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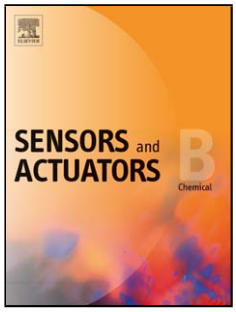

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Rapid qualitative and quantitative detection of beef fillets spoilage based on Fourier transform infrared spectroscopy data and artificial neural networks

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Running title: Monitoring meat spoilage with FTIR and neural networks

Abstract

A machine learning strategy in the form of a multilayer perceptron (MLP) neural network was employed to correlate Fourier transform infrared (FTIR) spectral data with beef spoilage during aerobic storage at chill and abuse temperatures. Fresh beef fillets were packaged under aerobic conditions and left to spoil at 0, 5, 10, 15, and 20°C for up to 350 hours. FTIR spectra were collected directly from the surface of meat samples, whereas total viable counts of bacteria were obtained with standard plating methods. Sensory evaluation was performed during storage and samples were attributed into three quality classes namely fresh, semi-fresh, and spoiled. A neural network was designed to classify beef samples to one of the three quality classes based on the biochemical profile provided by the FTIR spectra, and in parallel to predict the microbial load (as total viable counts) on meat surface. The results obtained demonstrated that the developed neural network was able to classify with high accuracy the beef samples in the corresponding quality class using their FTIR spectra. The network was able to classify correctly 22 out of 24 fresh samples (91.7%), 32 out of 34 spoiled samples (94.1%), and 13 out of 16 semi-fresh samples (81.2%). No fresh sample was misclassified as spoiled and vice versa. The performance of the network in the prediction of microbial counts was based on graphical plots and statistical indices (bias and accuracy factors, standard error of prediction, mean relative and mean absolute percentage residuals). Results demonstrated good correlation of microbial load on beef surface with spectral data. The results of this work indicated that the biochemical fingerprints during beef spoilage obtained by FTIR spectroscopy in combination with the appropriate machine learning strategy have significant potential for rapid assessment of meat spoilage.

Keywords: Artificial neural networks, Aerobic storage, Beef fillets, FTIR, Machine learning, Meat spoilage.

1. Introduction

In most developed countries meat consumption is very high mainly due to its high nutritional value in the human diet. The great variability in raw meat in terms of chemical composition, technological and chemical attributes results in highly variable end products which are marketed without a desired and controlled level of quality [1]. In order to maintain quality standards, control procedures must be carried out on meat comprising chemical analyses, instrumental methods, organoleptic evaluation, and molecular screening methods. More than fifty methods have been used for the detection of microbiologically spoiled or contaminated meat (e.g. organoleptic, microbiological, and physico-chemical), all of which are well documented [2]. However, these techniques are invasive, time consuming, labour intensive, demand highly trained personnel, and thus they are unsuitable for online application and routine analysis [3-7]. The lack of general agreement on the early signs of incipient spoilage for meat makes more difficult the task to evaluate it objectively, mainly due to changes in the technology of meat preservation (e.g. vacuum, modified atmospheres, etc.). The use of microbial metabolites as a consequence of microbial activity in meat has been continuously recognized as a potential means for assessing meat quality [4, 6, 8]. The attempts that have been made over the last two decades to associate given metabolites with microbial spoilage of meat have not been very much appreciated, due to low understanding of the underlying phenomena [6].

Recently, some interesting analytical approaches using mathematical equations have been applied to describe the kinetics of ephemeral/specific spoilage organisms (E(S)SO) with the purpose to predict spoilage of various foods [7, 9]. Other approaches are based on the use of biosensors (enzymatic reactor systems), electronic noses (array of sensors), and vibrational spectroscopy methods (e.g. FTIR, Raman spectroscopy) [10-12] for the same purpose. In contrast to conventional methods, Fourier transform infrared (FTIR) spectroscopy is rapid,

non-invasive, requires no specific consumable or reagent permitting users to collect full spectra in a few seconds allowing simultaneous assessment of numerous meat properties [4-5, 13]. The basic concept underlying this method stipulates that as bacteria grow on meat, they utilize nutrients and produce by-products that cause spoilage. The quantification of these metabolites represents a fingerprint characteristic of any biochemical substance, providing thus information about the type and the rate of spoilage [2, 13]. The integration of the FTIR Attenuated Total Reflectance biosensors or other biosensors in tandem with an information platform would 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.

The application of advanced statistical methods (e.g. discriminant function analysis, clustering analysis, partial least square regression, chemometrics) and intelligent methodologies (neural networks, fuzzy logic, evolutionary algorithms, genetic programming) can be used as qualitative indices rather quantitative since their primary target is to distinguish objects or groups or populations [14-15]. Nowadays, machine learning strategies are based on supervised learning algorithms [16]. The last mentioned approach together with the development of artificial neural networks (ANN) could be used effectively in the evaluation of meat spoilage. Interest in using artificial neural networks in food science is increasing in the last years, as they have shown promising results in several applications such as sensory analysis, pattern recognition, classification, microbial predictions, and food process optimization [17-21]. Essentially, ANNs are computing algorithms that attempt to imitate the computational capabilities of large, highly connected networks of relatively simple elements such as neurons in the human brain [22]. They contain a series of mathematical equations that are used to simulate biological processes such as learning and memory. Their development first involves a learning process that adaptively responds to the input variables according to a

learning rule. The network has the ability to learn from its environment and adapt to it similar to its biological counterparts [23]. An ANN normally has no restriction on the type of relationship between the growth parameters (input patterns) and the desired output. In contrast to conventional models in which a mathematical equation must be stated beforehand, ANNs directly explore the knowledge contained in the input-output patterns by adjusting the highly nonlinear topology, as the input-output patterns are repeatedly presented to the network [24].

The aim of the present study was to build upon previous experience undertaken in our laboratory on beef spoilage using FTIR spectroscopy and develop an artificial neural network that could (i) discriminate between different quality classes of beef fillets during aerobic storage at chill (0, 5°C) and abuse (10, 15, and 20°C) temperatures, and (ii) predict the microbial load on the surface of meat samples directly from FTIR spectral data.

