



Analytical Methods

Monitoring of physical–chemical and microbiological changes in fresh pork meat under cold storage by means of a potentiometric electronic tongue

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ABSTRACT

This work describes the correlation found along 10 days between potentiometric measurements obtained by using an electronic tongue and the variation in certain physicochemical, microbial and biochemical parameters measured on a whole piece of pork loin stored under refrigeration. The electronic tongue consists of a set of six electrodes made of Au, Ag, Cu, Pb, Zn and C, and a reference electrode. Through the use of various multivariate analysis techniques, such as: PCA and two types of artificial neural networks (i.e. multilayer perceptron (MLP) and fuzzy ARTMAP) it was found that it is possible to determine the time elapsed in relation to the degradation of the loin by using simple potentiometric measurements. Additionally, in the same pork sample used to measure redox potentials with the electronic tongue, the following parameters were also determined; pH, microbial count, concentrations of inosine 5'-monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx). Through the use of PLS analysis, it was found a rather good correlation between pH and the potentiometric data. Also a remarkable correlation was observed between the measures carried out with the electronic tongue and the so-called *K*-index that simultaneously measures the variation in the adenosine triphosphate (ATP) degradation products. These results suggest that this simple, or a similar electronic tongue, could be useful for the undemanding qualitative or semi-quantitative evaluation of freshness in meat samples in a wide range of situations.

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1. Introduction

Meat freshness is a rather complex concept, which includes different microbiological, physicochemical and biochemical attributes and that is related with two different processes. One, desired, is known as aging that is determined by the period of storage that meat (especially beef meat) needs in order to reach the optimum state of consumption; whereas the other, also related with storage, deals with meat spoilage due to bacterial growth and autolysis. Traditionally, there have been two methods to evaluate meat freshness; one consists of a sensory test controlling organoleptic attributes with the help of experts and the other is the chemical or biochemical determination of the concentration of target bio-indicators. The former is rapid but expensive, whereas for the latter

a large number of reported studies try to relate freshness and the concentration or presence of certain species. For instance, the determination of biogenic amines, trimethylamine or volatile amines have been proposed as a suitable method to determine meat spoilage and as an index for meat freshness (Edwards, Dainty, & Hibbard, 1983; Hurst, 1990; Yano, Yokoyama, & Karube, 1996). On the other hand, the determination of degradation products of ATP has also proved to be useful for the determination of meat degradation (Yano, Yokoyama, Tamiya, & Karube, 1996). Other methods for evaluating aging rely for instance on the measurement of gaseous components released from food using semiconductor gas sensors (Funazaki et al., 1995), or in the use of electromagnetic sensors, particularly microwave sensors (Clerjon & Damez, 2007). All these analytical techniques currently used to study freshness have proved their validity; however, in general, they are slow and need relatively sophisticated tools. This makes that the application of these techniques in the industry is rather limited, since they are not suitable for rapid monitoring, require highly skilled operators, are relatively expensive and time-consuming and in

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general are designed for in-laboratory use only. One important consequence is that current methods cannot evaluate correct meat freshness in the market when it is sold. Additionally, from a quality assurance viewpoint it would be advantageous to develop non-destructive sensing methods able to inspect a large number of samples on-line. In fact, the development of simple, undemanding and non-destructive new analytical tools that additionally would be low-cost and that could be applied in a wide range of situations for monitoring food freshness without requiring highly specialised personnel is still an unresolved goal.

One of the protocols that fulfil most of these requirements is related with the use of the so-called electronic noses and electronic tongues. These are tools that have been introduced recently, inspired in the mode in which mammals recognise food through the senses of olfaction and taste (Toko & Cambridge University Press, 2000). These techniques are especially appealing when it is important to characterise complex attributes of the whole sample rather than to know the exact concentration of certain analytes. Electronic tongues and noses do not use specific but unspecific sensors, that are nevertheless able to respond in some way differentially toward a group of related chemical species and whose global response can be related sometimes with certain parameters or characteristics. The unspecific sensors are usually integrated in an array, i.e. the electronic tongue, and their response is commonly analysed by suitable pattern recognition algorithms.

