Food Research International 44 (2011) 3264–3271

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Contribution of Fourier transform infrared (FTIR) spectroscopy data on the quantitative determination of minced pork meat spoilage

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ARTICLE INFO

Article history:
Received 13 June 2011
Accepted 14 September 2011

Keywords:
Aerobic storage
FTIR
Meat spoilage
Minced pork meat
PLS
Predictive microbiology

ABSTRACT

The aim of this work was to investigate the feasibility of Fourier transform infrared (FTIR) spectroscopy to quantify biochemical changes occurring in fresh minced pork meat in the attempt to monitor spoilage. For this reason, partial least squares (PLS) models were constructed to correlate spectral data from FTIR with minced pork meat spoilage during aerobic storage of meat samples at different storage temperatures (0, 5, 10, and 15 °C). Spectral data were collected from the surface of meat samples in parallel with microbiological analysis to enumerate the population of total viable counts, *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria and *Enterobacteriaceae*. Qualitative interpretation of spectral data was based on sensory evaluation, using a three point hedonic scale, discriminating meat samples in three quality classes, namely fresh, semi-fresh and spoiled. The purpose of the developed models was to classify minced pork samples in the respective quality class, and also to correlate the population dynamics of the microbial association with FTIR spectra. The obtained results demonstrated good performance in classifying meat samples in one of the three pre-defined sensory classes. The overall correct classification rate for the three sensory classes was 94.0% and 88.1% during model calibration and validation, respectively. Furthermore, PLS regression models were also employed to provide quantitative estimations of microbial counts during meat storage. The performance was based on graphical plots and statistical indices (bias factor, accuracy factor, standard error of calibration, standard error of prediction, and correlation coefficient). The values of the bias factor were close to unity for all microbial groups indicating no systematic bias of the models. Moreover, the calculated values of the accuracy factor showed that the average deviation between predictions and observations was 7.5% and 7.9% for total viable counts and *Pseudomonas* spp. and 10.7% and 11.3% for lactic acid bacteria and *B. thermosphacta*. Finally, correlations above 0.80 between observed and estimated counts were observed for both training and test data sets.

1. Introduction

Pork meat production amounts to almost 50% of total meat production in Europe and according to a recent survey (FAO, 2007) 75% of consumers choose pork once a week in their diet. In general, minced meat is appreciated by consumers for its convenience, although its shelf-life is limited because of the large exposed surface area that favours spoilage (Limbo, Torri, Sinelli, Franzetti, & Casiraghi, 2010). Currently, more than 50 methods have been employed for the characterization of microbiologically spoiled or contaminated meat (Byun et al., 2003; Ellis & Goodacre, 2001; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). However, these methods have certain disadvantages as they are time-consuming, destructive, require highly trained personnel, provide retrospective information, and hence are unsuitable for on-line monitoring (Dainty, 1996; Ellis, Broadhurst, & Goodacre, 2004; Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002; Liu, Lyon, Windham, Lyon, & Savage, 2004; Nychas, Drosinos, & Board, 1998; Nychas et al., 2008). Consequently, there is a need to develop fast and reliable systems to determine safety and quality of meat products for the benefit of the public and also minimise unnecessary economic losses, as one fourth of global food supply is lost through microbial activity alone (Huis in't Veld, 1996).

In the last decades, lifestyle regarding meat consumption patterns has changed and consumers have become more demanding especially in terms of higher (intrinsic) quality, increased functional and nutritional properties, increased awareness between food and health, better animal welfare and care for the environment (Nychas et al., 2008; Verbeke, Perez-Cueto, de Barcellos, Krystallis, & Grunert, 2009). As a result, meat production systems have changed and the industry faced the need to adopt novel technologies for meat production, packaging, and distribution. To this end, several non-invasive methods based on mechanical, optical, dielectrics, X-rays, spectroscopy, and nuclear magnetic resonance have been researched as potential meat safety/quality sensors depending

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doi:10.1016/j.foodres.2011.09.012

on the various meat textural and structural attributes (Ammor, Argyri, & Nychas, 2009; Argyri, Panagou, Tarantilis, Polysiou, & Nychas, 2010; Balasubramanian, Panigrahi, Logue, Gu, & Marchello, 2009; Damez & Clerjon, 2008; Ellis, Broadhurst, Clarke, & Goodacre, 2005; Prieto, Roehe, Lavín, Batten, & Andrés, 2009; Rajamäki et al., 2006). The principle underlying this approach is based on the assumption that the metabolic activity of microorganisms on meat results in biochemical changes with the concurrent development of metabolic by-products which may be indicative of spoilage. The quantification of these metabolites constitutes a characteristic fingerprint of any biochemical substance, thus providing information on the type and rate of spoilage (Ellis & Goodacre, 2001; Nychas et al., 2008).