2. Materials and methods

2.1 Sample preparation

Fresh deboned pieces of beef were purchased from a local meat retail outlet and transported under refrigeration to the laboratory within 30 min. On arrival, the samples were prepared by cutting the meat pieces into portions (40 mm wide x 50 mm long x 10 mm thick) and maintained at 4°C for 1 h until use. The portions were subsequently placed into 90 mm Petri dishes and stored at 0, 5, 10, 15, and 20˚C in high-precision (±0.5°C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan) for an overall period of 350 h, depending on storage temperature, until spoilage was pronounced. Meat samples were not subjected to any prior pre-treatment such as fat and connective tissue removal, or inoculation with selected species of bacteria. For the FT-IR measurements, a thin slice of the aerobic upper surface of the fillet was excised and used for further spectral analysis.

2.2 Microbiological analyses

For microbiological analysis a portion (40 mm wide x 50 mm long x 10 mm thick) was added to 150 ml sterile quarter strength Ringer's solution, and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature (*ca.* 20°C). Further decimal dilutions were prepared with the same diluent, and duplicate 0.1 ml samples of three appropriate dilutions were spread in triplicate on plate count agar (PCA 4021452; Biolife, Italy) for counts of total viable bacteria (TVC), which was incubated at 30ºC for 48 h. Duplicate samples from each storage temperature were analyzed at appropriate time intervals to allow for efficient kinetic analysis of total viable counts. Specifically, meat samples stored at 0 and 5°C were analyzed every 24 h, whereas samples stored at 10, 15, and 20°C were analyzed every 8, 6, and 4 h, respectively. Growth data from plate counts were log transformed and fitted to the primary model of Baranyi and Roberts [25] using the in-house program DMFit (Institute of Food Research, Norwich, UK) to determine the kinetic parameters of microbial growth (maximum specific growth rate and lag phase duration).

2.3 Sensory analysis

Sensory evaluation of meat samples was performed during storage according to Gill and Jeremiah [26] by a sensory panel composed 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 sample tested. The sensory evaluation was carried out in artificial light and the temperature of all samples was close to ambient. 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. Each sensory attribute was scored on a three-point hedonic scale corresponding to: 1=*Fresh*; 2=*Marginal*; and 3=*Spoiled*. Score of 1.5 was

characterized as *Semi-fresh* and it was the first indication of meat spoilage. Odour characteristics of beef fillets, as determined by special samples kept frozen and thawed prior to each sensory evaluation, were considered as fresh. 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. Overall, 74 meat samples were assessed by the sensory panel and classified into the selected three groups as fresh (n = 24), semi-fresh (n = 16), and spoiled (n = 34).

2.4 FT-IR/ATR spectroscopy

Meat samples were analyzed in parallel to the microbiological and sensory analyses. FT-IR spectra were collected using a ZnSe 45° ATR (Attenuated Total Reflectance) crystal on a Nicolet 6700 FT-IR 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 aerobic upper surface of the meat 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 4,000 to 400 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 Pre-treatment of the data and neural network development

The FT-IR spectra collected between 1800 and 1000 cm^{-1} were initially submitted to smoothing based on the Savitzky-Golay algorithm. Subsequently, mean-centred and standardized spectral data were subjected to principal components analysis (PCA). The PCA is an unsupervised method that transforms a large number of potentially correlated factors into a small number of orthogonal (uncorrelated) factors (i.e. principal components), reducing thus the size of the initial dataset and optimizing the feature vector [27]. Since the raw spectral data could not be used because of the strong correlation among the variables (wavenumbers), the uncorrelated principal components from PCA analysis were employed for this purpose. The variables (wavenumbers), for which the communality values of the first three PCs were higher or equal to 0.6 were considered as significantly explaining the variance of the spectral data, and hence they were considered as potential wavenumbers associated with the biochemical changes during meat spoilage. The wavenumbers that were selected from the first PCA to be significant in this data set ranged from 1718 to 1203 cm^{-1} and 1020 to 1001 cm^{-1} and were selected for further analyses. A second PCA with the selected variables (wavenumbers) revealed the Principal Components (PCs) that significantly contributed to the variance of the data set. In our case, the total variance (100%) of the data set could be explained by 37 principal components (PCs) from which the first five were extracted and used as input to the developed neural network, accounting for 98.08% of cumulative variance observed in the experiment (data not shown).

A multilayer perceptron (MLP) network based on backpropagation was developed to determine the applicability of neural networks as a meat quality classifier. The network

consisted of an input layer with seven input nodes for storage temperature, sampling time, and the five principal components (Fig. 1). The output layer consisted of two nodes, one for the quality class (Fresh, Semi-fresh, Spoiled), and another for the predicted total viable counts of the meat sample. The class membership of a single sample pattern was coded in a numerical format by assigning 1 for "Fresh" samples, 2 for "Semi-fresh", and 3 for "Spoiled" samples with a cut-off value of 0.5. In order to keep the neural network as simple as possible one hidden layer was selected with a varying number of neurons. The network configuration was approached empirically by testing different possibilities (i.e. neurons in the hidden layer, learning rate, and momentum) and selecting the one that provided the best classification accuracy. In a fully interconnected network, all neurons in the hidden layer are connected to all neurons in the input and output layers, but no connections are allowed between neurons in one layer or from one neuron to itself, or directly between input and output layer neurons. The hidden neurons are in fact the elements in a neural network that provide high degree of nonlinearity (24). In these networks each node receives signals through connections with other nodes or the outside world in the case of the input layer. The net input to node *j* has the form:

$$I_j = \sum_{i=1}^{n} w_{ij} \cdot x_i + \theta_j \qquad (1)$$

where x_i are the inputs, w_{ij} are connection weights associated with each input/node and θ_j is the bias associated with node *j*. The output from each node is used as an input in a nonlinear transfer function:

$$O_j = f(I_j) \qquad (2)$$

The most commonly used transfer functions are sigmoidal, hyperbolic tangent and linear function. In our work the sigmoidal and hyperbolic tangent were selected as transfer functions in both hidden and output layers. All inputs were normalized in the range from 0.1 to 0.9 and

-1 to +1 for sigmoidal and hyperbolic tangent functions, respectively, to avoid saturation problems in their performance due to different value ranges of the inputs.