An easy way to build up an electronic tongue is through the use of a set of electrodes using potentiometric or voltammetric (Holmin, Spangeus, Krantz-Rulcker, & Winquist, 2001) electrochemical techniques. In fact, several electronic tongues based on electrochemical sensors have been developed (Gallardo, Alegret, & del Valle, 2005). Among them, those relying in potentiometric measurements have been the most widely used employing for instance ion-selective electrodes (Mimendia et al., 2010). A suggestive alternative to avoid the employment of membrane-containing sensors is the use of simple metallic wires as suitable electrodes in electronic tongue devices (Lvova et al., 2006). Electronic tongues with metallic electrodes are very simple to prepare and easy to use. The response with this kind of electrodes in the potentiometric mode is based on the spontaneous polarisation of the metals and other elements in the presence of certain chemical species (Soto et al., 2006). In relation to the latter, for instance, freshness in pork has been evaluated by means of simple solid electrodes (i.e. Pt, CuS and Ag₂S) able to detect certain compounds responsible for the initial stage of the process of putrefaction of meat (Kaneki et al., 2004).

As it has been reported, changes in the redox potential of electrodes such as those used here (i.e. metal electrodes and graphite) is a rather unspecific process and a number of variations in the composition and characteristics in post-mortem meat may affect to the electrode potential. For instance, these changes are among others, changes in pH, redox potential, the reaction of certain compounds with the surface of the electrodes (typically reaction of thiol containing molecules on gold or silver electrodes), physisorption processes, etc. All these changes can cause a significant modification of the potential of the electrodes. However, it is this rather unspecific behavior that makes metallic electrodes suitable for the fabrication of electronic tongues. Thus, others and we have recently reported that arrays of metal-based electrodes could be used as a simple mode to develop such devices. In fact, we have recently been able to evaluate 'fish freshness' using a simple set of electrodes (Barat et al., 2008). In order to advance further in the concept of using metal and metal-like electrodes for the development of suitable electronic tongues, able to relate the unspecific measurement with rather complex attributes such as freshness, we have followed the potential changes occurring on a piece of pork loin.

Within this background, we report herein, the results obtained by the use of an electronic tongue made with metallic electrodes (Au, Ag, Cu, Pb and Zn) and graphite for the analysis of the evolution of pork loins stored throughout time. A discussion in relation to the suitability of the method and a correlation of data from the electronic tongue with classical analysis including measurements of pH, microbial count and the determination of the concentration of certain ATP-degradation compounds is included.

2. Materials and methods

2.1. Raw material

All the experiments using the electronic tongue and the biochemical determinations were carried out on a three whole pieces of pork loin. The pork loins were obtained from a local slaughterhouse with 1 day post-mortem. Samples were obtained from female pigs (crossbreeds Landrace × Large White) sacrificed with an average live weight of 130 kg. During the experimental process, the meat was stored under refrigeration at a temperature of 4 °C. Every day, two slices of each loin were cut, one for the potentiometric measurements and one more for the corresponding biochemical analysis (*vide infra*).

2.2. Electrochemical measurement, data acquisition and computing tools

The complete measurement equipment was built up for this specific application and was formed by several blocks including: the electrodes, the signal conditioning system, the data acquisition system and tools for the multivariate analysis of the data. Each part of the equipment is detailed below.

2.3. Electrodes

The electronic tongue consists of a set of six electrodes of different materials: i.e. gold, silver, copper, lead, zinc and graphite. The length of each electrode was about 3 cm whereas the thickness varies depending on the material used. The electrodes were soldered to a printed circuit board that served to support to the whole system. Through a central hole in the board a calomel reference electrode was also incorporated to the system. A similar set of electrodes and configuration has been used previously in other applications (Gil et al., 2008).

