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ORIGINAL PAPER

Evaluation of the Spoilage of Raw Chicken Breast Fillets Using Fourier Transform Infrared Spectroscopy in Tandem with Chemometrics

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Abstract The aim of this work was to evaluate the potential of Fourier transform infrared (FTIR) spectroscopy as a rapid and accurate technique to detect and predict the onset of spoilage in fresh chicken breast fillets stored at 3, 8, and 30 °C. Chicken breasts were excised from carcasses at 6 h post-mortem; cut in fillets; packed in air; stored at 3, 8, and 30ºC; and periodically examined for FTIR, pH, microbiological analysis, and sensory assessment of freshness. Partial least squares regression allowed estimations of total viable counts (TVC), lactic acid bacteria (LAB), *Pseudomonas* spp., *Brochothrix thermosphacta*, *Enterobacteriaceae* counts and pH, based on FTIR spectral data. Analysis of an external set of samples allowed the evaluation of the predictability of the method. The correlation coefficients (R^2) for prediction were 0.798, 0.832, 0.789, 0.810, 0.857, and 0.880, and the room mean square error of prediction were 0.789, 0.658, 0.715, 0.701, 0.756 log cfu g^{-1} and 0.479 for TVC, LAB, *Pseudomonas* spp., *B. thermosphacta*, *Enterobacteriaceae*, and pH, respectively. The spectroscopic variables that can be linked and used by the models to predict the spoilage/freshness of the samples, pH, and microbial counts were the absorbency values of 375 wave numbers from 1,700 to 950 cm^{-1}. A principal component analysis led to the conclusion that the wave numbers that ranges from 1,408 to 1,370 cm^{-1} and from 1,320 to 1,305 cm^{-1} are strongly connected to changes during spoilage. These wave numbers are linked to amides and amines and may be considered potential wave numbers associated with the biochemical changes during spoilage. Discriminant analysis of spectral data was successfully applied to support sensory data and to accurately bound samples freshness. According to the results presented, it is possible to conclude that FTIR spectroscopy can be used as a reliable, accurate, and fast method for real time freshness evaluation of chicken breast fillets during storage.

Keywords Chicken breast spoilage · IR spectroscopy · PLS-R · FTIR · Chemometrics

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Introduction

The consumer demand for chicken meat is increasing, due in part to dietary health considerations (Ellis et al. 2002; Sahar et al. 2011). Distribution chain agents, retailers, and consumers ask for long shelf life as well as good quality and safety throughout the entire shelf life period. This is a challenge to the meat industry as they have to optimize the processes in order to achieve the best shelf life (Nychas et al. 2008; Walker and Betts 2000).

Chicken meat is a highly perishable food; therefore, it becomes important to improve quantitative measurements of spoilage in order to monitor the quality of the meat (Guevara-Franco et al. 2010; Lin et al. 2004, Sahar et al. 2011). This can be affected by factors such as health, age, sex, chicken carcasses condition at the time of slaughter, type of packaging, and storage conditions used (Huis in't Veld 1996; Jiménez et al. 1997). After slaughtering, raw chicken meat could deteriorate in 4 to 10 days, even when stored under refrigeration (Jiménez et al. 1997; Lin et al. 2004; Sahar et al. 2011).

The freshness can be defined by various indicators, including microbial enumeration methods, such as total viable counts (TVC), volatile compound analysis, total volatile basic nitrogen (TVB-N), biogenic amines index, measurement of lipid oxidation (Balamatsia et al. 2006, 2007), and sensory evaluation, which is the most frequently used method for freshness evaluation of meat and meat products in industry and retail deliveries (Guevara-Franco et al. 2010; Nychas et al. 2008).

However, most of these methods have the disadvantage of being invasive, often expensive, time consuming, and, in the case of sensory analysis, is unsuitable for online monitoring and may not provide rapid results. Thus, it is important to develop new rapid detection methods of meat spoilage to guarantee its safety and quality (Archer 1996; Ellis et al. 2002; Guevara-Franco et al. 2010; Lin et al. 2004; Sahar et al. 2011).

The application of Fourier transform infrared spectroscopy (FTIR) analysis to monitor meat spoilage is not new, and it has been reported by previous researchers (Ellis et al. 2002, 2004; Ellis and Goodacre 2001; Papadopoulou et al. 2011). Ellis et al. (2002) showed that the microbial count and spoilage in comminuted chicken stored at room temperature can be monitored by FTIR, suggesting the beginning of spoilage when microorganisms have reached 10^7 cfu g^{-1}. In those studies, the application was specified on comminuted beef, pork, and poultry meats.

FTIR was also successfully used to distinguish microbial cells producing biochemical fingerprints, leading to identification of bacterial species and strains (Mossoba et al. 2003; Naumann et al. 1991; Rodriguez-Saona et al. 2001) and has been successfully employed to correlate spectral data to microbial counts (Ellis et al. 2002; Ellis and Goodacre 2001) with the objective of TVC prediction. However, no effort was made to take into account other specific microbial groups which are representative of the microbial dynamics during meat spoilage, such as *Pseudomonas* spp., LAB, *B. thermosphacta*, and *Enterobacteriaceae* (Doulgeraki et al. 2012). *B. thermosphacta* is psychrotrophic; commonly linked with fresh meats spoilage; and has the ability to grow during storage in air, vacuum, and modified atmosphere packaging. Consequently, it is a substantial meat colonizer and an important portion of the spoilage microbiota, being occasionally the dominant organism (Labadie 1999; Nychas et al. 2008).

Numerous studies have already applied near infrared spectroscopy (NIR) for microbial identification and quantification both in isolated systems (membranes and water solution) and food (Sousa Marques et al. 2013; Tito et al. 2012).

NIR and short wavelength (SW) spectroscopy was used in conjunction with multivariate statistics for the determination of *Enterobacteriaceae* on chicken fillet (Feng et al. 2013) and for the assessment of freshness in packaged sliced chicken breasts (Grau et al. 2011). Despite the excellent work presented by Alexandrakis et al. (2009) for the detection of spoilage of intact chicken breast muscle using NIR and FTIR, to our knowledge, the potential of FTIR has not been fully evaluated for qualitative and quantitative assessment of spoilage of chicken breast fillets stored at both chill and abusive temperatures. Also, there is scarce data on FTIR potential to predict pH and LAB, *B. thermosphacta*, and *Pseudomonas* spp. population in chicken breast fillets.

The objective of this study was to explore the potential of FTIR as a rapid and accurate method to detect and predict the onset of spoilage in fresh chicken breast fillets stored at 3, 8, and 30 °C. TVC, LAB, *B. thermosphacta*, *Pseudomonas* spp., and *Enterobacteriaceae* counts and pH, associated with chicken breast fillets spoilage and its relation with FTIR spectral data, were studied for several storage times and temperatures.

A further objective of this work was to demonstrate the possibility of building PLS-R-based models in view to predict fresh chicken breast spoilage from measured IR spectra. Correlation of spectral data with freshness/spoilage categories defined by the sensory panel is presented.

Material and Methods

Sampling

Chicken breasts were excised from carcasses at 6 h (t0) postmortem and were cut in fillets of 3×4×1 cm, weighing approximately 20 g, and packed in air overwrapped with polyethylene (PE) film. Following packaging, samples were stored at 3, 8, and 30ºC and examined at intervals of 96, 168, 240, and 336 h for the first two temperatures and at 7, 24, and 48 h of storage for samples kept at 30ºC.

The experiment was repeated six times over a period of a few months. At each sampling point, two samples were analyzed for different parameters, specifically, spectroscopic, microbial, physical-chemical determinations, and sensory analysis. Therefore, a total of 144 samples were analyzed.