The standard backpropagation algorithm for network training is based on the steepest-descent gradient approach applied to the minimization of the error function defined as:

$$E = \frac{1}{2}\sum_{s=1}^{3} d_{qs} - y_{out,s}^{\ 2} \quad (3)$$

where d_q represents the desired network output for the q^{th} input pattern in the s network layer and y_{out} is the network output. The generalized delta rule was applied for adjusting the weights of the feedforward networks in order to minimize equation 3. The rule for adjusting weights was given by the following equation:

$$w_{ij}^{s}\ t+1 = w_{ij}^{s}\ t + \eta\delta_j^s y_j^s + \alpha\Delta w_{ij}^{s}\ t \quad (4)$$

where η is the learning rate parameter, α the momentum term, and δ the negative derivative of the total square error with respect to the neuron's output.

The entire database consisted of 74 meat spectral patterns corresponding to different storage temperatures and sampling times. As the number of observations was small, separation of the dataset into training and testing subsets (hold-out method) would further reduce the number of data and would result in insufficient training of the network. Therefore, in order to improve the robustness of classification, the leave-1-out cross validation technique was employed to evaluate the performance of the developed network. The classification accuracy of the MLP network was determined by the number of correctly classified samples in each sensory class divided by the total number of samples in the class. The performance of the neural network in the prediction of total viable counts for each meat sample analyzed was determined by the bias (B_f) and accuracy (A_f) factors [28], the mean relative percentage residual (MRPE) and the mean absolute percentage residual (MAPR) [29], and finally by the root mean squared error (RMSE) and the standard error of prediction (SEP) [30]. The MLP

network was developed in MATLAB version 7.0 code (Mathworks, Inc., Massachusetts, USA).

3. Results

The population dynamics of total viable counts (TVC) during beef fillet storage at different temperatures is presented in Figure 2, whereas the estimated kinetic parameters after fitting with the primary model of Baranyi and Roberts are shown in Table 1. Lag phase was observed only at 0 and 5°C, while a progressive increase of maximum specific growth rate (μ_{max}) values with storage temperature was evident. The aerobic plate counts of meat samples indicated that the total microflora ranged from 2.9-3.3 $\log_{10}$ cfu cm^{-2} at the onset of storage (fresh samples) to 8.7-9.4 $\log_{10}$ cfu cm^{-2} for samples characterised as spoiled.

Typical FTIR spectral data in the range of 1800 to 1000 cm^{-1} collected from fresh and spoiled beef fillet samples stored at 5°C for 10 days are shown in Figure 3. These spectra can be employed to obtain metabolic snapshots (fingerprints) of beef fillets during storage at different temperatures in an attempt to monitor meat spoilage. The temperature of 5°C was chosen as a typical chill storage temperature for meat. The comparison of FTIR spectra could provide information on certain biochemical changes occurring during meat spoilage. Hence, based on Figure 2, a major peak at 1640 cm^{-1} due to the presence of moisture (O-H stretch) with an underlying contribution from amide I in the meat sample was apparent, whereas a second peak at 1550 cm^{-1} appeared due to the absorbance of amide II (N-H bend, C–N stretch). A second amide vibration was shown at 1400 cm^{-1} (C–N stretch), followed by amide III peaks at 1315 and at 1240 (C-N stretch, N-H bend, C-O stretch, O=C-N bend). The peaks at 1460, 1240 and 1175 cm^{-1} can be attributed to fat (C=O ester). Finally, the peaks arising from 1025 to 1140 could be absorbance due to amines (C-N stretch) [4-5, 13, 31, 32].

An MLP neural network based on back propagation was used to classify beef fillet samples into three sensorial categories (fresh, semi-fresh, spoiled) from the metabolic fingerprints of FTIR spectral data after dimensionality reduction with principal components analysis. The classification performance of the MLP network with variable number of neurons in the hidden layer and different transfer functions (logistic sigmoid and hyperbolic tangent) is presented in Fig. 4. The learning rate (n = 0.10) and momentum (α = 0.20) parameters were selected to ensure that the convergence of the learning process was achieved. Generally, the classification performance of the network obtained for the meat samples stored at different temperatures and cross validated with leave-1-out method was lower when the selected transfer function was hyperbolic tangent despite the fact that the algorithm converged faster. The highest overall correct classification with hyperbolic tangent transfer function (86.5%) was obtained with 20 neurons in the hidden layer (Fig. 4b), however within the individual classes performance was low, especially for semi-fresh meat samples (62.5%). The best performance of the classifier was obtained with 10 neurons in the hidden layer and a logistic sigmoid transfer function (Fig. 4a) providing a 90.5% overall correct classification, which within the selected classes corresponded to 91.7%, 94.1%, and 81.3% for fresh, spoiled, and semi-fresh meat samples, respectively. The classification accuracies obtained from this network, designated as 7-10-2, are presented in the form of a confusion matrix in Table 2. The sensitivities (i.e. how good the network is at correctly identifying the positive samples) for fresh and spoiled meat samples were 91.7% and 94.1%, respectively, representing 2 misclassifications out of 24 fresh meat samples, and also 2 misclassifications out of 34 spoiled samples. In the case of semi-fresh samples the respective figure was somehow lower (81.2%). In this case 3 samples out of 16 were misclassified, 1 as fresh and 2 as spoiled. The specificity index (i.e. how good the network is at correctly identifying the

negative samples) was also high especially in fresh and spoiled samples, indicating satisfactory discrimination between these two classes (Table 2).