Fourier transform infrared (FTIR) spectroscopy has substantial potential as a quantitative control method in the food industry (Van de Voort, 1992) and it has been successfully employed so far to tackle authentication and adulteration problems in various foods (Edelman, Diewok, Schuster, & Lendl, 2001; He, Rodriguez-Saona, & Giusti, 2007; Kelly, Petisco, & Downey, 2006; Rohman & Che Man, 2010; Vlachos et al., 2006). FTIR analysis is rapid, non-invasive, requires minimum sample pre-treatment, no specific consumables or reagents, and in conjunction with attenuated total reflectance (ATR) technology permits users to collect full spectra in a few seconds, allowing simultaneous assessment of numerous meat properties (Ammor et al., 2009; Ellis et al., 2004, 2002). The integration of FTIR-ATR spectroscopy with an appropriate information platform could result in the development of an expert system that would be able to qualitatively and/or quantitatively discriminate between meat samples based on extracted pre-processing features (Berrueta, Alonso-Salces, & Héberger, 2007). Chemometric methods (e.g. principal components analysis, hierarchical cluster analysis, discriminant function analysis, partial least squares regression) in parallel with computational biology approaches based on soft computing (e.g. artificial neural networks, genetic algorithms, support vector machines) have been applied as data mining techniques in bioprocess data (Cevallos-Cevallos, Reyes-De-Corcuera, Etxeberria, Danyluk, & Rodrick, 2009; Charaniya, Hu, & Karypis, 2008; Goodacre, 2000). These approaches could provide rapid information related to the contribution of the ephemeral spoilage organisms (ESO) in meat or discriminate meat with regard to (i) type of muscle and (ii) spoilage (Balasubramanian et al., 2009; Dainty, 1996; Ellis et al., 2005; Mataragas, Skandamis, Nychas, & Drosinos, 2007; Verouden, Westerhius, Werf, & Smilde, 2009).

As meat freshness is important to consumers, the meat industry and retailers, the purpose of the present study was to investigate the feasibility of FTIR spectroscopy in conjunction with chemometric analysis to evaluate the quality of minced pork meat stored aerobically at different isothermal conditions (0, 5, 10, and 15 °C). Another aim was to examine possible correlation between FTIR spectral data and population dynamics of different bacterial groups on the surface of meat samples during storage.

2. Materials and methods

2.1. Sample preparation and experimental design

Fresh minced pork (pH 5.6–5.8) obtained from different carcasses was purchased immediately after grinding from the central meat market in Athens and transported under refrigeration to the laboratory within 30 min. The meat was divided in portions of 50 g and packed aerobically in Styrofoam trays which were subsequently wrapped manually with air-permeable polyethylene plastic film. The underlying objective of the treatment was to simulate the pre-packaged meat available in retail outlets. Samples were randomly divided into groups and stored under controlled isothermal conditions at 0, 5, 10, and 15 °C in high precision (±0.5 °C) incubators (MIR-153, Sanyo Electric Co., Osaka, Japan) for up to 340 h, depending on storage temperature, until spoilage was pronounced (intense discoloration and presence of off-odours). For the analysis, each sample was divided into two portions (25 g), one portion was used for microbiological analysis and the other for FTIR analysis. It was assumed that the microbial population in the first portion of the meat would be representative of the microbial population in the other portion of the meat subjected to FTIR analysis. Samples stored at 0 and 5 °C were analyzed approximately every 24 and 12 h, respectively, whereas samples stored at 10 and 15 °C were analyzed every 6–7 h. From each sampling occasion, two replicate samples were withdrawn to undergo microbiological and FTIR analysis and two additional samples were employed in sensory evaluation.

2.2. Microbiological analyses

To estimate the number of viable cells, minced meat samples (25 g) were weighed aseptically, added to sterile quarter strength Ringer's solution and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature. Serial dilutions were prepared with the Ringer solution and duplicate 0.1 or 1 ml samples of the appropriate dilutions were spread or mixed on the following media: Plate Count Agar (PCA, Biolife 4021452, Milano, Italy) for total viable counts, incubated at 30 °C for 48–72 h; Pseudomonas Agar Base selective supplement (PAB, Biolife 401961, Milano, Italy) for *Pseudomonas* spp., incubated at 25 °C for 48–72 h; Streptomycin Thallous Acetate–Actidione Agar (STAA, Biolife 402079, Milano, Italy) for *Brochothrix thermosphacta*, incubated at 25 °C for 72 h; Violet Red Bile Glucose Agar (VRBGA, Biolife, 402185, Milano Italy) for *Enterobacteriaceae* counts, overlaid with the same medium and incubated at 37 °C for 18–24 h; de Man–Rogosa–Sharp medium with pH adjusted at 5.7 (MRS, Biolife, 4017282, Milano, Italy) for lactic acid bacteria, overlaid with the same medium and incubated at 30 °C for 48–72 h. Growth data from plate counts were log transformed and fitted to the primary model of Baranyi and Roberts(1994) using the program DMFit (available at www.combase.cc) to determine the kinetic parameters of microbial growth (maximum specific growth rate and lag phase duration). In parallel with microbiological analyses, the pH value of minced pork meat was recorded with a digital pH meter (Metrohm pH Lab, Switzerland), the glass electrode of which was immersed in the homogenised meat sample after the end of microbiological analysis.

2.3. Sensory analysis

Sensory evaluation of pork samples was performed during storage as previously reported (Argyri et al., 2010) by a sensory panel of five members (staff from the laboratory) at the same time intervals as for microbiological analyses. The same trained persons were used in each evaluation and all were blinded to the meat sample tested. Sensory evaluation was carried out under controlled conditions of light, temperature and humidity. Meat samples were left to reach ambient temperature prior to analysis. The descriptors selected were based on the perception of colour, smell, and taste. The first two descriptors were assessed before and after cooking for 20 min at 180 °C in a preheated oven, while the last descriptor was evaluated only after cooking. A freshly thawed and cooked meat sample, stored at −20 °C, was also presented to the panellists as a control sample. A three class evaluation scheme was employed in this experiment. The first class (Fresh) corresponded to the absence of off-flavours, equal to the reference sample; the second class (Semi-fresh) corresponded to the presence of slight off-flavours but not spoiled (still acceptable quality); and the third class (Spoiled) corresponded to clearly off-flavours development (unacceptable quality). Semi-fresh was the first indication of meat spoilage (incipient spoilage) in which the sample was marginally accepted. Putrid, sweet, sour, or cheesy odours were regarded as indicative of microbial spoilage and classified the samples as spoiled. Bright colours typical of fresh oxygenated meat were considered fresh, whereas a persistent dull or unusual colour rendered the sample spoiled (Ammor et al., 2009; Argyri et al., 2010; Skandamis & Nychas, 2002). Overall, 134 minced pork meat samples

were scored by the taste panel and discriminated into the defined groups as fresh (30), semi-fresh (32), and spoiled (72).