2.4. Signal conditioning system

The electrical signals generated by the electrodes have characteristics, such as their small scale and high output impedance, which make them very sensitive to electrical noise. For this reason, a signal conditioning system is included in the measurement equipment. This contains two parts; one with a very high input impedance and very low current of polarisation that was made with electrometric amplifiers LMC6001 (www.national.com) and a second part containing an active low pass filter in order to eliminate noise signals that come from the electrical network.

2.5. Data acquisition system

The signals from the signal conditioning system are input to the data acquisition system whose mission is to capture the electrical signals coming from different sensors and, once these are suitably adapted, to store them. The data acquisition system additionally enables the display of information in real time. An Adlink PCI-9112 card (www.nudaq.com) was used in the computer that was

also equipped with the VEE-Pro software (Agilent Technologies, Santa Clara, CA, www.home.agilent.com) that allows both viewing the data on the computer screen and storing the data for subsequent processing.

2.6. Multivariate analysis of data

With the response signals from the different electrodes a matrix of data was obtained that was used to perform multivariate analysis. The MATLAB® (R2007 MathWorks) analysis program was used to apply techniques of pattern recognition that allow to visualise if the data taken from the electrodes were correlated with some parameters (in our case, for instance, the day of measurement, vide infra). One of the techniques most commonly employed for this purpose is the use of principal component analysis (PCA) algorithms that transform input variables (usually strongly correlated) in a smaller number of non-correlated variables, allowing its graphical representation in two (or three) dimensions of the two (or three) principal components.

To classify the measurements, in order to make a correlation between the data and the day in which the data were taken, artificial neural networks were used. There are various types of artificial neural networks, the best known being the so-called multi-layer perceptron (MLP) algorithm. For the operation of the MLP neural network two stages are required, an initial training that establishes the value of the weights and thresholds of each neuron within the network and a second stage of testing, where new entries and values different from those in the training process are used.

MLP networks have been widely used in electronic tongue systems (Panagou, Sahgal, Magan, & Nychas, 2008), but also have shown some limitations (e.g. they are difficult to train when a low number of samples are available or when there is an uneven number of samples per category (Llobet, Hines, Gardner, Bartlett, & Mottram, 1999)). On the other hand, artificial neural networks based on the so-called Adaptive Resonance Theory (ART) have been developed to tackle some of the problems found with the MLP. ART is basically a method that seeks to provide answers to some of the problems of stability and plasticity that normally affect MLPs and additionally can be easily trained with a reduced number of samples. Based on this theory various algorithms, such as the Fuzzy Artmap have been developed and implemented recently in electronic noses and tongues (Carpenter, Grossberg, Markuzon, Reynolds, & Rosen, 1992).

2.7. Potentiometric measurement method

The experiments with the electronic tongue consisted of the measurement of the electrode potential during a period of 10 days, which usually corresponds to the maximum time of storage for fresh pork meat at a temperature of 4 °C. Measurements were performed every day except days 5 and 6. Each day two slices of each loin were cut ($n = 3$); one of them was used to make potentiometric measurements, whereas biochemical analyses were carried out on the other two.

To achieve homogeneity in all measurements, both ends of the loins were removed before sampling and we were careful at making slices of similar size and to puncture in the same area of each slice (centre). The samples belong to the Longissimus dorsi muscle that is a muscle of homogeneous structure.

One of the main problems when using metallic electrodes is the possible occurrence of response or baseline drift that could lead to obtain irreproducible data or to mistakenly believe that drift variations are changes in meat aging. In order to control these possible drifts, each day the potential of the electrodes, when placed on a 'standard solution' of distilled water buffered at pH 7, was measured.

Data gathering in pork meat was carried out by sticking on the meat sample the set of electrodes and the reference electrode. Data were taken for no less than 5 min in order to allow the electrodes to reach the equilibrium. The data measured were automatically stored on the computer in an ASCII-file type. The file obtained included one data point for each of the electrodes, the value of which was determined by the arithmetic mean of the potential of the electrode when the steady-state was reached in order to damp possible influences of electrical noise or random variations in the measurements. With data from all the measurements performed, a result matrix was made. The matrix was formed by 6 columns, one for each of the electrodes and by 24 rows (3 measurements per day during 8 days).