The plot of predicted versus observed total viable counts (Fig. 5) showed reasonably good distribution around the line of equity ($y = x$), with the majority of data (*ca.* 78%) included within the ± 1 log unit area, although some over-prediction was evident in the case of fresh meat samples, especially with low observed initial counts. The performance of the MLP network is also presented in Figure 6 where the % relative error of prediction is depicted against the observed microbial population. Based on this plot, data were almost equally distributed above and below 0, with approximately 88% of predicted microbial counts included within the ± 20% RE zone. It needs to be emphasized though that the network over-estimated the bacterial population for certain fresh samples, especially at lower observed microbial counts, corresponding to low temperature (0°C) and short storage time. The performance of the MLP network to predict total viable counts in meat samples in terms of statistical indices is presented in Table 3. Based on the calculated values of the bias factor (B_f) it can be inferred that the network under-estimated total viable counts in semi-fresh and spoiled samples ($B_f < 1$), whereas for fresh samples over-estimation of microbial population was evident ($B_f > 1$). In addition, the values of the accuracy factor (A_f) indicated that the predicted total viable counts were 18.1%, 12.2%, and 8.4% different (either above or below) from the observed values for fresh, semi-fresh, and spoiled meat samples, respectively. The mean relative percentage residual index (MRPR) also confirmed the under-prediction for semi-fresh and spoiled samples (MRPR > 0) and over-prediction for fresh samples (MRPR < 0), whereas the values of mean absolute percentage residual (MAPR), representing the average deviation between observed and predicted counts, verified the information provided by the accuracy factor. The standard error of prediction (SEP) index is a relative typical deviation of the mean prediction values and expresses the expected average error associated

with future predictions. The lower the value of this index is, the better the capability of the network to predict microbial counts in new meat samples. The value of the index was less than 10% in spoiled samples indicating good performance of the network for microbial count predictions in this class (Table 3). Comparable results were observed for semi-fresh samples (SEP 13.6%), but for fresh samples the index gave higher values as the network over-estimated microbial counts for some fresh samples, particularly those stored at 0°C and for short storage time (Fig. 5).

4. Discussion

A major challenge facing the meat industry today is to obtain reliable information on meat quality throughout the production, distribution, and storage chains, and turn this information into decision support systems which would ultimately provide a guaranteed quality of meat products for consumers [1]. The metabolomic concept in food microbiology which has been introduced recently [14] improved the concept of using single biochemical indicators as proposed in late 80s and 90s [3, 33-36]. Chemometrics (e.g. principal components analysis-PCA, hierarchical cluster analysis-HCA, discriminant function analysis-DFA, partial least square regression-PLSR), in parallel with machine learning approaches based on soft computing (e.g. artificial neural networks-ANN, genetic algorithms, support vector machines-SVM) have been applied as data mining techniques in bioprocess data [16, 37]. These approaches could rapidly provide information related to the contribution of the ephemeral spoilage organisms (ESO) in meat or to the categorization of meat with regard to (i) type of meat and (ii) spoilage [3, 15, 38, 39]. Ellis and co-workers [4, 5] have been the pioneers stipulating that FTIR spectroscopy can be used directly on the surface of food to produce biochemical interpretable ‘‘fingerprints” (metabolic snapshots), enabling thus early detection of microbial spoilage of chicken breast and beef rump steaks.

In this work, FTIR spectroscopy was employed to obtain metabolic fingerprints of beef fillets during storage in aerobic conditions at five different storage temperatures (0, 5, 10, 15, and 20°C). A machine learning approach was then followed to develop a pattern recogniser based on a simple multi-layer perceptron (MLP) neural network, in an attempt to classify meat samples in three quality classes (Fresh, Semi-fresh, Spoiled) as judged previously by a taste panel. The classification performance of the MLP network was very good for fresh and spoiled samples with correct classification rates exceeding 91% (Table 2) after leave-1-out cross validation of the dataset. It is characteristic that no fresh samples was misclassified as spoiled and vice versa, indicating that the biochemical fingerprints provided by FTIR spectral data could discriminate these two classes quite accurately. Lower percentages were obtained for semi-fresh samples (*ca.* 81%) with erroneous classifications in the other two classes. It must be emphasized however that the number of examined samples within each class was not equally distributed, due to the different spoilage rate of beef samples at the different temperatures assayed (Table 2). This may have affected the learning process of the neural network, which is basically a data driven approach [40], and thus could account for the lower classification accuracies observed for this class. It is also worth noting that the logistic sigmoid transfer function employed in the neurons of the hidden layer gave higher classification accuracies compared with the hyperbolic tangent transfer function (Fig. 4), despite the fact that the latter results in faster convergence of the training algorithm [24]. It is worth-noting that initially two independent neural networks were developed for the prediction of either quality class or TVC counts, with lower prediction accuracies each (data not shown). Moreover, as both output parameters are not independent, in the sense that quality class is related to microbiological counts and vice versa, a network that would combine both outputs would be more efficient. The relatively lower accuracies obtained in the semi-fresh class could also be attributed to the performance of the sensory evaluation process, as the difference

between “fresh” and “semi-fresh” class is sometimes not very clear. So further improvement on prediction could be based on better training of sensory evaluation panels in combination with the development of an improved/standardised protocol for meat assessment.

The application of machine learning to correlate FTIR spectral data with meat spoilage is not new and it has been tackled in the past [2, 4, 5]. However, in these works, the focus was given on the rapid detection of bacterial spoilage, in terms of microbiological analyses, whereas no attempt was made to correlate spectral data with quality classes defined by sensory assessment of the samples. In addition, spoilage was monitored in only one storage temperature (room temperature), whereas in our work five different storage temperatures have been assayed (0, 5, 10, 15 and 20°C). In this way spoilage has been monitored not only at abuse temperatures but also at chill temperatures. Concerning TVC counts indicating beef spoilage it was found by sensory evaluation that the respective values ranged from 7.0-8.2 $\log_{10}$ cfu cm^{-2}, depending on storage temperature. In a previous work undertaken in our lab [13], FTIR snapshots were taken into account for the characterization of minced beef samples into the same quality classes using linear discriminant function analysis (DFA) analysis. Results showed that the classification accuracies of the MLP classifier were better compared with DFA in the characterization of meat samples, indicating the advantage of ANN approach in tackling complex, non-linear problems as meat spoilage.