2.4. FTIR-ATR spectroscopy

Meat samples (8 cm × 1 cm × 0.5 cm) were analyzed in parallel to the microbiological and sensory analysis. FTIR spectra were collected using a ZnSe 45° ATR (Attenuated Total Reflectance) crystal on a Nicolet 6700 FTIR Spectrometer equipped with a DLaTGS (deuterated L-alanine doped triglycene sulphate) Detector with KBr beamspliter. The samples were placed on the ZnSe ATR crystal so that the meat sample was in intimate contact with the crystal, and then pressed with the machine's gripper in order to obtain the best possible contact with the crystal. The ZnSe ATR crystal was capable of 12 external reflections, with the evanescent field effecting a depth of 1.01 μm. The spectrometer was controlled by Omnic Software-version 7.3 to collect spectra over the wavenumber range of 4000 to 650 cm^{-1}, by accumulating 100 scans with a resolution of 4 cm^{-1}. The collection time for each sample spectrum was 2 min. Each sample was analyzed in duplicate and results are displayed as mean value of both measurements. Reference spectra were acquired by collecting a spectrum from the cleaned blank crystal prior to the presentation of each sample replicate. At the end of each sampling, the crystal surface was cleaned with detergent, washed with distilled water, dried with lint-free tissue, cleaned with ethanol, and finally dried with lint-free tissue at the end of each sampling interval.

2.5. Data analysis

Data analysis was performed using multivariate methods included in the Unscrambler software (version 9.7, CAMO, Norway). Specifically, FTIR spectral data collected between 1800 and 900 cm^{-1} were initially standardized using standard normal variate (SNV) transformation. SNV was applied to minimize the effect of baseline shift and other interferences that may impair multivariate calibrations (Sinelli, Barzaghi, Giardina, & Cattaneo, 2005). Subsequently, mean centred and standardized data were subjected to partial least squares (PLS) analysis to investigate the relationships between FTIR spectral data and quality class as well as microbiological data. PLS is a supervised multivariate calibration technique that aims to define the relationship between a set of predictor data **X** (independent variables, FTIR data in this case) and a set of responses **Y** (dependent variables, sensory class or microbial counts). The PLS method projects the initial input–output data down into a latent space, extracting a number of principal components, also known as latent variables (LV) with an orthogonal structure, while capturing most of the variance in the original data. The first LV conveys the largest amount of information, followed by the second LV and so forth. The optimal number of LVs is determined with the help of the residual variances to model useful information and avoid over-fitting of the data (Nielsen, Esaiassen, Heia, & Sigernes, 2002).

For qualitative analysis, PLS-discriminant analysis (PLS-DA) was used to develop models allowing the discrimination of meat samples in the selected sensory classes. For class discrimination, the so-called one-of-many encoding was employed (Berrueta et al., 2007). Specifically, a 'dummy' **Y** matrix was constructed with zeros and ones, containing as many columns as there as classes. A meat sample was assigned the value of 1 for the class it belongs and 0 for the rest. In this way, three PLS-DA models were developed, one for each class. The closer the element of a certain column is to 1 and the elements of the other columns to 0, the more likely a sample is a member of this particular class. Model performance was established by cross-validation using the leave-one-out procedure for the prediction of sensory class of minced meat samples. The classification accuracy was determined by the number of correctly classified samples in each sensory class divided by the total number of samples in the class.

In addition, PLS regression (PLS-R) models were built for the quantitative analysis of the population of total viable counts, *Pseudomonas* spp. *B. thermosphacta*, and lactic acid bacteria, using the FTIR responses of the samples as input variables and the counts of each individual microbial group as output variables. The performance of the PLS-R models were quantified by the calculation of the bias (B_f) and accuracy (A_f) factors (Ross, 1996), the standard error of calibration (SEC), standard error of prediction (SEP), and correlation coefficient (r) between the observed and predicted counts. SEC and SEP were defined as follows (Hernández Gómez, Wang, Hu, & García Pereira, 2007):

$$SEC = \sqrt{\frac{1}{I_c - 1}\sum_{i=1}^{Ic}(\hat{y}_i - y_i)^2} \quad (1)$$

$$SEP = \sqrt{\frac{1}{I_p - 1}\sum_{i=1}^{Ip}(\hat{y}_i - y_i - bias)^2} \quad (2)$$

where, $\hat{y}_i$ is the predicted value of the ith observation, y_i is the measured value of the ith observation, I_c is the number of observations in the calibration set, and I_p is the number of observations in the validation set. Bias systematic difference between predicted and observed counts is given by (Hernández Gómez et al., 2007):

$$Bias = \frac{1}{I_p}\sum_{i=1}^{Ip}(\hat{y}_i - y_i) \quad (3)$$

In addition, the bias (B_f) and accuracy (A_f) factors were expressed as follows:

$$B_f = 10^{(\sum \log(\hat{y}_i/y_i)/n)} \quad (4)$$

$$A_f = 10^{(\sum |\log(\hat{y}_i/y_i)|/n)} \quad (5)$$

where n is the number of observations.