While the temperature of 3ºC was chosen as correct storage temperature for chicken breast fillets and the exposition limit for selling is 4ºC, the value of 8 °C is the typical extreme high temperature of a home refrigerator. The temperature of 30ºC is an extreme value that was used to reach a wide band of experimental conditions.

Microbial Analysis

The number of microorganisms potentially associated with chicken meat spoilage was counted, namely LAB, *B. thermosphacta*, *Enterobacteriaceae*, *Pseudomonas* spp., and TVC. *Escherichia coli* was also evaluated in our study as a pathogenic microorganism, but was not considered in this paper because it is not considered a specific spoilage microorganism, therefore, not relevant in the presented work.

Meat cuts were sampled aseptically at each interval. Samples were homogenized with tryptone salt (tryptone

0.1 % and NaCl 0.85 %) in a Stomacher for 90 s. Serial decimal dilutions were prepared in the same solution for microbiological determinations.

TVC were obtained on Plate Count Agar (CM325, OXOID, England) (30ºC, 72 h); LAB on double layer on Man Rogosa Sharpe agar (CM361, OXOID, England) (30ºC, 72 h); *Enterobacteriaceae* on double layer Violet Red Bile Glucose agar (CM485, OXOID, England) (37ºC, 24 h); *B. thermosphacta* on Streptomycin Thallous Acetate and Actidione agar (CM881, SR151, OXOID, England) (25ºC, 48 h); and *Pseudomonas* spp. on selective Cetrimide, Fucidin, and Cephaloridine agar (CM0559, SR0103, OXOID, England) (25ºC, 48 h), according to ISO 4833 (2003), NF V 04-503 (1988), ISO 21528-2 (2004), ISO 13722 (1996), and NF V04-504 (1998).

The enumeration of *Enterobacteriaceae* (ISO 21528-2, 2004) and *Pseudomonas* spp. (NF V04-504 1998) was performed by biochemical tests and oxidase test.

In case the microorganism counts were below the detection limit, the result was considered to be zero for statistical purposes.

Physical-Chemical Measurements: pH

The pH was measured directly in the muscle using a penetration electrode with a pH meter (Crison Instruments, Spain) and was evaluated in duplicate immediately after opening the packages.

FTIR Measurement

Infrared spectra were collected in a FTIR spectrometer (Mattson, Unicam Research Series, USA) equipped with a single reflection attenuated total reflection (ATR) module (Golden Gate, UK), a DLaTGS detector, and a KBr beamspliter. The equipment is connected to a computer and controlled by WinFirst Software.

For the spectroscopic measurements, the samples were placed on top of the ATR crystal, which was kept at 30ºC, ensuring that the aerobic surface of the meat was in close contact with the crystal and then pressed with the gripper. Assuming that meat is mainly composed of water, calculation using equation 2.7 of Stuart (2004) showed that the evanescent field was probing a depth of approximately 1.0 μm. All infrared spectra were recorded from 900 to 2,000 cm^{-1}, co-adding 128 interferograms at a resolution of 2 cm^{-1}. The collection time for each sample spectrum was approximately 2 min. These spectra were subtracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR base was carefully cleaned in situ by scrubbing with ethanol (99.9 %) and dried with soft tissue before measuring the next sample. The cleaning method was verified by collecting a background spectrum and compared to the previous one. These spectra were recorded as absorbance values at each data point. For each sampling occasion, two replicate samples were analyzed by FTIR, each replicate was measured twice and the spectra averaged.

Mathematical Treatment

Spectral data collected between 900 and 2,000 cm^{-1} were initially submitted to smoothing based on the Savitzky-Golay algorithm (Savitzky and Golay 1964). Afterward, mean-centered and standardized spectra were subjected to a principal component analysis (PCA) to inspect differences between samples. The PCA transforms the large number of potentially correlated factors into a smaller number of uncorrelated factors (principal components, PCs), and thus reduces the size of the data set (Abdi and Williams 2010).

For qualitative analysis, principal components contributing to the variance of the data set were subjected to discriminant analysis (DA) in an attempt to predict the likelihood of a sample belonging to a previously defined group. Since the raw spectral data could not be used because of the strong correlation between the wave numbers, uncorrelated PCs resulting from PCA were employed. DA is a statistical method used to find a linear combination of structures which characterizes or separates classes of objects or observations (McLachlan 2004). The resulting arrangement may be used as a linear classifier or dimensionality reduction priori to classification.

For quantitative analysis, the measured microbial, physical-chemical, and sensory parameters, factors considerably contributing to the variance of the data set, were regressed using partial least squares regression (PLS-R) onto the referred variables (Liang and Kvalheim 1996; Wentzell and Montoto 2003). This multivariate calibration technique, sometimes called factor analysis, transform the original variables (FTIR spectra absorbencies) into the new ones (known as latent variables), which are linear combination of original variables (Miller and Miller 2005).

The method relied on two steps, the so-called calibration and cross-validation steps. In the calibration step, a mathematical model was built to establish a correlation between the matrix of FTIR spectra (predictor variables, X) and the concentration of analytes of interest (response variables, Y) used a set of observations usually named calibration set. In the cross-validation step, the developed calibration model was used to calculate the concentration of samples not used to set up the model (De Luca et al. 2009). The dependent variable (Y) was either the sensory classes for DA or the measured pH and bacterial counts for PLS-R analysis.

The relative performance of the established model is accessed by the root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and multiple coefficient of determination or regression coefficient (R^2) (Divya and Mishra 2007). The model selected is then used to determine the concentration of the

samples in an independent prediction set. The predictive ability of the model is evaluated from the root mean square of prediction (RMSEP). The lower the RMSEP value, the higher the degree of accuracy of the prediction result provided by the calibration model (Corgozinho et al. 2008).

PCA, DA, and PLS-R calculations were performed using the Excel-based XLSTAT V2006.06 package (Addinsoft, Inc, NY, USA) and statistical software Unscrambler V9.6 package (Camo, Oslo, Norway).

Sensory Analysis

Sensory evaluation of chicken breast fillets was performed during storage by a panel of eight members (with a minimum of six members per session) consisting of graduate professors and staff of the lab with experience on meat sensory evaluation by virtue of having participated in several meat sensory panels over the last years.

Sensory evaluation was carried out under controlled conditions of light (white fluorescent lamps) in sensory booths. The presentation of samples in each session was randomized and evaluation data were collected on a separate profile sheet. Each evaluation was carried out once. Chicken breast samples were assessed immediately after opening the package. Meat quality was rated on a non-structured scale extending from 0 to 15 cm ISO 4121 (2003), based on the perception overall assessment of freshness (0=extremely spoiled, 15=extremely fresh).

Mean scores of the examined samples by the panelists were used to include each sample in one of the three defined categories of freshness: fresh (>10 cm), semi-fresh (>5 and ≤10 cm), and spoiled (≤5 cm), according to the stage of freshness/spoilage.

In this work, the classification of chicken meat samples by a panel of sensory analysis in three distinct freshness/spoilage categories is a more lifelike approach to consumer insight about meat spoilage which was used by Papadopoulou et al. (2011). A third category of "semi-fresh" has been introduced between "fresh" and "spoiled" categories, representative of the early stage of spoilage where the meat has developed slight off-odors but it is still acceptable for consumption.