Another challenge from the microbiological perspective would be the implementation of machine learning approaches to correlate FTIR spectral data to bacterial counts on meat samples. As reported in previous works [5], spectra collected from the surface of beef contained biochemical information that could be correlated with the spoilage status of the samples. In this way, expensive and time-consuming microbiological analysis could be replaced in the long term by an on-line system based on spectroscopic data, providing rapid, non-invasive, and low cost microbiological analyses [4, 7]. To investigate this issue, the MLP

classifier was designed with two nodes in the output layer, one corresponding to the sensorial class of beef fillets, and another one for the prediction of microbial counts for each sampling time and storage temperature, based on TVC measurements. The comparison of observed and predicted bacterial counts, based on calculated statistical induces and plots (Figs. 5-6, Table 3), presented reasonably good agreement, showing that the developed neural network approach could be used effectively to assess the spoilage condition of beef fillets. The plots of the estimates versus observed bacterial counts were within *ca.* 1 $\log_{10}$ cfu cm^{-2} from the line of equity, which is comparable with a value of *ca.* 0.5 $\log_{10}$ cfu cm^{-2} for beef steaks reported previously [5]. These results were also confirmed by the percent relative error index (%RE) between observed and predicted values (Fig. 6) with the exception of three samples corresponding to fresh beef fillets with low initial counts. The calculated validation indices showed acceptable performance of the developed neural network in predicting total viable counts of beef samples directly from FTIR spectral data. The values of the bias factor (B_f) were close to unity indicating good agreement between predictions and observations when the three quality classes were taken together (Table 3). However, within classes, underestimation was evident for spoiled and semi-fresh samples, while TVC for fresh samples were overestimated. However, the calculated values B_f are within the range of 0.9 to 1.0 or 1.0 to 1.05 which are considered adequate [41], whereas other authors have accepted B_f values of between 0.75 and 1.25 as being acceptable for spoilage microorganisms [42]. Generally, the highest prediction accuracy of the neural network was observed in the case of spoiled samples as this class presented the lowest values of indices compared to the other two classes. Concerning the values of the accuracy factor (A_f) it has been reported [43] that an increase of 0.15 (15%) would be acceptable for each independent variable included in model development. Therefore, in our study, with only one independent variable (temperature) we would expect A_f up to 1.15, which is in good agreement with the calculated values for the

three classes and the overall model as well (Table 3). The mean relative percentage residual and the mean absolute percentage residual are statistics similar to the bias and accuracy factors [44] which provided similar information as the other two indices about the performance of the neural network.

4. Conclusion

In conclusion, these data demonstrate the utility of the analytical approach based on FTIR spectroscopy which in combination with an appropriate machine learning strategy (artificial neural networks) could become an effective tool for monitoring beef fillets spoilage during aerobic storage at chill and abuse temperatures. 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 also could be used to predict satisfactorily the microbial load directly from the sample surface.

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George-John Nychas is Professor at the Food Science and Technology Department of the Agricultural University of Athens, and also Director of the Laboratory of Microbiology and Biotechnology of Foods. In the last 20 years he has coordinated and/or participated in 30 EU projects, in the area of food safety, food microbial ecology, microbial physiology of pathogenic and spoilage bacteria, natural antimicrobial systems, metabolomics, mathematical (predictive) modeling and risk analysis.

Figure Legends

Fig. 1. Schematic structure of the developed neural network. The input layer contains the incoming signals of the network corresponding to storage temperature, time, and the values of the five principal components. The output layer contains two nodes, one for the predicted quality class (Fresh, Semi-fresh, Spoiled) of meat samples and one for total viable counts. w_{ij} : synaptic weights with *i* being the index of the input signal neuron and *j* being the output signal neuron; b: bias term.

Fig. 2. Changes of total viable counts (TVC) obtained from beef fillets stored under aerobic conditions at 0°C (◆), 5°C (■), 10°C (●), 15°C (▲), and 20°C (×). Data points are values from duplicate meat samples after incubation at 30°C for 48 h. Lines represent growth curves fitted with the Baranyi primary model.

Fig. 3. Typical FTIR spectra in the range of 1800 to 1000 cm^{-1} collected from fresh (black line) and spoiled (red line) beef fillets stored at 5°C for 10 days.

Fig. 4. Classification performance of neural networks with variable number of neurons in the hidden layer according to logistic sigmoid (a) and hyperbolic tangent activation transfer functions.

Fig. 5. Comparison of total viable counts (TVC) of beef fillets generated by the ANN model against experimentally observed values during storage at aerobic conditions (F: fresh; SF: semi-fresh; S: spoiled meat samples).

Fig. 6. Percent relative errors between observed and predicted by the neural network total viable counts (TVC) during storage of beef fillets at aerobic conditions (F: fresh; SF: semi-fresh; S: spoiled meat samples).