The database ($n = 134$ spectra) was partitioned into a training and test dataset. The measurements of the first replication of the experiment for each sampling day and storage temperature were gathered in the training dataset ($n = 67$) and the remaining measurements of the second replication were gathered in the test dataset ($n = 67$). Test data were not employed in any step of model development but they were used exclusively to determine its performance. Before the calibration, pre-processed spectral data were subjected to PCA analysis for dimensionality reduction, and the number of PCs (also known as latent variables in PLS analysis) explaining most of the variability in the data set were subsequently used as independent variables (**X**) in model development. This is a standard procedure in PLS analysis. The dependent variable (**Y**) was either the sensory classes for PLS-DA analysis or the counts of the different microbial groups for PLS-R analysis.

3. Results and discussion

3.1. Development of microbial association

The microbiological analysis showed that the initial microbiota of minced pork consisted of *Pseudomonas* spp., *B. thermosphacta*, lactic acid bacteria and *Enterobacteriaceae*. The population dynamics of these groups and their contribution to the final microbiota were greatly affected by storage temperature, as exemplified by the evolution of total viable counts (Fig. 1). Pronounced lag phase was evident in all microbial groups at 0 °C, the duration of which was greatly reduced or not observed at all at higher temperatures. A progressive increase of maximum specific growth rate (μ_{max}) was observed for all members of the microbial association with increasing storage temperature (Table 1).

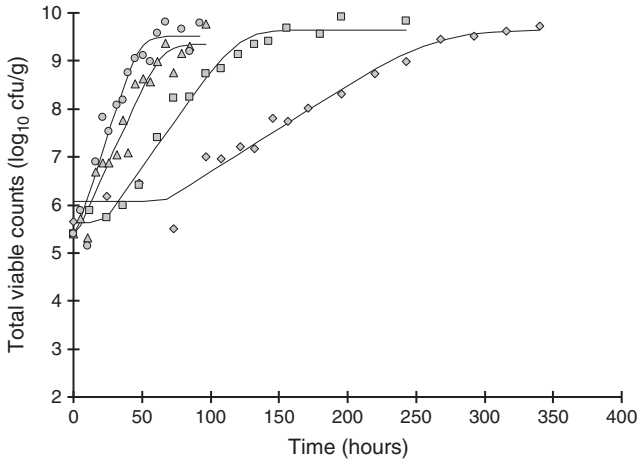

Fig. 1. Changes of total viable counts (tvc) obtained from minced pork stored aerobically at 0 °C (♦), 5 °C (■), 10 °C (▲), and 15 °C (●). Lines represent growth curves fitted with the Baranyi and Roberts primary model.

In general, aerobic storage of minced pork allowed high final population levels regardless of storage temperature, with *Pseudomonas* spp. being the dominant microorganism followed by *B. thermosphacta*, whereas lactic acid bacteria and *Enterobacteriaceae* remained at lower levels. A major effect of storage temperature was observed on the level of *Enterobacteriaceae*, whose growth was greatly retarded at 0, 5, and 10 °C but not at 15 °C (Table 1). Aerobic storage accelerated spoilage due to the fast growth of *Pseudomonas* spp. that became the dominant microorganism at all temperatures. Similar results have been reported previously for beef and pork meat (Ercolini, Russo, Nasi, Ferranti, & Villani, 2009; Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Sakala et al., 2002; Skandamis & Nychas, 2001, 2002; Tsigarida, Skandamis, & Nychas, 2000).

Table 1
Estimated kinetic parameters of different microbial groups in pork minced meat stored aerobically at various temperatures.

Microorganism	T (°C)	Lag phase (h)	y_0^a ($\log_{10}$ cfu/g)	y_{end}^b ($\log_{10}$ cfu/g)	μ_{max} (h^{-1})[c]	R^2
Total viable counts	0	46.1	5.81	9.68	0.039	0.953
Pseudomonas spp.		17.3	5.52	9.88	0.036	0.964
B. thermosphacta		48.6	4.20	8.16	0.043	0.945
Lactic acid bacteria		192.4	4.53	6.33	0.071	0.765
Enterobacteriaceae		268.9	3.43	–[d]	0.074	0.825
Total viable counts	5	13.7	5.37	9.66	0.090	0.973
Pseudomonas spp.		2.8	5.17	9.63	0.089	0.971
B. thermosphacta		14.1	3.58	7.90	0.136	0.986
Lactic acid bacteria		–	3.56	7.39	0.061	0.980
Enterobacteriaceae		34.4	3.05	7.55	0.063	0.967
Total viable counts	10	–	5.29	9.36	0.143	0.933
Pseudomonas spp.		–	5.30	9.59	0.132	0.969
B. thermosphacta		–	3.70	7.77	0.163	0.968
Lactic acid bacteria		–	3.69	7.20	0.145	0.875
Enterobacteriaceae		19.8	3.02	7.75	0.156	0.955
Total viable counts	15	–	5.27	9.52	0.200	0.929
Pseudomonas spp.		0.88	5.12	9.52	0.211	0.947
B. thermosphacta		–	3.63	7.75	0.252	0.952
Lactic acid bacteria		–	3.56	7.54	0.229	0.939
Enterobacteriaceae		–	3.30	8.21	0.249	0.977

[a,b]Initial and final population estimated by the Baranyi model.
[c]Maximum specific growth rate.
[d]Not observed.

