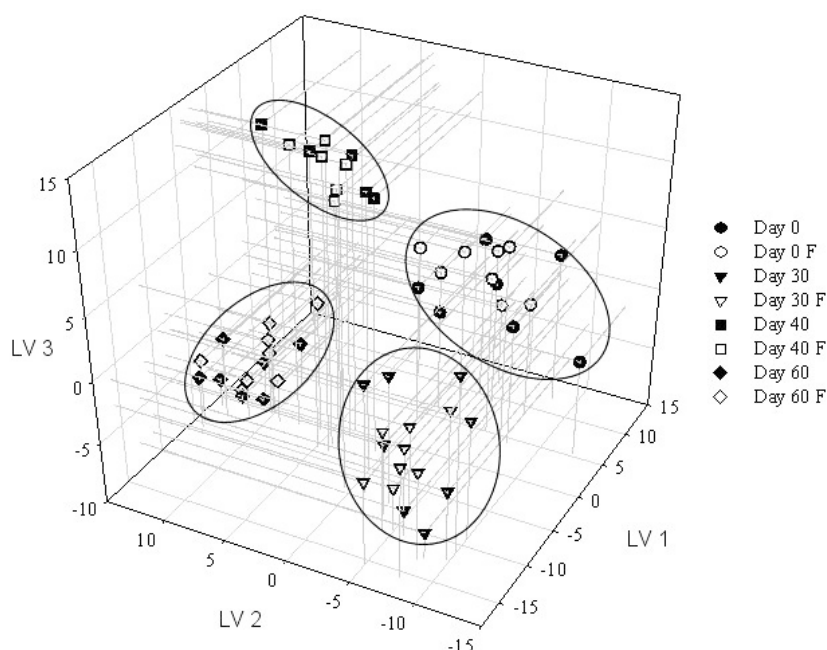


Figure 4. Predicted cooked ham samples by PLS-DA study using the second derivative of the spectra. The samples with "F" mean that they were packed

slight, or negligible, increases in the optimal prediction errors with the SW-NIR method. The results in the Table 2 were obtained from packed and unpacked (opened) samples, thus this model could be applied with success to either of these cases.

Correlation between image analysis (non-destructive analysis) and the physico-chemical and microbiological analysis (destructive analysis)

The relationship between the second derivative spectra and the values obtained from the destructive parameters with a significant influence of the storage time (TVB-N, pH, and log (CFU/g)) are shown in Table 3. This study was done in order to analyse in detail each sample evolution because samples with the same storage time manifest different freshness losses. PLS setting an upper limit of 20 PLS factors ( $LV_s$ ) (BHARATI & CHAMPAGNE

2004) was employed. In both products, the values of  $R^2 C$ ,  $R^2 CV$ , and  $R^2 P$  were high for all parameters although they were slightly higher for turkey ham using lower  $LV_s$ . The greatest changes in the freshness losses with the turkey samples (greater variation of the physico-chemical parameters evaluated, Table 2) could explain the better results for this product. Despite the study of the spectra and their second derivatives, no definitive identification of any of the peaks or troughs could be related to a specific structure; however, the models developed for the prediction of TVB-N, pH, and microbial counts are expected to provide realistic results. This could be explained by the fact that the parameters represent indirect measurements and therefore no specific absorbance bands can be recognised in the SW-NIR region.

Table 2. Statistical results of the Partial Least Squares Discriminant Analysis (PLS-DA) of turkey ham and cooked ham for the second derivative of the spectra

	$LV_s$	Time (days)	RMSEC	$R^2$ calibration	RMSECV	$R^2$ cross-validation	RMSEP	$R^2$ prediction
Cooked ham	3	0	0.075	0.977	0.131	0.96	0.118	0.959
		30	0.062	0.977	0.116	0.96	0.12	0.904
		40	0.079	0.961	0.131	0.922	0.138	0.899
		60	0.065	0.952	0.131	0.893	0.158	0.899
Turkey ham	3	0	0.068	0.981	0.102	0.969	0.087	0.978
		30	0.066	0.978	0.100	0.962	0.095	0.952
		40	0.065	0.974	0.114	0.943	0.118	0.946
		60	0.072	0.947	0.116	0.909	0.133	0.919

$LV_s$  – PLS factor; RMSEC – root mean-square error calibration; RMSECV – root-mean-square error of cross-validation; RMSEP – root mean-square error prediction

Table 3. Statistical results of the square regression (PLS) for the second derivative of spectra and results from destructive analysis

		RMSEC	$R^2$ calibration	RMSECV	$R^2$ cross-validation	RMSEP	$R^2$ prediction
Cooked ham	TVB-N	0.215	0.994	0.781	0.949	1.023	0.866
	log (CFU/g)	0.094	0.992	0.322	0.948	0.488	0.893
	pH	0.167	0.995	0.060	0.963	0.086	0.893
Turkey ham	TVB-N	0.361	0.994	1.113	0.949	0.666	0.98
	log (CFU/g)	0.200	0.994	0.626	0.957	0.425	0.984
	pH	0.021	0.992	0.070	0.948	0.081	0.950

The prediction results for an independent test set are expressed as both the root mean square error (RMSE) and the determination coefficient ( $R^2$ ) for calibration (C), cross-validation (CV), and prediction (P) models; the number of LV<sub>s</sub> in all cases was 3

## CONCLUSION

The loss of freshness in cooked ham and turkey ham has mainly been associated with an increase in the microbial loads (CFU/g) and TVB-N values and a decrease in pH values although these cannot be used to define the consumer deadline. The image spectra obtained from visible and SW-NIR spectroscopy were related to the storage time of the samples. A PLS-DA model was developed independently for packaged or unpackaged samples using the second derivative of the spectra. So the technique developed could be applied to monitoring the freshness of commercial packed cooked ham and turkey ham as a non-destructive technique.

Further studies will be needed to check the spectra obtained from samples of different commercial brands in order to evaluate more precisely the efficiency of the method.

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