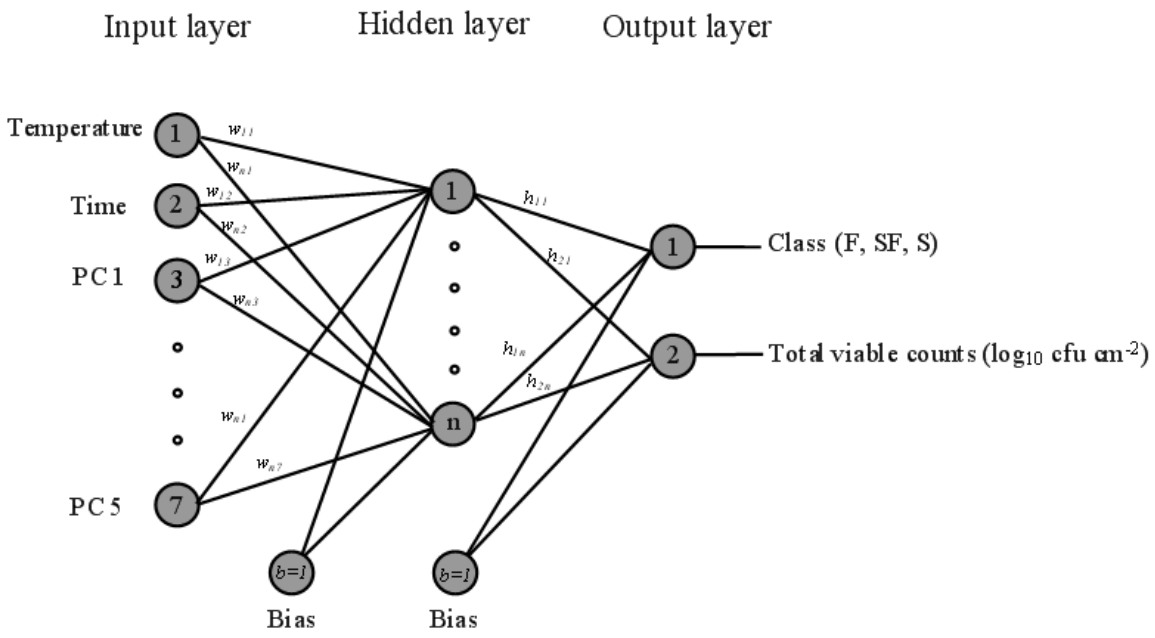

Fig. 1.

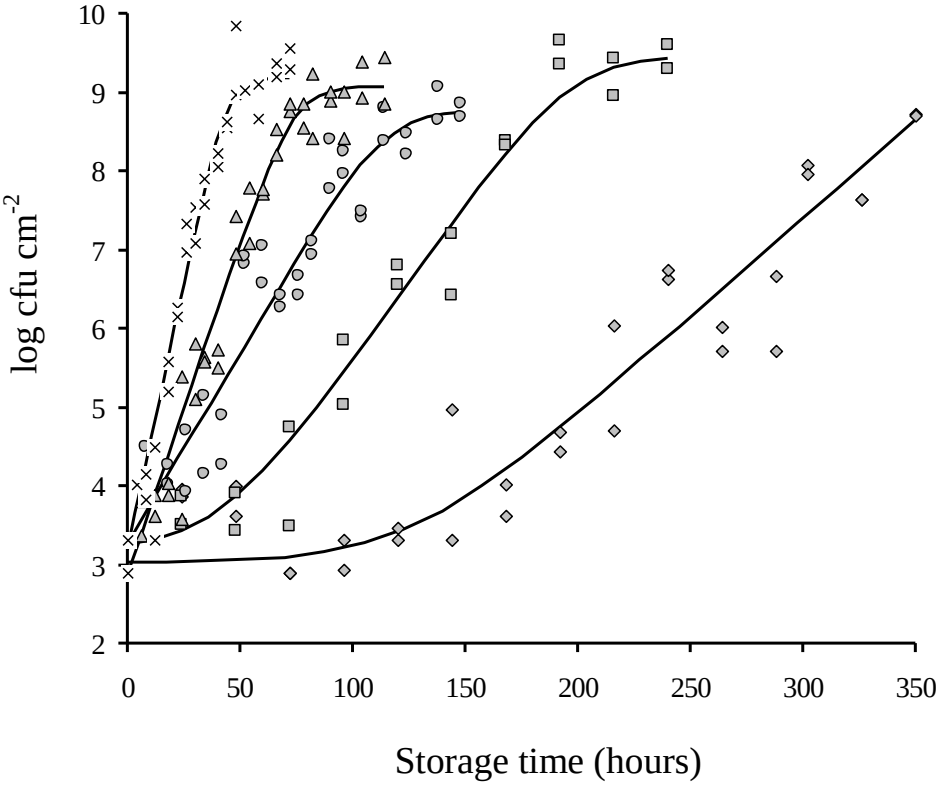

Fig. 2.

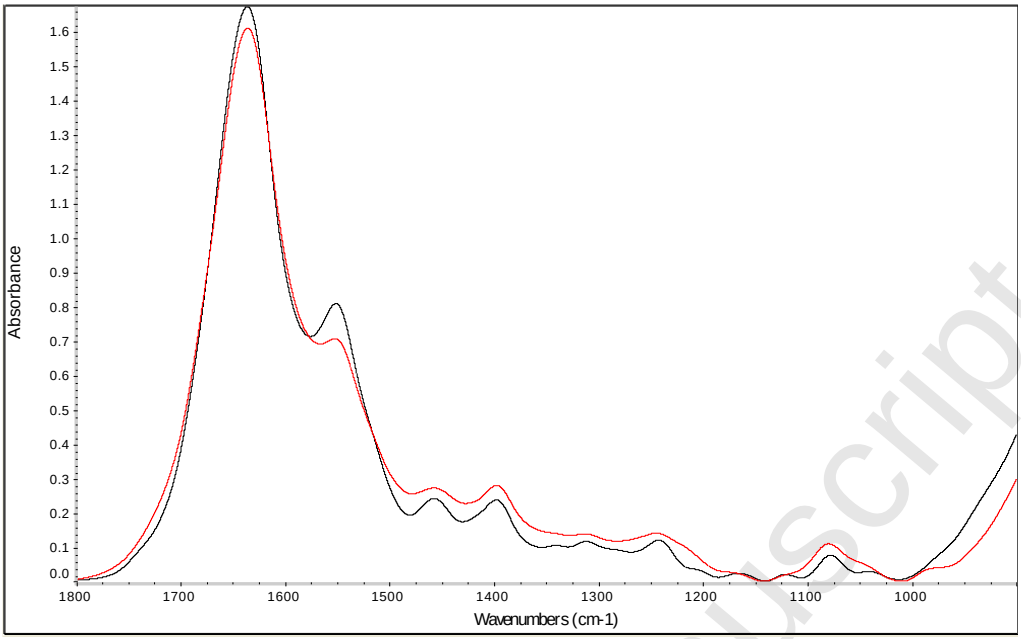

Fig. 3.