The time required by the sensory panel to consider a meat sample as semi-fresh or spoiled differed for each temperature. Specifically, the sensory panel judged a sample as semi-fresh after 108, 48, 21.5 and 16 h at 0, 5, 10 and 15 °C respectively. In addition, at the time of sensory rejection (meat characterized as spoiled) the mean value of total viable counts was *ca.*8 $\log_{10}$cfu/g at all storage temperatures. This observation is in agreement with previous researchers who concluded that bacterial counts of 7–8 $\log_{10}$cfu/g can cause noticeable off-odours and slime (Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008; Koutsoumanis, Stamatiou, Skandamis, & Nychas, 2006), whereas others have reported that proteolytic changes do not occur until bacterial counts reach approximately 9 $\log_{10}$cfu/cm^2 (Brooks et al., 2008). No correlation could be established between the pH values and the spoilage detection time and as indicated by previous researchers the use of pH as an indicator of spoilage or remaining shelf-life in meats would be inadequate (Ellis et al., 2004, 2002). In general, pH started to increase only when bacterial counts reached *ca.*8 $\log_{10}$cfu/g, which is comparable with Ellis et al. (2004). This group reported that in the case of poultry meat pH values fluctuated prior to spoilage and started to increase only when bacterial counts reached 8 $\log_{10}$cfu/g.

3.2. Spoilage monitoring using FTIR spectral data

Typical FTIR spectral data in the range of 1800–900 cm^{-1} collected from fresh and spoiled meat samples stored at 0 °C are shown in Fig. 2. Spectral data could serve as metabolic snapshots (fingerprints) of minced pork providing information on biochemical changes occurring during meat storage in an attempt to monitor spoilage. Thus a major peak appeared at 1640 cm^{-1} due to moisture (O–H) and contribution of amide I bands of the proteins. A second peak was observed at 1550 cm^{-1} due to the absorption of amide II (C–N stretch), followed by a third peak at 1745 cm^{-1} corresponding to fat (C=O ester carbonyl stretching vibration of triglycerides). Other minor intensity peaks were observed at 1458 cm^{-1} (fat, CH_2 scissoring), 1398 cm^{-1} (amides, C–N stretch), 1311 cm^{-1} and 1246 cm^{-1} (amines, C–N stretch), 1166 cm^{-1} and 1090 cm^{-1} (amines, free amino acids, C–N stretch) (Ammor et al., 2009; Ellis et al., 2002; Osorio et al., 2009). It needs to be noted however, that in Fig. 2 there were no obvious feature peaks to reflect the difference in quality between fresh and spoiled meat samples during storage at 0 °C. Therefore, chemometrics were introduced to analyze the spectra and establish the qualitative and quantitative relationship between FTIR data and meat spoilage.

PLS-discriminant analysis (PLS-DA) was used to set up a calibration model for sensory quality prediction of minced meat samples. The method of full cross-validation, following the leave-one-out procedure, was used to determine the maximum number of latent variables (LVs) to ensure the predictive ability and avoid over-fitting of the data. From a preliminary plotting of the data one sample in the training dataset was characterized as outlier and removed from further analysis (data not shown) resulting in 66 samples of minced pork meat. It is often appropriate in spectroscopy applications to refine the PLS model by selecting wavenumbers and for this reason the b-coefficients (regression coefficients) provide the accumulated picture of the most important wavenumbers contributing to spoilage. Fig. 3 presents the b-coefficients over the entire spectral range 1800–900 cm^{-1} with open symbols indicating the selected intervals considered in calculations. Therefore, data evaluation focused on the following selected regions in the spectrum: 971–973 (corresponding to P–O bond) , 985–997 (corresponding to proteins), 1016–1041 (corresponding to C–O–C, glycosidic linkage), 1292–1371 (corresponding to amines and Amide III group), 1388–1486 (corresponding to C–H bending, fat), 1529–1540 (corresponding to Amide II, protein), and 1697–1726 cm^{-1}, (corresponding to fat, Amide I and H–O–H bending from water) (Ellis et al., 2004; Pedersen, Morel, Andersen, & Engelsen, 2003).

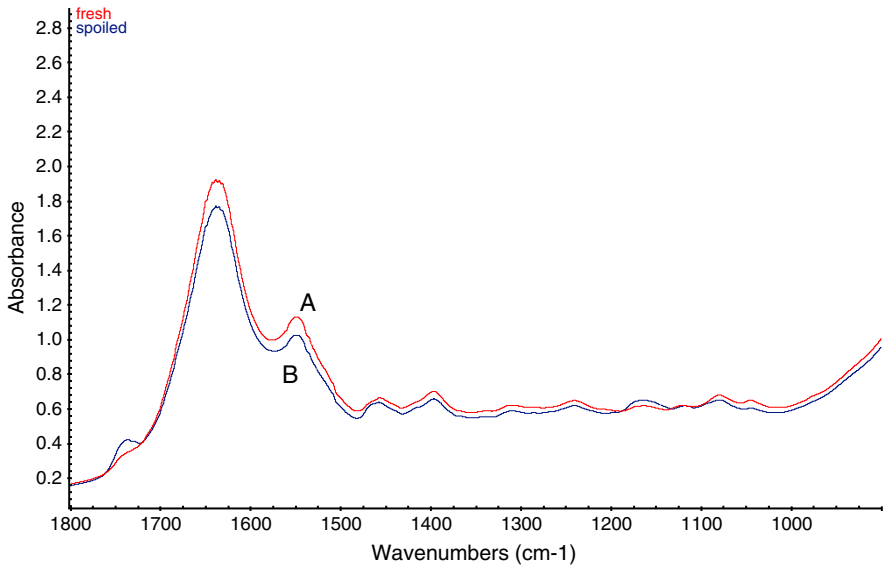

Fig. 2. Typical FTIR-ATR spectra of meat in the range of 1800–900 cm^{-1} stored at 0 °C at the beginning of storage (A: fresh, 24 h) and after 292 h (B: spoiled).