Results and Discussion

Microbial and Sensory Data

The TVC of chicken breast samples indicated that the total microflora was 4.2±0.5 log cfu g^{-1} at the onset of storage (samples considered as fresh) to 9.8±0.1 log cfu g^{-1} for samples characterized as completely spoiled. The hedonic mark "fresh", "semi-fresh" and "spoiled" was attributed to samples having average TVC of 5.1±1.3, 7.6±1.5, and 8.7±1.1 log cfu g^{-1}, respectively. Actually, depending on the storage parameters, samples were rejected by the sensory panel (classified as Spoiled) at a TVC between 7.7 and 9.9 log cfu g^{-1}. Therefore, a reasonable correlation was observed between measured TVC levels and the sensory assessment of freshness classification attributed by the sensory panel. These results are in agreement with Nychas and Tassou (1997) and Sahar et al.(2011), referring that the general

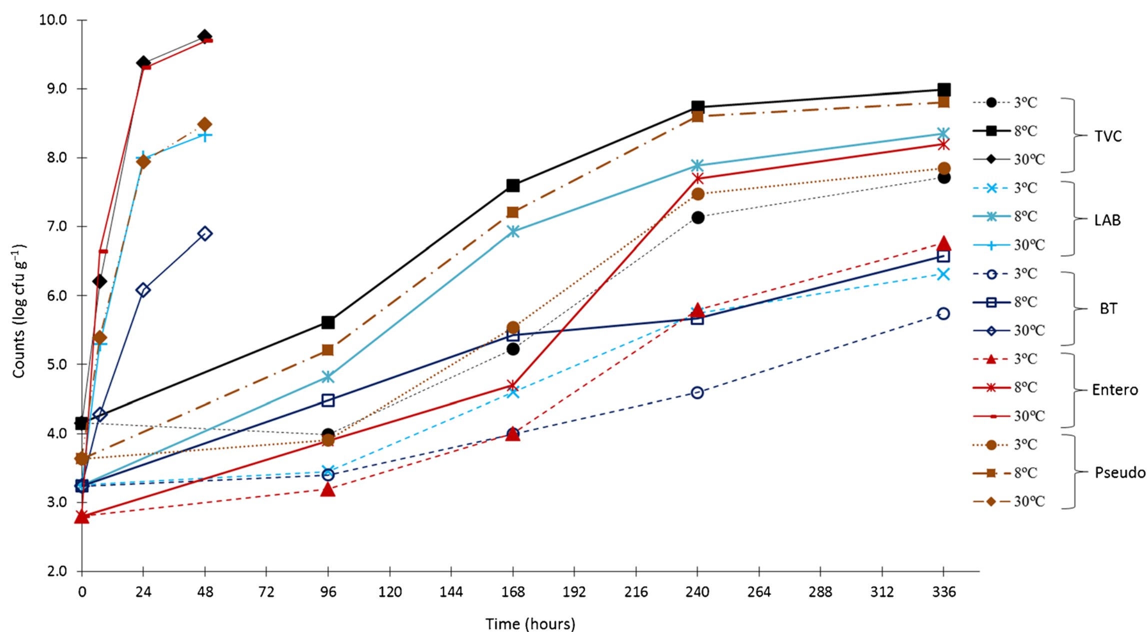

Fig. 1 Time evolution of total viable counts (*TVC*), lactic acid bacteria (*LAB*), *B. thermosphacta* (*BT*), *Enterobacteriaceae* (*Entero*), and *Pseudomonas* spp. (*Pseudo*) for chicken breast fillets stored aerobically at 3, 8, and 30ºC

sensory spoilage of chicken fillets stored under aerobic conditions is detectable when the TVC is around 8 log cfu g^{-1}.

The microbiological analysis indicated that *Pseudomonas* spp., LAB, *B. thermosphacta*, and *Enterobacteriaceae* are the initial microbiota of chicken breast fillets. The time evolution of the population of these groups depends on the storage temperature, as illustrated in Fig. 1. The temperatures of storage were found to affect the microbial development and the competition between microbial groups of the meat and consequently, the spoilage process, as referred previously by Nychas et al. (2008). It was perceived that *Pseudomonas* spp. constituted the predominant population at 3 and 8 °C, presenting a fast increase at 8°C.

At 3°C, the pattern of spoilage is dominated by the slow proliferation of *Pseudomonas* spp., reaching levels near 5 log at 168 h and 7 log cfu g^{-1} at 240 h, while the counts of LAB and *Enterobacteriaceae* are around 4 log cfu g^{-1} at 168 h, and did not achieved 7 log cfu g^{-1} at the end of the storage time. Different species of these microorganisms have different minimum temperatures of development ranging between 1.3 and 8.7 °C (Crowley et al. 2005). At 8°C, *Pseudomonas* spp. predominated the microbial population with average levels of 8.81 log cfu g^{-1} at the end of the storage. The LAB counts are always slightly lower compared to the previous. Higher levels of *Enterobacteriaceae* are obtained at 268 h, reaching 8.2 log cfu g^{-1} at the end of storage.

According to Labadie (1999), *Pseudomonas* spp. are always dominant after a few days storage at temperatures ranging between 0 and 7 °C in any type of meat (Ercolini et al. 2009; Molin and Ternström 1982). *Pseudomonas* spp. are followed by the development of LAB, *Enterobacteriaceae*, and *B. thermosphacta*.

Lin et al. (2004) reported that early stages of the spoilage of chicken samples packed in air were mainly associated with *Pseudomonas* spp. and, after the oxygen depletion, LAB were the dominant microorganisms on the chicken meat. However, in our work, *Pseudomonas* spp. counts are always higher than LAB counts for 3 and 8°C, indicating that *Pseudomonas* spp. grows faster in aerobic storage under refrigeration than other bacteria, remaining predominant in the last stage of spoilage. This was also referred by other authors (Gill and Newton 1977; Dainty and Mackey 1992; Borch et al. 1996).

This can be justified because in general, meat spoilage is connected to the composition of the microbial population and also the nature of substrates (glucose, lactate, among others) present in meat (Nychas and Skandamis 2005; Nychas et al. 2008). After depletion of glucose, spoilage under aerobic atmosphere is most frequently associated with the catabolism of nitrogenous compounds and free amino acids by *Pseudomonas* spp. (Gill 1986; Nychas et al. 2008). It was recognized that in those conditions, the free amino acids increased during storage, revealing consistency with the counts of bacteria (Nychas et al. 2008).

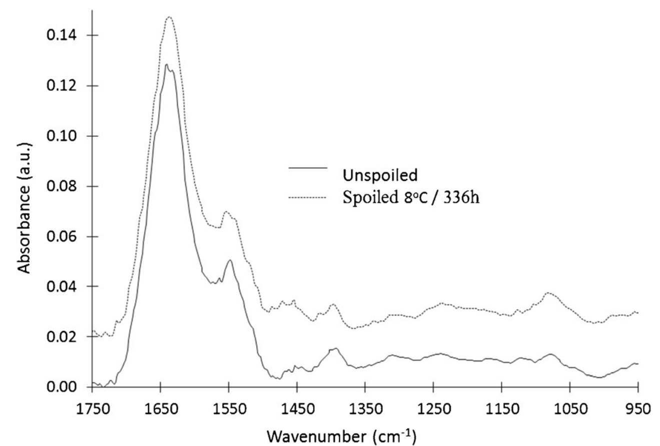

Fig. 2 FTIR spectra collected from fresh and spoiled chicken breast samples stored at 8ºC for 336 h. These spectra correspond to 3.7 and 9.4 log cfu g^{-1} of TVC and to 5.7 and 6.5 pH values, respectively

A major effect of storage temperature was observed on the level of *Enterobacteriaceae*, whose growth was greatly increased at 30 °C, being the dominant microorganism since they are often more metabolically active at these temperatures (ICMSF 2000; Holt et al. 1994). They are followed by *Pseudomonas* spp. and LAB, whereas *B. thermosphacta* remained at lower levels. While at refrigerated temperatures the *Enterobacteriaceae* counts were below *Pseudomonas* spp. and LAB counts, a result similar to those published (Borch et al. 1996; Russo et al. 2006), at abusive temperatures (30 °C), no data was found in literature to compare with. However, Ridell e Korkeala (1997) refers that any temperature