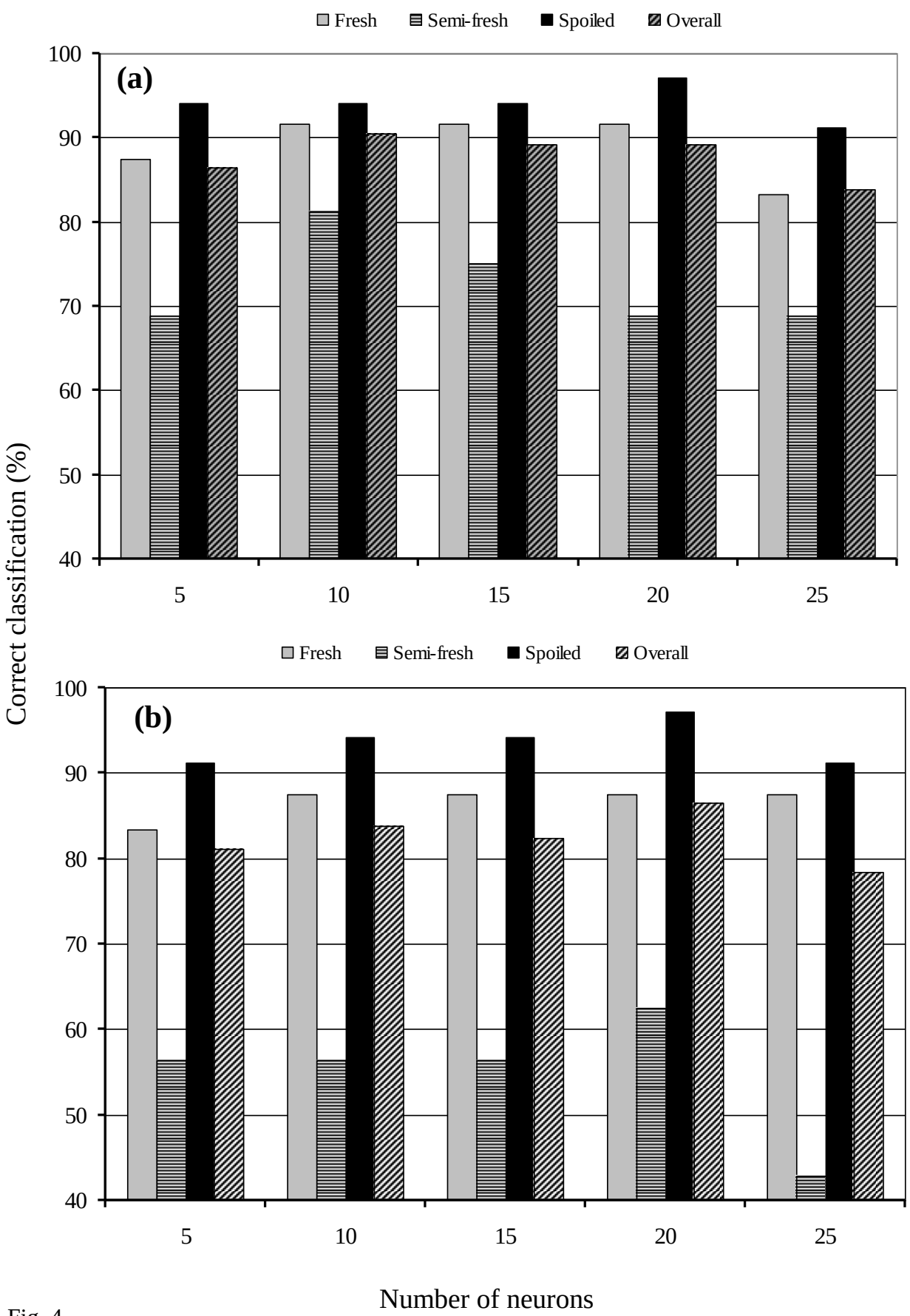

Fig. 4.

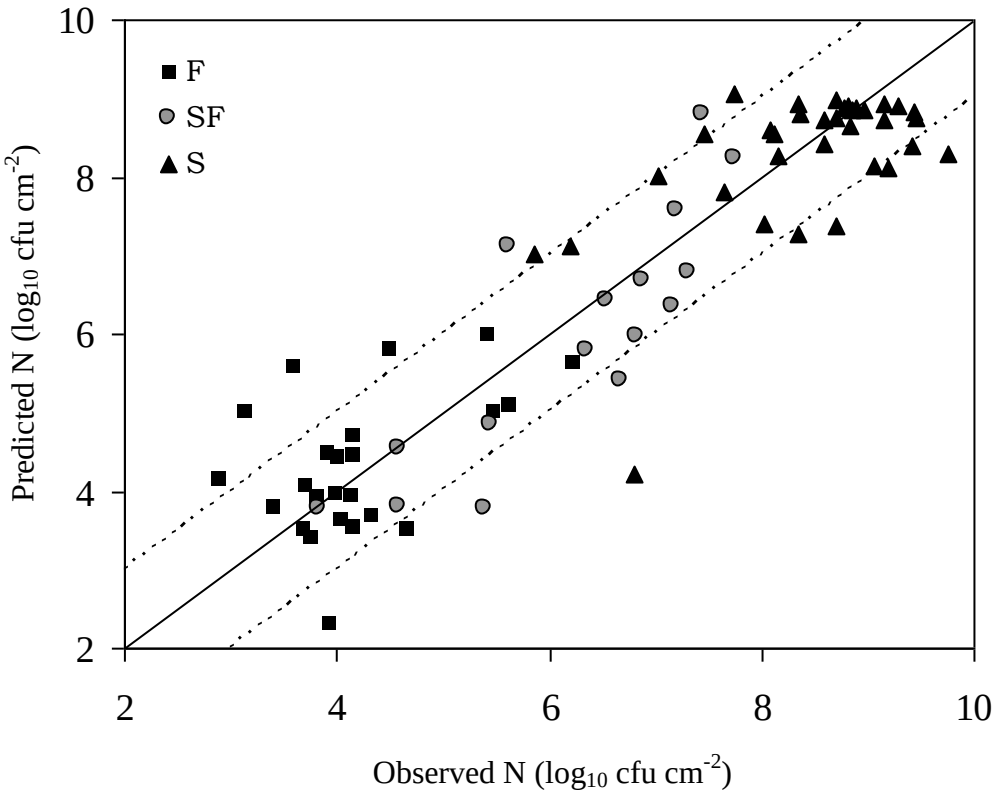

Fig. 5.