3.3. Sensory class evaluation

Good classification was obtained in all sensory classes demonstrating the effectiveness of the method as a rapid screening technique to monitor minced pork spoilage (Table 2). Specifically, a number of 6 LVs was finally selected as input variables in PLS-DA model development, presenting the highest correct classification (%) in the training (94.0%) and test (88.1%) datasets. For the training dataset, the PLS-DA approach provided 100% correct classification for spoiled samples, whereas for fresh and semi-fresh the respective number was 93.3% and 86.7%, respectively, representing 1 misclassification out of 15 fresh samples and 2 misclassifications out of 15 semi-fresh samples. However, for the test dataset the calculated percentages were lower, which is not unusual as these data were not provided at all in the model during training, but used as unknown cases for prediction. Specifically, classification rate for fresh, semi-fresh and spoiled samples was 86.7, 87.5, and 88.9%, respectively (Table 2). The percentage of erroneous predictions in the safe side (i.e., fresh meat characterized as semi-fresh or spoiled and semi-fresh meat characterized as spoiled) was 2.98% corresponding to 2 cases out of 67 meat samples. In addition, the misclassified cases in the dangerous side (i.e., semi-fresh or spoiled meat characterized as fresh, and spoiled meat characterized as semi-fresh) was 8.95% corresponding to 6 cases out of 67 meat samples. It needs to be noted however, that no fresh minced meat sample was characterized as spoiled and vice versa, indicating that the biochemical fingerprints provided by FTIR spectral data could discriminate these two classes quite accurately. The lack of complete clustering among the different sensory groups could be due to the uncertainty of sensory evaluation and the variability of the samples. Sensory science employs human senses as instruments of measures, consequently like any other instrument the performance of each panellist could be affected by systematic or random errors (i.e., uncertainty)

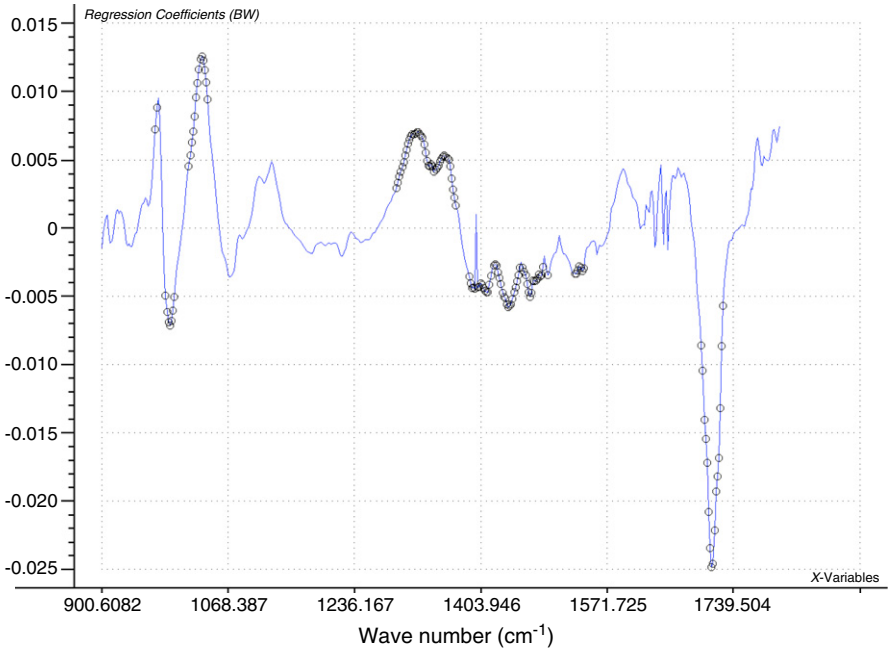

Fig. 3. Plot of b-coefficients (regression coefficients) over the entire spectral range 1800–900 cm^{-1}. The selected intervals of wavenumbers considered in calculations are marked with open circle symbols.

Table 2
Confusion matrix of the PLS-DA model regarding sensory quality discrimination of minced pork meat samples based on FTIR spectral data.

From/to	Fresh	Semi-fresh	Spoiled	Total	Correct (%)
Training (n = 66)					
Fresh	14	1	0	15	93.3
Semi-fresh	2	13	0	15	86.7
Spoiled	0	0	36	36	100.0
Testing (n = 67)					
Fresh	13	2	0	15	86.7
Semi-fresh	2	14	0	16	87.5
Spoiled	0	4	32	36	88.9

Overall correct classification (accuracy) for train and test data sets: 94.0% and 88.1%, respectively. Training of the PLS-DA model was carried out with 66 samples, as one 'semi-fresh' sample was removed from the database as outlier.

that influence the panel agreement (Versari, Parpinello, Chinnici, & Meglioli, 2011). So further improvement on prediction could be based on better training of the taste panel in combination with the development of an improved/standardized protocol for meat assessment. It must also be emphasized that the number of examined samples within each class was not equal due to the different spoilage rate of minced pork at different storage temperatures resulting in a variable number of samples in each class. Thus the number of spoiled samples was greater than twice the number of fresh or semi-fresh samples. This may have affected the training process which is basically a data driven approach, and could thus account for the lower classification accuracies observed in certain classes.