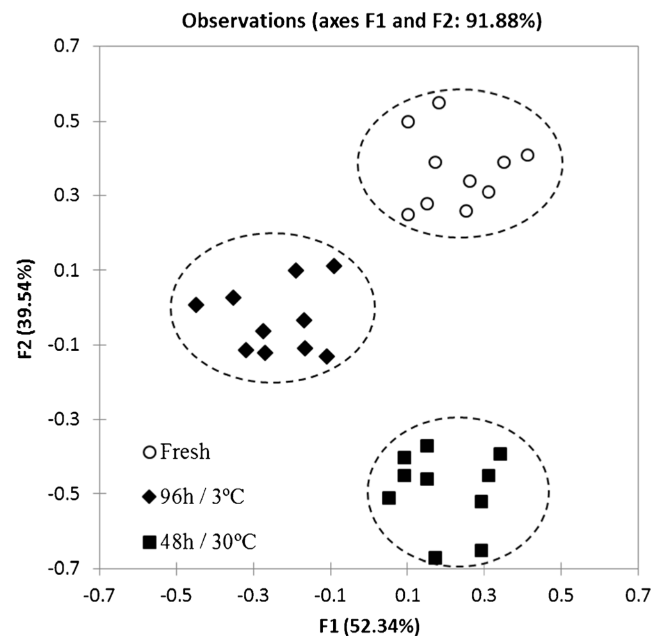

Fig. 3 PCA based comparison of spectral data obtained from fresh chicken fillets (day 0) and stored 96 h at 3ºC and 48 h at 30ºC (all batches)

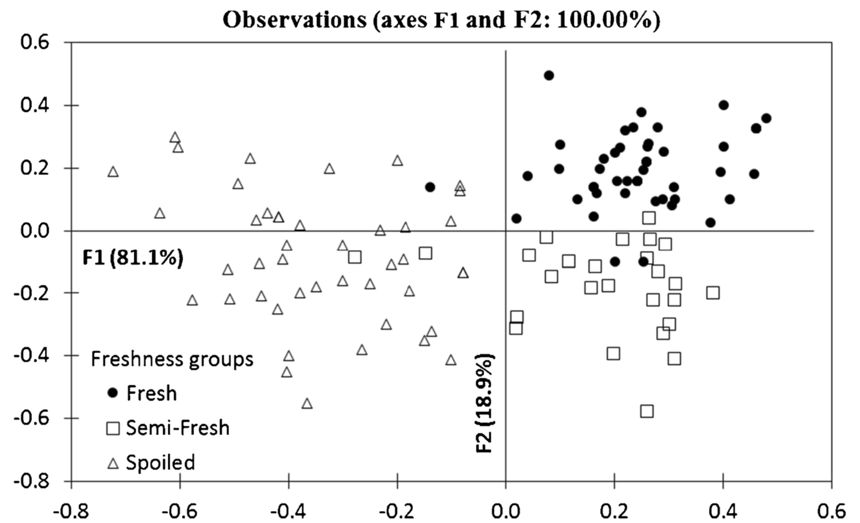

Fig. 4 Observations diagram determined by discriminant factors F1 and F2 for FTIR-ATR spectral data for different chicken breast meat freshness groups: *Fresh*, *Semi-fresh*, *Spoiled*

increase during storage, transportation, and distribution can lead to an increase in *Enterobacteriaceae* counts.

Note also that *Enterobacteriaceae* showed the lowest counts in initial microflora, and presented a long *lag* phase on samples stored at 3 and 8 ºC, particularly evident at 3 ºC. At 8 ºC, in the final stages of storage, this microbial group are a good competitor with LAB.

B. thermosphacta counts augmented slower than other microorganisms, appearing always in levels lower than LAB. In fact, according to Holzapfel (1998), this microorganism appear to be more prevalent in pork and lamb than in chicken meat.

Through the TVC, the meat was considered spoiled on the following hours: 240 h at 3 °C, and 168 h at 8 °C, though at 30 °C, the onset of spoilage is about 7 h.

FTIR Measured Spectra

The infrared spectra can provide information on biochemical changes occurring during spoilage (Ellis et al. 2002). Representative FTIR spectral data in the range of 1,750 to 950 cm^{-1} collected from fresh and spoiled chicken breast samples stored at 8 ºC for 336 h are shown in Fig. 2. These spectra correspond to 3.7 and 9.4 log cfu g^{-1} of TVC and to 5.7 and 6.5 pH values, respectively. A major peak at 1,639 cm^{-1} due to the presence of water (O–H stretch) with a simultaneous contribution from amide I in the samples is obvious. A second peak at 1,550 cm^{-1} was due to the absorbance of amide II (N–H bend, C–N stretch). A second amide vibration can be seen at 1,398 cm^{-1} (C–N stretch). Amide III peaks at 1,314 and at 1,238 cm^{-1} (C–N stretch, N–H bend, C–O stretch, O=C–N bend). The peaks at 1,460, 1,240, and 1,175 cm^{-1} can be attributed to fat (C O ester). Finally, the peaks arising from 1,025 to 1,140 cm^{-1} could be absorbance due to amines (C–N stretch) (Ellis et al. 2002; Ammor et al. 2009).

The small differences between the spectra are due to biochemical changes in the meat due to a combination of autolytic and microbiological proteolysis of meat muscle proteins. An intensification in absorption at certain wavelengths, with storage time, corresponding to amides and amines was already reported by Alexandrakis et al. (2009), suggesting the production of free amino acids and peptides. Hydrolysis of proteins points to the production of metabolites related to spoilage such as ammonia and volatile amines.

Preliminary Analysis of the Spectral Data Set

Smoothed, mean-centered, and standardized FTIR spectral data expressing various combinations of storage time and

Table 1 Confusion matrix for the calibration and cross-validation datasets based on the sensory panel discrimination and FTIR spectral data

From/To	Calibration					Cross-validation				
	Fresh	Semi-fresh	Spoiled	Total	Correct (%)	Fresh	Semi-fresh	Spoiled	Total	Correct (%)
Fresh	46	3	1	50	92.0	34	15	1	50	68.0
Semi-fresh	1	24	1	26	92.3	2	20	4	26	76.9
Spoiled	0	2	42	44	95.5	0	11	33	44	75.0
Total	47	29	44	120	93.3	36	46	38	120	73.3

Observed classifications in rows. Predicted classifications in columns

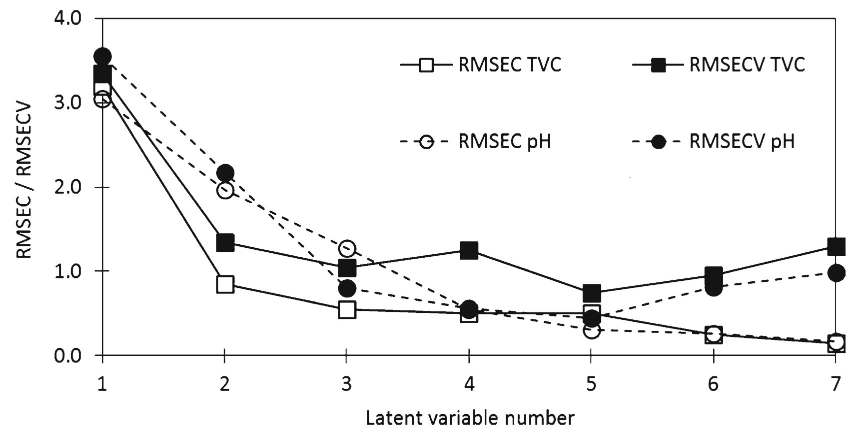

Fig. 5 *RMSEC* and *RMSECV* as a function of latent variable number for *TVC* and *pH*

temperature were subjected to PCA. It was found that the variance of the data set, based on 550 original variables (wave numbers), could be explained by 87 principal components, among which the first eight principal components explain approximately 97.6 % of the total variance.