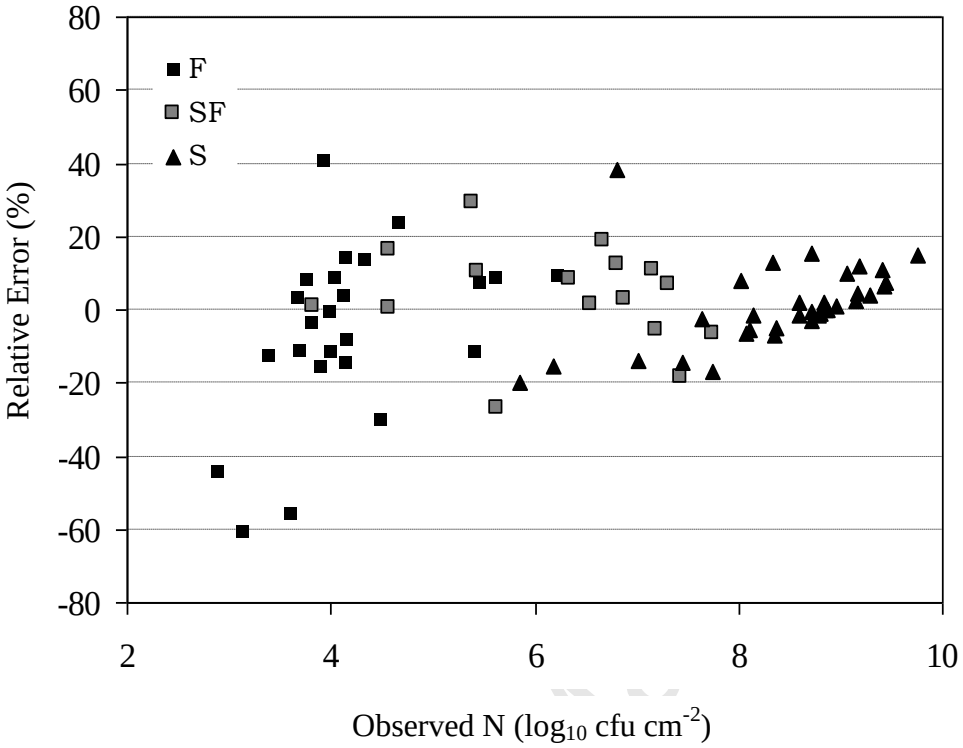

Fig. 6.

Table 1

Estimated kinetic parameters of total viable counts (TVC) by the Baranyi model as a function of storage temperature (initial counts 3.10 ± 0.30 $\log_{10}$ cfu cm^{-2}).

Temperature (°C)	μ_{max} (h^{-1})[a]	Lag phase (h)	y_0 ($\log_{10}$ cfu cm^{-2})[b]	y_{end} ($\log_{10}$ cfu cm^{-2})[c]		Standard error of fit	R^2
				Observed	Predicted		
0	0.057	125.2	3.03	8.71[f]	-[e]	0.445	0.953
5	0.091	42.5	3.26	9.44	9.47	0.384	0.974
10	0.111	-[d]	3.26	8.77	8.78	0.522	0.924
15	0.194	-	2.87	9.15	9.07	0.338	0.975
20	0.312	-	3.17	9.42	9.18	0.278	0.982

[a] maximum specific growth rate
[b, c] initial and final total viable counts estimated by the Baranyi model
[d] not observed
[e] not computed as fitted curve presented no upper asymptote
[f] mean value from two independent experiments

Table 2

Confusion matrix of the 7-10-2 MLP classifier performing the task of discrimination of meat samples based on the leave-1-out cross validation method.

True class	Predicted class			Row Total ($n_{i.}$)	Sensitivity (%)
	Fresh	Semi-fresh	Spoiled		
Fresh (n=24)	**22**	2	0	24	91.7
Semi-fresh (n=16)	1	**13**	2	16	81.2
Spoiled (n=34)	0	2	**32**	34	94.1
Column Total ($n_{.j}$)	23	17	34	74	
Specificity (%)	95.6	76.5	94.1		

Overall correct classification (accuracy): 90.5%.

Table 3

Performance of the 7-10-2 MLP classifier for the prediction of total viable counts in meat samples (fresh, semi-fresh, spoiled, overall) analyzed by FTIR.

Statistical index	Mathematical expression	Fresh	Semi-fresh	Spoiled	Overall
Bias factor (B_f)	$10^{\sum \log(P/O)/n}$	1.031	0.951	0.982	0.991
Accuracy factor (A_f)	$10^{\sum \lvert\log(P/O)\rvert/n}$	1.181	1.122	1.084	1.123
Mean Relative Percentage Residual (MRPR %)	$\frac{1}{n}\cdot\sum\frac{100\cdot\ O-P}{O}$	-5.572	3.971	1.082	-0.451
Mean Absolute Percentage Residual (MAPR %)	$\frac{1}{n}\cdot\sum\frac{100\cdot\lvert\ O-P\ \rvert}{O}$	17.564	11.078	7.869	11.708
Root Mean Squared Error (RMSE)	$\sqrt{\frac{\sum(O-P)^2}{n}}$	0.872	0.846	0.835	0.850
Standard Error of Prediction (SEP %)	$\frac{100}{\overline{O}}\cdot\sqrt{\frac{\sum(O-P)^2}{n}}$	20.861	13.622	9.917	12.937