The application of FTIR analysis to monitor meat spoilage is not new and it has been reported by previous researchers (Ellis & Goodacre, 2001; Ellis et al., 2004, 2002). It needs to be noted that in those studies the focus was given on (i) beef and poultry meat, whereas pork has not received so far the same level of attention, and (ii) emphasis was given on spoilage in terms of microbiological analyses based mainly on total viable counts. In the aforementioned cases, meat sample was characterized as spoiled when the counts exceeded a certain threshold, normally 7–8 $\log_{10}$ cfu/g, but no attempt was made to correlate spectral data with quality classes defined by a sensory taste panel. In addition spoilage was monitored in one storage temperature (room temperature), whereas in this work four different storage temperatures (0, 5, 10, and 15 °C) have been assayed including both abuse and chill temperatures. Moreover, the characterization of minced meat samples by the taste panel in three distinct sensory classes is a more realistic approach following consumer perception about meat spoilage. Thus, apart from the well-defined "fresh" and "spoiled" classes, an intermediate class of "semi-fresh" has been determined indicating the early stage of spoilage where the meat has developed slight off-odours but it is still acceptable for consumption.

3.4. Correlation of microbiological data with FTIR spectra

PLS regression (PLS-R) models were built for the counts of tvc, *Pseudomonas* spp., *B. thermosphacta* and lactic acid bacteria using FTIR responses as input variables and the microbial counts as output variables. It needs to be noted that no regression model was attempted for *Enterobacteriaceae* because this microbial group presented a long lag phase duration (Table 1) at all temperatures and growth was initiated after the samples had been characterized as spoiled by the sensory panel. The models were developed so that the first replication of the experiment was used in the training process and the second replication in

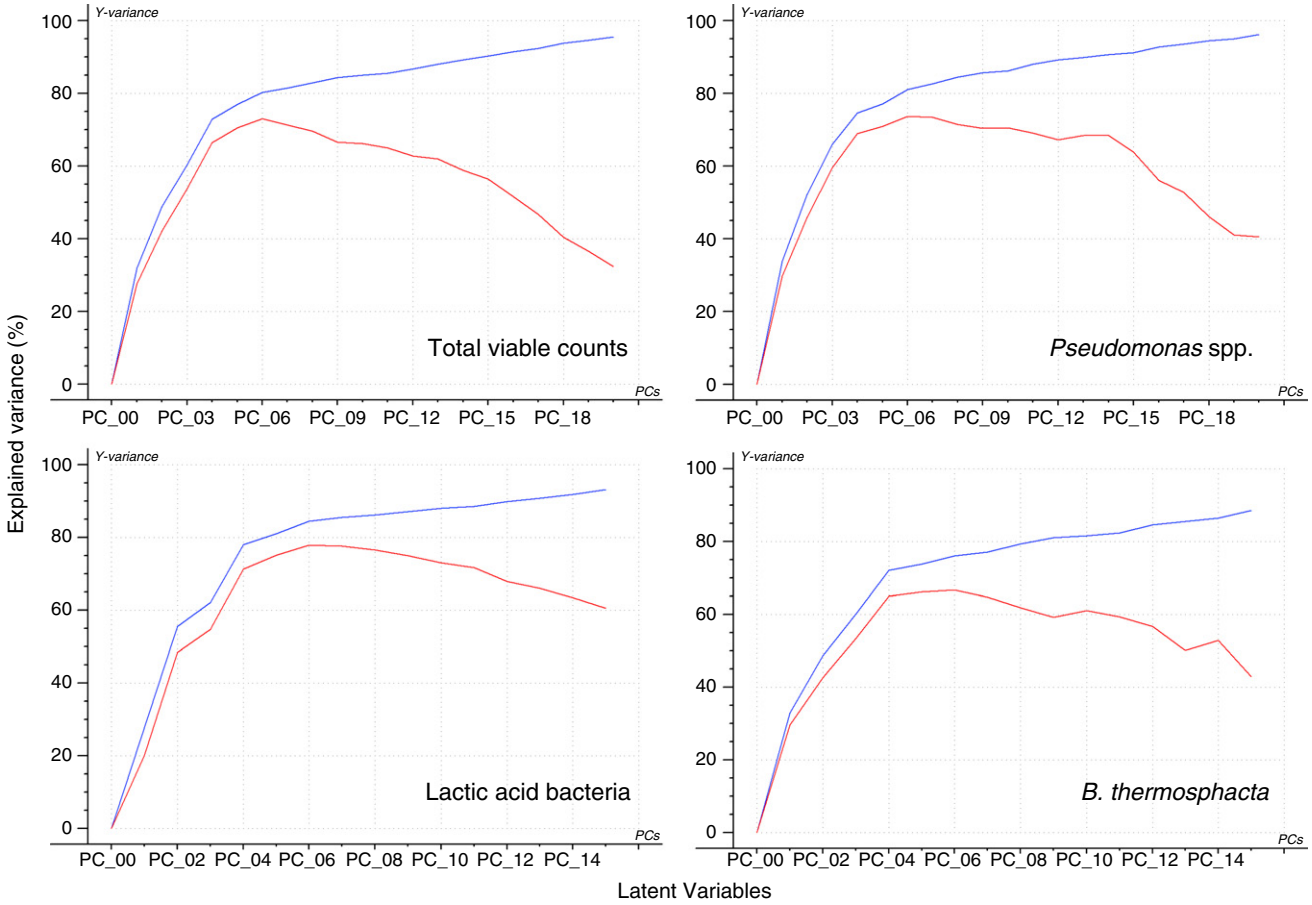

Fig. 4. Optimization of the PLS regression (PLS-R) models using latent variables (LVs) ranging from 1 to 20 for the training (blue line) and test (red line) datasets after leave-one-out cross-validation. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