In statistics, communality is defined as the sum of the squared factor loadings for all factors for a given original variable. It is the variance in that variable accounted for by all the factors. In other words, the communality measures the percentage of variance in a given variable explained by all the factors jointly and may be interpreted as the consistency of the indicator (Abdi and Williams 2010). By definition, the initial value of the communality in PCA is 1. Small communality values after extraction indicate variables that do not fit well with the factor solution and should be dropped from the analysis (Field, A.P. 2005). According to Stevens (2002), a lower limit of 0.6 should be used.

Following a procedure outlined by Nychas and Tassou (1997), wave numbers for which the communality value of each principal components was higher than 0.6 were considered important to explain the variance of the spectral data set and were then considered as potential wave numbers associated with the biochemical changes happening during spoilage of chicken meat (Argyri et al. 2010). These wave numbers extended, approximately, from 1,700 to 950 cm^{-1} (375 variables), which were then selected for additional analyses.

A new PCA was then performed using wave numbers from 1,700 to 950 cm^{-1}, which showed that the variance could be explained by 38 principal components among which the first five principal components explain approximately 98.9 % of the total variance. It should be noticed that wave numbers for which the communality value of each principal components out of the first five was higher than 0.9 ranged from 1,408 to 1,370 cm^{-1} and from 1,320 to 1,305 cm^{-1}. These wave numbers are connected to amides and amines (Ammor et al. 2009) and were considered potential wave numbers associated with the biochemical changes during spoilage.

Table 2 Quality parameters of the multivariate models for quantification of total viable counts, lactic acid bacteria, *Pseudomonas* spp., *B. thermosphacta* and *Enterobacteriaceae* counts, and pH (RMSE microbial counts in log cfu g^{-1})

	Factors	Equation			R^2			RMSE (log cfu g^{-1})		
		Calibration	Validation	Prediction	Calibration	Validation	Prediction	Calibration	Validation	Prediction
TVC	5	y=0.834x+ 1.225	y=0.824x+ 1.273	y=0.834x+ 1.225	0.898	0.863	0.798	0.512	0.751	0.789
LAB	6	y=0.996+ 0.037	y=0.9787x+ 0.067	y=1.024x− 0.052	0.950	0.880	0.832	0.581	0.599	0.658
Pseudomonas spp.	5	y=1.090x− 0.411	y=1.028x− 0.130	y=0.9752x+ 0.029	0.946	0.813	0.789	0.601	0.699	0.715
Brochothrix thermosphacta	5	y=0.989x+ 0.085	y=0.991x+ 0.052	y=1.052x− 0.321	0.834	0.798	0.810	0.591	0.641	0.701
Enterobacteriaceae (at 30ºC)	4	y=1.008x− 0.080	y=1.038x− 0.171	y=0.872x+ 0.610	0.963	0.927	0.857	0.625	0.702	0.756
pH	5	y=0.901x+ 0.611	y=0.929x+ 0.416	y=0.965x+ 0.241	0.882	0.806	0.880	0.312	0.452	0.479

TVC total viable counts, *LAB* lactic acid bacteria

Meat Storage Supervising Using Spectroscopic Data

PCA was then performed using wave numbers from 1,700 to 950 cm^{-1}, to compare the spectra obtained from fresh breast fillets (day 0) and those obtained from the fillets stored in acceptable conditions (96 h at 3 ºC, corresponding to an average TVC of 3.5±0.6 log cfu g^{-1}) and in abusive conditions (48 h at 30 ºC, corresponding to an average TVC of 9.8±0.1 log cfu g^{-1}). For each batch, the three groups are distinctly separate from one another, Fig. 3, with F1 and F2 principal components describing 52.34 and 39.54 % of the variation, respectively.

Analysis of the fresh samples, located in the upper half of the observations diagram, shows that the batches (fillets purchased and analyzed on the same day) are different from each other. This batch effect could be the result of a number of factors ranging from differences in the chicken age or health to differences in handling such as temperature fluctuations during transport (Koutsoumanis and Taoukis 2005).

The batch variability adds features to the spectral data set that is not related to the number of bacteria present on the meat after spoilage (Nychas et al. 2008). To minimize batch variability on the model, it would be interesting to include as many batches as possible. There were five batches in this work, and it is expected that increasing the number of batches to ten or more would improve the performance of the model.

The spectral data from 1,700 to 950 cm^{-1} was then subjected to a discriminant analysis based on the known membership of each sample analyzed as defined by the sensory panel (chicken meat freshness groups: fresh, semi-fresh, spoiled), constituting the dependent variable.

In Fig. 4, shown is the observation diagram as defined by discriminant factors F1 and F2, which explained the total variance. The classification in Table 1, resulting from the discriminant analysis, provided 93.3 % correct classification for the calibration set and 73.3 % correct classification when cross-validated. Albeit 26.7 % of samples were not cross-validated, spoiled samples were never classified as fresh.

PLS-R Models for Prediction of Microbiological Data and pH Based on Spectral Data Set

PLS-R calibration were carried out in order to evaluate the possibility of predicting the microbiological counts and pH (*Y*, response variables) from the knowledge of the predictor variables (*X*, absorbencies at wave numbers from 1,700 to 950 cm^{-1}). The PLS regression was performed on the same frequency regions used for DA.

The quality of the fitting was scrutinized by the root mean square error of calibration (RMSEC), multiple coefficient of determination or regression coefficient (R^2, where R is the correlation factor), and by the root mean square error of cross-validation (RMSECV). To validate the developed PLS-R models, Leave-One-Out cross-validation (LOOCV) method was applied to a subset of 120 samples (the remaining 24 samples were used to determine the ability of the methodology to predict new samples). In this technique, one sample at a time is randomly excluded. Then, the properties of the removed sample was predicted with a model constructed with the remaining samples (the training set). This procedure was repeated until each sample was excluded once (Picard and Cook 1984).

The capability of the models to predict the microflora population and pH for external samples was inspected by the RMSEP (Corgozinho et al. 2008; Divya and Mishra 2007).

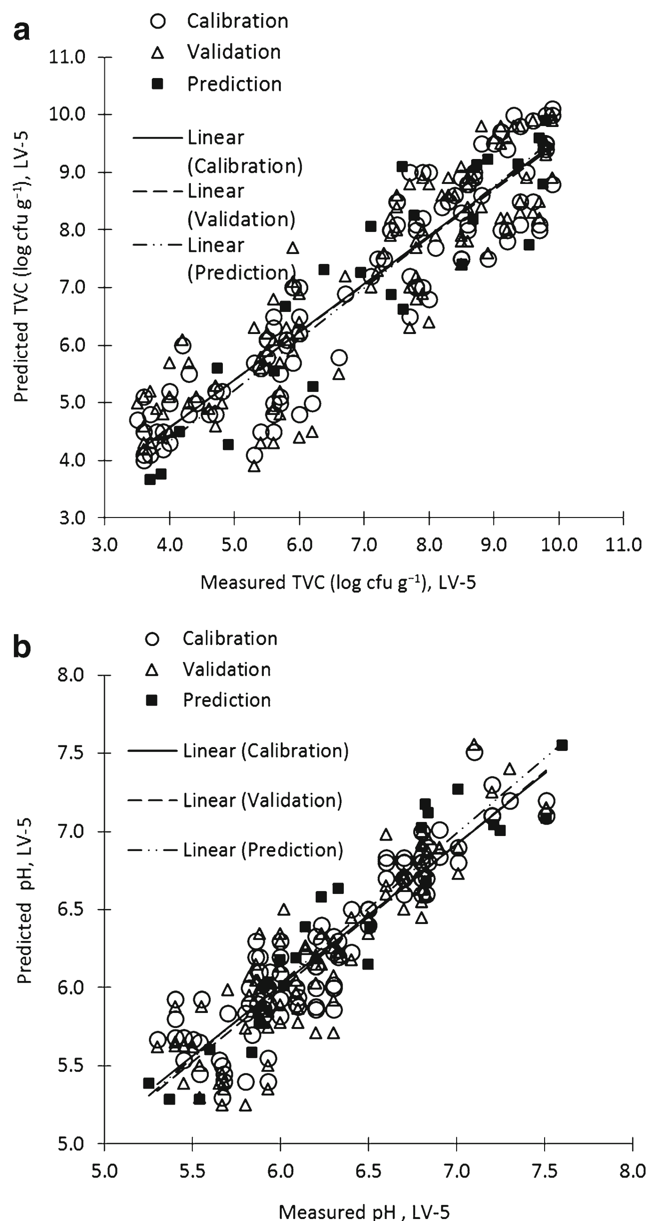

Fig. 6 Correlation between the observed and the estimated values obtained from FTIR spectra for: **a** TVC and **b** pH, (LV-latent variable)

The confirmation and validation of the analysis region used for developing the PLS model were performed by computing the predicted residual error sum of squares (PRESS) values for different latent variable (LVs). The PRESS value is a direct measure on how well a calibration predict the value of samples left out during a cross-validation (Smith 2002).

In order to exemplify the typical behavior of the calculated RMSEC and RMSECV, Fig. 5 represents its values as a function of the latent variable number for TVC and pH PLS regression models. The optimum number of latent variables, between four and six for the models developed in this work (see Table 2), is the lowest that minimizes the RMSECV, which becomes stable thereafter. This confirms that the spectral region used for developing the models for quantification exhibits significant correlation with microflora population and pH values.

The accuracy and the performance of the models which correlate the actual and estimated values obtained from FTIR spectra is illustrated in Fig. 6a, b for TVC and pH values, respectively and in Fig. 7a–d for LAB, *Pseudomonas* spp., *B. thermosphacta*, and *Enterobacteriaceae*, respectively. The correlation of the spectral data with the pH of the samples allows a more complete characterization of the meat using only one spectroscopic measurement.

Good relationships were found between the spectral data and the microbiological analysis. Table 2 resumes the quantitative performance of the multivariate calibrations determined in this work in terms of the R^2 coefficients, RMSEC, RMSECV, and RMSEP.

For TVC, LAB, and *Pseudomonas* spp., using the values of Table 2, it can be seen that the predicted values for the external set are higher or similar than the measured values, except for

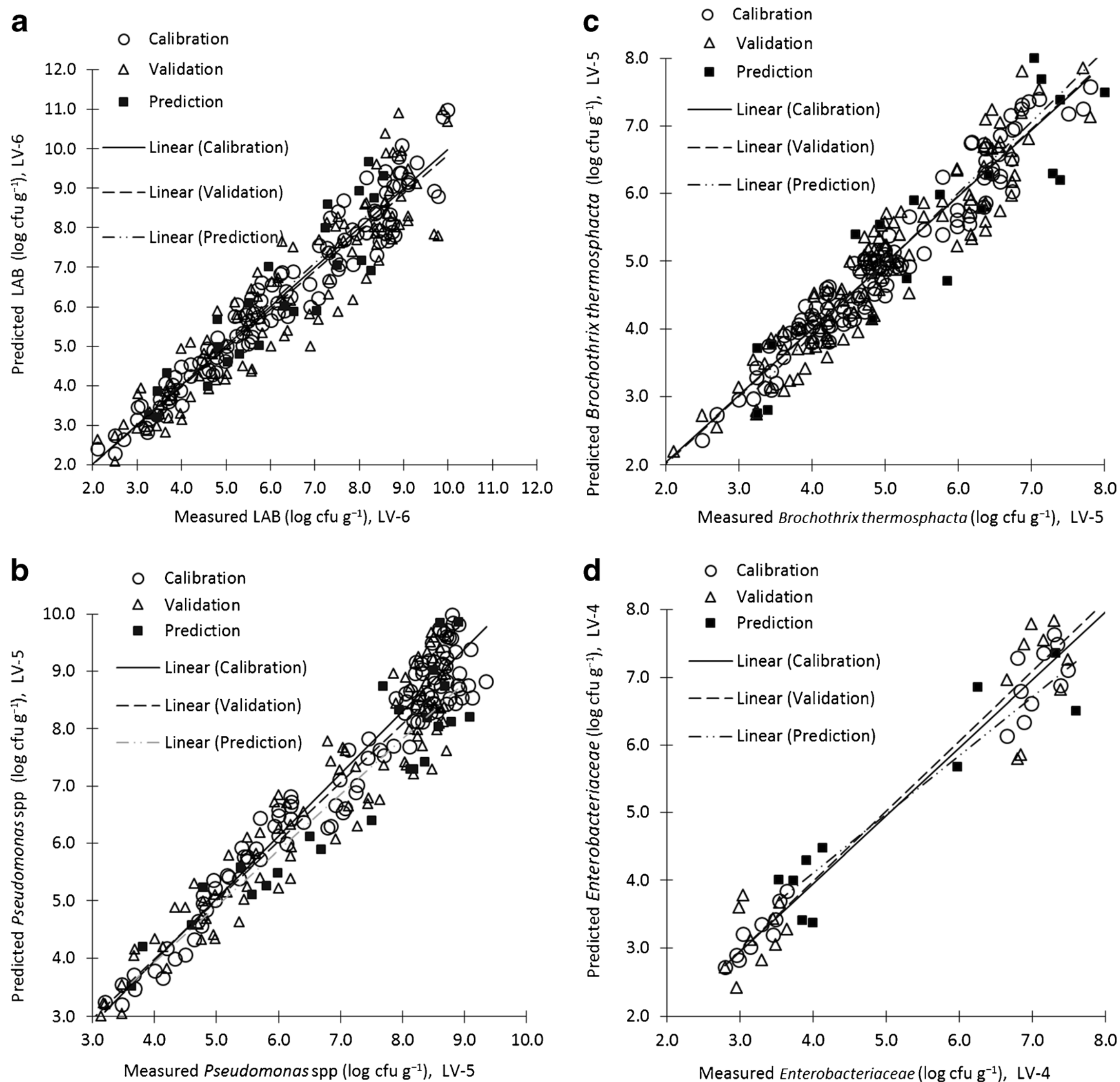

Fig. 7 Correlation between the observed and the estimated values obtained from FTIR spectra for: **a** LAB, **b** *Pseudomonas* spp., **c** *B. thermosphacta*, and **d** *Enterobacteriaceae* (LV-latent variable)

B. thermosphacta. The slopes are, in general, different from 1, maybe because the FTIR spectroscopic measurement are made only at the surface and microbial counting method uses the full samples of meat. Therefore, given the inhomogeneity of this matrix, the experimental procedure through this work is responsible for an offset between the two measurements. In any case, the FTIR models for predicting microflora population provide an early warning of high bacteria loads. This is an interesting feature when dealing with public health.

It needs to be noted that no reasonable regression model was attained for *Enterobacteriaceae* with all temperatures; R^2 values of 0.7 or less and RMSEP higher than 2 log cfu g^{-1} were typical. This is an expected result as this microbial group presented a long *lag* phase at 3 and 8 ºC, in agreement with the work by Papadopoulou et al. (2011). However, at 30ºC, the *lag* phase was negligible.

The high value of R^2 and the low values of RMSEC and RMSECV indicate good performance and precision of PLS-R models. Additionally, to obtain a robust calibration model, the number of regression factors used should be the lowest as possible (Hui-shan et al. 2006). The bias factors of the different regressions were found close to unity, indicating no major structural deviation of the models, i.e., systematic over or underprediction of the microbial counts (Miller and Miller 2005).