testing. A critical aspect in PLS model development is the determination of the optimum number of LVs. Thus, using too few variables results in insufficient model, but using too many variables unnecessary overfitting occurs (Lin et al., 2004). Fig. 4 illustrates that the explained variance of the test dataset increases sharply within the first six LVs and then starts to decrease progressively. So, a number of 6 LVs was finally selected presenting the highest percentage of explained variance during model development for each individual microbial group. Good relationships were found between the results of FTIR and the microbiological analysis. The performance indices of the PLS-R models for model building and testing are presented in Table 3. The bias factors of the different microbial groups were close to unity indicating no structural deviation of the models, i.e., systematic over or under-prediction of the microbial counts. Moreover, based on the values of the accuracy factor, the average deviation between predictions and observations was 7.5% and 7.9% for total viable counts and *Pseudomonas* spp. and 10.7% and 11.3% for lactic acid bacteria and *B. thermosphacta*. Correlations above 0.80 were also found in the PLS-R models for both training and test data sets. The best correlations between predicted and observed counts for individual microbial groups were found for lactic acid bacteria and *Pseudomonas* spp. These observations were also graphically verified by the comparison of the observed vs. predicted counts plots (Fig. 5). FTIR has been successfully employed in the past to correlate spectral data to microbiological counts (Ellis et al., 2004, 2002) but the efforts have been focused on the prediction of total viable counts, which are not always representative of the microbial dynamics during meat spoilage, whereas no attempt has been undertaken to correlate other microbial groups. In a recent work (Rajamäki et al., 2006), the applicability of an electronic nose was investigated to develop PLS models to predict the counts of lactic acid bacteria, aerobic mesophilic bacteria, anaerobic and facultative anaerobic bacteria and *Enterobacteriaceae* during modified atmosphere packaged poultry meat. The authors reported correlations above 0.70 between observed and predicted counts concluding that an electronic nose analysis could offer a powerful and rapid tool for the quality evaluation of meat products and the microbiological counts of different microbial groups could be predicted on the basis of the electronic nose responses.

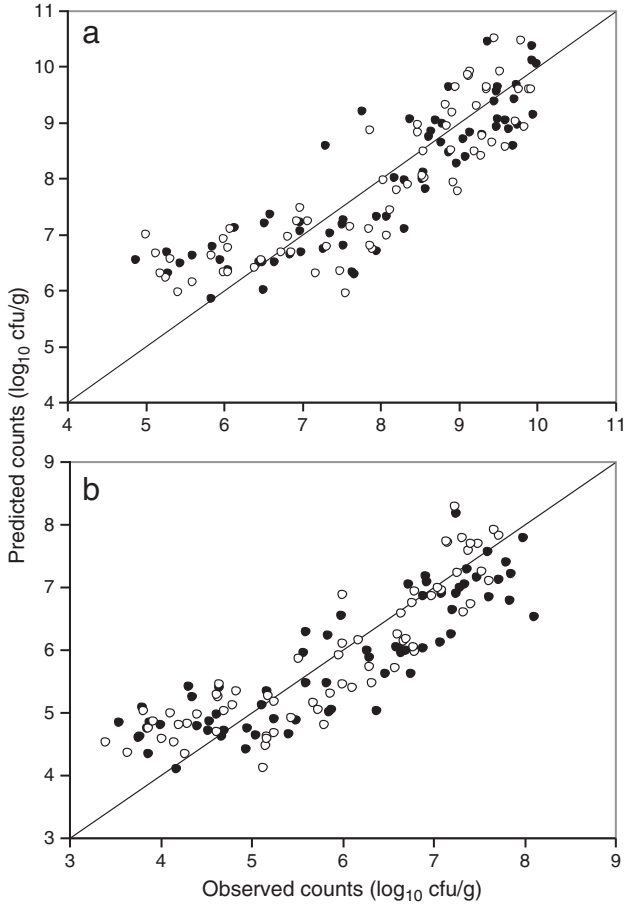

Fig. 5. Comparison between observed and predicted counts of *Pseudomonas* spp. (a) and lactic acid bacteria (b) by the PLS regression model based on FTIR spectral data (open symbols: training data; solid symbols: test data).

Table 3
Performance indices of the PLS regression (PLS-R) model correlating the microbial counts in minced pork meat samples on the basis of FTIR spectral data.

Microorganism	B_f	A_f	SEC	SEP	r_{train}	r_{test}
Total viable counts	0.984	1.075	0.781	0.674	0.895	0.880
Pseudomonas spp.	0.998	1.079	0.764	0.714	0.854	0.865
B. thermosphacta	1.018	1.113	0.834	0.804	0.808	0.826
Lactic acid bacteria	0.990	1.107	0.594	0.670	0.878	0.870

B_f : bias factor; A_f : accuracy factor; SEC: standard error of calibration; SEP: standard error of prediction; r : correlation coefficient.

4. Conclusion

The results obtained in this study demonstrated that FTIR spectral data could become an interesting tool to monitor minced pork spoilage through the measurement of biochemical changes occurring in meat substrate. The collected spectra could be considered as biochemical fingerprints containing valuable information for the discrimination of meat samples in quality classes corresponding to different spoilage levels, and could also be used to correlate the population of the different microbial groups directly from the sample surface. However, it must be stressed that the developed PLS models can only be as good as the experimental data provided, and errors for example in the sensory or microbiological analysis could result in a poorly functioning model. For this reason, further research is required for the method to be eligible and able to be updated with the novel packaging and preservation techniques that are raising and sequentially change the time course and character of spoilage.

Acknowledgements

The authors acknowledge the Symbiosis-EU (www.symbiosis-eu.net) project (no.211638) financed by the European Commission under the 7th Framework Programme for RTD. The information in this document reflects only the authors' views and the Community is not liable for any use that may be made of the information contained therein.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.foodres.2011.09.012.

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