The values of R^2 and RMSEC, showing the quality of models, were 0.898/0.512 for the TVC and 0.882/0.312 for the pH, respectively. Regarding the bacterial groups LAB, *Pseudomonas* spp., *B. thermosphacta*, and *Enterobacteriaceae*, the corresponding values of R^2 and RMSE were 0.950/0.581, 0.946/0.601, 0.834/0.591, and 0.963/0.625, respectively.

When the data is subjected to cross-validation, the quality of the models for prediction decrease; R^2 and RMSE becomes 0.863/0.751 for the TCV and 0.806/0.452 for pH, 0.880/0.599 for LAB, 0.813/0.699 for *Pseudomonas* spp., 0.798/0.641 for *B. thermosphacta*, and 0.927/0.702 for *Enterobacteriaceae*, respectively.

The PLS-R calibration model was also used to calculate the microflora population in external samples (samples not used to build the models). Figures 6 and 7 mentioned above also show the scatter plot for the relationship between actual and FTIR predicted values of the microflora and pH. The values of R^2 and RMSEP obtained are 0.798/0.789 for the TCV and 0.880/0.479 for pH, 0.832/0.658 for LAB, 0.789/0.715 for *Pseudomonas* spp., 0.810/0.701 for *B. thermosphacta*, and 0.857/0.756 for *Enterobacteriaceae*, respectively.

Conclusions

The different microbial groups that potentially contributed to the spoilage of chicken breast studied depends on the storage temperature. Aerobic storage of chicken meat allowed high final population levels regardless of storage temperature, with *Pseudomonas* spp. being the dominant microorganism followed by LAB and *Enterobacteriaceae*, whereas *B. thermosphacta* remained at lower levels. At 30ºC, *Enterobacteriaceae* showed the highest values, being largely predominant.

Infrared spectroscopy was used to obtain spoilage fingerprints of chicken breasts fillets during storage at different temperatures in an attempt to quantitatively monitor the process.

The FTIR-ATR technique was found to represent an inexpensive and rapid instrument for monitoring raw chicken fillets spoilage through measurement of biochemical changes happening in the chicken matrix instead of using classical counting bacteria colonies. According to Ellis et al. (2002), an infrared spectrum may be considered a metabolic pattern that can be straightforwardly converted to valuable data related to degree of spoilage.

From the PCA, it was concluded that the wave numbers from 1,408 to 1,370 cm^{-1} and from 1,320 to 1,305 cm^{-1}, linked to amides and amines, are strongly connected to spoilage dynamics.

However, to set up empirical models to predict the spoilage/freshness of the samples, pH, and microbial counts, the absorbency values of 375 wave numbers from 1,700 to 950 cm^{-1} were utilized. The authors are involved in trying to build calibration models based in a discrete number of wave numbers.

The collected infrared spectra contain valuable information, allowing the discrimination of meat samples in quality categories corresponding to different spoilage levels, and could also be used to correlate the population of the different microbial groups, particularly *Pseudomonas* spp., LAB, and *Enterobacteriaceae* directly from the sample surface. Supplementary systematic work must be carried out to expand the spectral data for more accurate results.

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Compliance with Ethics Requirements Helena Vasconcelos, Cristina Saraiva, and José Manuel M. M. de Almeida declare that they have no conflict of interest.

The present work does not contain any studies with human or animal subjects.

References

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *Wiley Interdisciplinary Reviews Computational Statistics, 2*, 43–459.

Alexandrakis, D., Downey, G., & Scannell, A. G. M. (2009). Rapid non-destructive detection of spoilage of intact chicken breast muscle using near-infrared and Fourier transform mid-infrared spectroscopy and multivariate statistics. *Food and Bioprocess Technology, 5*, 338–347.

Ammor, M. S., Argyri, A., & Nychas, G.-J. E. (2009). Rapid monitoring of the spoilage of minced beef stored under conventionally and active packaging conditions using Fourier transform infrared spectroscopy in tandem with chemometrics. *Meat Science, 81*, 507–514.

Archer, D. L. (1996). The validation of rapid methods in food microbiology. *Food Control, 7*, 3–4.

Argyri, A., Panagou, E. Z., Tarantilis, P. A., Polysiou, M., & Nychas, G.-J. E. (2010). Rapid qualitative and quantitative detection of beef fillets spoilage based on Fourier transform infrared spectroscopy data and artificial neural networks. *Sensors and Actuators B: Chemical, 145*, 146–154.

Balamatsia, C. C., Paleologos, E. K., Kontominas, M. G., & Savvaidis, I. N. (2006). Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4 degrees C: possible role of biogenic amines as spoilage indicators. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 89*, 9–17.

Balamatsia, C. C., Patsias, A., Kontominas, M. G., & Savvaidis, I. N. (2007). Possible role of volatile amines as quality-indicating metabolites in modified atmosphere-packaged chicken fillets: correlation with microbiological and sensory attributes. *Food Chemistry, 104*, 1622–1628.

Borch, E., Kant-Muemans, M.-L., & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology, 33*, 103–120.

Corgozinho, C. N. C., Pasa, V. M. D., & Barbeira, P. J. S. (2008). Determination of residual oil in diesel oil by spectrofluorimetric and chemometric analysis. *Talanta, 76*, 479–484.

Crowley, H., Cagney, C., Sheridan, J. J., Anderson, W., MacDowell, D. A., Blair, I. S., et al. (2005). *Enterobacteriaceae* in beef products from retail outlets in the Republic of Ireland and comparison of the presence and counts of *E. coli* O157:H7 in these products. *Food Microbiology, 22*, 409–414.

Dainty, R. H., & Mackey, B. M. (1992). The relationship between the phenotypic properties of bacteria from chilled-stored meat and spoilage processes. *Journal of Applied Bacteriology Symposium supplement, 73*, 103S–114S.

De Luca, M., Oliverio, F., Loele, G., & Ragno, G. (2009). Multivariate calibration techniques applied to derivative spectroscopy data for analysis of pharmaceutical mixtures. *Chemometrics and Intelligent Laboratory Systems, 96*, 14–21.

Divya, O., & Mishra, A. K. (2007). Combining synchronous fluorescence spectroscopy with multivariate methods for the analysis of petrol–kerosene mixtures. *Talanta, 72*, 43–48.

Doulgeraki, A. I., Ercolini, D., Villani, F., & Nychas, G.-J. E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology, 157*, 130–141.

Ellis, D. I., & Goodacre, R. (2001). Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. *Trends in Food Science and Technology, 12*, 414–424.

Ellis, D. I., Broadhurst, D., Kell, D. B., Rowland, J. J., & Goodacre, R. (2002). Rapid and quantitative detection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. *Applied and Environmental Microbiology, 68*, 2822–2828.

Ellis, D. I., Broadhurst, D., & Goodacre, R. (2004). Rapid and quantitative detection of the microbial spoilage of beef by Fourier transform infrared spectroscopy and machine learning. *Analytica Chimica Acta, 514*, 193–201.

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., & Villani, F. (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Applied and Environmental Microbiology, 75*, 1990–2001.

Feng, Y. Z., Elmasry, G., Sun, D. W., Scannell, A. G. M., Walsh, D., & Morcy, N. (2013). Near-infrared hyperspectral imaging and partial least squares regression for rapid and reagentless determination of *Enterobacteriaceae* on chicken fillets. *Food Chemistry, 138*, 1829–1836.

Field, A. P. (2005). *Discovering statistics using SPSS* (2nd ed.). London: Sage.

Gill, C. O. (1986). The control of microbial spoilage in fresh meats. In A. M. Pearson & T. R. Dutson (Eds.), *Advances in meat research, chapter 2* (pp. 49–88). Westport: AVI Publishing.

Gill, C. O., & Newton, K. G. (1977). The development of aerobic spoilage flora on meat stored at chill temperatures. *The Journal of Applied Bacteriology, 43*, 189–195.

Grau, R., Sánchez, A. J., Girón, J., Iborra, E., Fuentes, A., & Barat, J. M. (2011). Nondestructive assessment of freshness in packaged sliced chicken breasts using SW-NIR spectroscopy. *Food Research International, 44*, 331–337.

Guevara-Franco, J. A., Alonso-Calleja, C., & Capita, R. (2010). Aminopeptidase activity by spoilage bacteria and its relationship to microbial load and sensory attributes of poultry legs during aerobic cold storage. *Journal of Food Protection, 73*, 322–326.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Facultatively anaerobic gram-negative roads. In Bergey's Manual of determinative bacteriology* (9th ed., p. 787). Baltimore: Williams & Wilkins.

Holzapfel, W.H. (1998). The Gram-positive bacteria associated with meat and meat products. In A. R. Davies, R. G. Board (Eds.) *The Microbiology of Meat and Poultry* (1st Edition), Chapter 2 (pp. 35-64). Springer-Verlag.

Huis in't Veld, J. H. J. (1996). Microbial and biochemical spoilage of foods: an overview. *International Journal of Food Microbiology, 33*, 1–18.

Hui-shan, L. U., Hui-rong, X. U., Yi-bin, Y., Xia-ping, F. U., Hai-yan, Y. U., & Hai-qing, T. (2006). Application Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. *Journal of Zhejiang University (Science), 7*, 794–799.

ICMSF (International Commission on Microbiological Specifications for Foods). (2000). *Microrganismos de los alimentos. Su significado y métodos de enumeración (2ª ed.)* (pp. 147–150). Zaragoza: Editorial Acribia.

ISO 13722 (1996). Meat and meat products. Enumeration of *Brochothrix thermosphacta* - Colony-count technique.

ISO 21528-2 (2004). International Organization for Standardization. Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae*. Part 2: Colony-count method.

ISO 4121 (2003). International Organization for Standardization. Sensory analysis. Guidelines for the use of quantitative response scales.

ISO 4833 (2003). International Organization for Standardization (2003). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30ºC.

Jiménez, S. M., Salsi, M. S., Tiburzi, M. C., Rafaghelli, R. C., Tessi, M. A., & Coutaz, V. R. (1997). Spoilage microflora in fresh chicken breast stored at 4ºC influence of packaging methods. *Journal of Applied Microbiology, 83*, 613–618.

Koutsoumanis, K. P., & Taoukis, P. (2005). Meat safety, refrigerated storage and transport: modeling and management. In J. N. Sofos (Ed.), *Improving the safety of fresh meat* (pp. 503–561). Cambridge: Woodhead.

Labadie, J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science, 52*, 299–305.

Liang, Y.-Z., & Kvalheim, O. M. (1996). Robust methods for multivariate analysis—a tutorial review. *Chemometrics and Intelligent Laboratory Systems, 32*, 1–10.

Lin, M., Al-Holy, M., Mousavi-Hesary, M., Al-Qadiri, H., Cavinato, A. G., & Rasco, B. A. (2004). Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm). *Letters in Applied Microbiology, 39*, 148–155.

McLachlan, G. J. (2004). *Discriminant Analysis and Statistical Pattern Recognition*. Wiley Interscience.

Miller, J. N., & Miller, J. C. (2005). *Statistics and Chemometrics for Analytical Chemistry (5th Edition)*. Edinburgh: Pearson.

Molin, G., & Ternström, A. (1982). Numerical taxonomy of psychrotrophic Pseudomonas. *Journal of General Microbiology, 128*, 1249–1264.

Mossoba, M. M., Al-Khaldi, S. F., Jacobson, A., Crowe, L. I. S., & Fry, F. S. (2003). Application of a disposable transparent filtration membrane to the infrared spectroscopic discrimination among bacterial species. *Journal of Microbiological Methods, 55*, 311–314.

Naumann, D., Helm, D., & Labischinski, H. (1991). Microbiological characterizations by FT-IR spectroscopy. *Nature, 351*, 81–82.

NF V04-503 (AFNOR) (1988). Viandes et produits à base des viande - Dénombrement des Bactéries lactiques.

NF V04-504 (AFNOR) (1998). Viandes et produits à base des viande - Dénombrement de *Pseudomonas* spp.

Nychas, G.-J. E., & Skandamis, P. N. (2005). Fresh meat spoilage and modified atmosphere packaging (MAP). In J. N. Sofos (Ed.), *Improving the safety of fresh meat* (pp. 461–502). Cambridge: CRC/Woodhead.

Nychas, G.-J. E., & Tassou, C. C. (1997). Spoilage process and proteolysis in chicken as detected by HPLC. *Journal of Science and Food Agriculture, 74*, 199–208.

Nychas, G. J. E., Skandamis, P. N., Tassou, C. C., & Koutsoumanis, K. P. (2008). Meat spoilage during distribution. *Meat Science, 78*, 77–89.

Papadopoulou, O., Panagou, E. Z., Tassou, C. C., & Nychas, G. J. E. (2011). Contribution of Fourier transform infrared (FTIR) spectroscopy data on the quantitative determination of minced pork meat spoilage. *Food Research International, 44*, 3264–3271.

Picard, R., & Cook, D. (1984). Cross-validation of regression models. *Journal of the American Statistical Association, 79*, 575–583.

Ridell, J., & Korkeala, H. (1997). Minimum growth temperatures of *Hafnia alvei* and other *Enterobacteriaceae* isolated from refrigerated meat determined with a temperature gradient incubator. *International Journal of Food Microbiology, 35*, 287–292.

Rodriguez-Saona, L. E., Khambaty, F. M., Fry, F. A., & Calvy, E. M. (2001). Rapid detection and identification of bacterial strains by Fourier transform near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 49*, 574–579.

Russo, F., Ercolini, D., Mauriello, G., & Villani, F. (2006). Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. *Food Microbiology, 23*, 797–802.

Sahar, A., Boubellouta, T., & Dufour, É. (2011). Synchronous front-face fluorescence spectroscopy as a promising tool for the rapid determination of spoilage bacteria on chicken breast. *Food Research International, 44*, 471–480.

Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry, 36*, 1627–1639.

Smith, B. C. (2002). *Quantitative spectroscopy: theory and practice*. Amsterdam: Academic.

Sousa Marques, A., Nicácio, J. T. N., Cidral, T. A., de Melo, M. C. N., & de Lima, K. M. G. (2013). The use of near infrared spectroscopy and multivariate techniques to differentiate *Escherichia coli* and *Salmonella* Enteritidis inoculated into pulp juice. *Journal of Microbiological Methods, 93*, 90–94.

Stevens, J. P. (2002). *Applied multivariate statistics for the social sciences* (4th ed.). Hillsdale: Erlbaum.

Stuart, B. (2004). Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons, Ltd.

Tito, N. B., Rodemann, T., & Powell, S. M. (2012). Use of near-infra red spectroscopy to predict microbial numbers on Atlantic salmon. *Food Microbiology, 32*, 431–436.

Walker, S. J., & Betts, G. (2000). Chilled foods microbiology. In M. Stringer & C. Dennis (Eds.), *Chilled foods (2nd Edition), Chapter 7* (pp. 162–166). Cambridge: Woodhead.

Wentzell, P. D., & Montoto, L. V. (2003). Comparison of principal components regression and partial least squares regression through generic simulations of complex mixtures. *Chemometrics and Intelligent Laboratory Systems, 65*, 257–279.