2.8. Analytical determinations

2.8.1. Measurement of pH

pH was determined in samples throughout the storage time by using a puncture pHmeter (micro pH 2000, Crison). pH was assayed in different points of the same section with no variation.

2.8.2. Microbiological analysis

Tenfold dilutions in 0.1% peptone water were prepared from each sample obtained from every container at every measurement day ($n = 3$) and 1 ml aliquots were plated in duplicate. Aerobic counts were determined by using Plate Count Agar (Merck). Duplicate pour plates were prepared per dilution and incubated at 28 °C for 48 h.

2.8.3. ATP breakdown compounds

The ATP-degradation compounds, consisting of IMP, Ino and Hx, were determined by HPLC. The extraction procedure was similar to that described by Burns and Kee (1985). Five grams of each sample were homogenised with 20 ml of 0.6 M of cold perchloric acid for 4 min at 4 °C by using a masticator (IUL Masticator, Barcelona, Spain). The obtained extract was centrifuged at 10,000g under cold conditions (4°C) for 20 min. The supernatant was filtered through glass wool and neutralised by adding solid potassium carbonate. The neutralised extract was kept in ice for 5 min and then centrifuged at 12000g in a refrigerated microcentrifuge for 10 min. The supernatant was stored at –28 °C prior to analysis.

An HPLC, model 1100, equipped with a diode array detector was used (Agilent Technologies, Palo Alto, CA). Nucleotides were separated in a LiCrospher 100 RP-18 column (150 × 4 mm) (Agilent Technologies) by using a program gradient between two solvents: 0.05 M dipotassium hydrogen phosphate buffer, pH 7.0 (A) and methanol. (B). Thus, after 8 min of isocratic elution with 100% solvent A, a 7 min gradient to 30% solvent B followed by a 10 min washing step with 50% solvent B was achieved. Before a new analysis, initial conditions (100% solvent A) were maintained for 15 min. Flow rate was 0.9 ml/min and separation was achieved at 30 °C. Injection volume was 10 µl. ATP-related compounds were monitored at 254 nm and their spectral signal in the range 190–350 nm was obtained to assure peak identification.

3. Results and discussion

3.1. Potentiometric measurements

The values of the potential of each electrode versus time (8 days of storage, from day 1 to day 10) are shown in Fig. 1. It can be seen that the range of variation of the potential depends on the type of electrode. The potential of silver and gold electrodes varies between 0 and –200 mV. Copper electrode has a fairly continuous behavior, in the –200 mV and –300 mV range, lead electrode be-

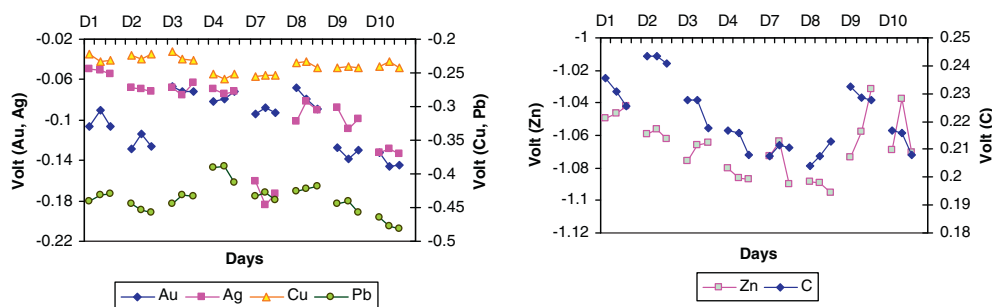


Fig. 1. Changes in the potential of the electrodes as a function of time in meat.

has similarly to copper but within the range -450 mV and -550 mV, whereas the zinc electrode shows responses between -1 V and -1.1 V. Finally, graphite (C) is the only electrode with positive potential and moves in the 180 – 250 mV range. A direct observation of the data in Fig. 1 gives no conclusive information about the evolution of sensor response with the increase in storage time of meat and, therefore, a more detailed study using multivariate analysis is required.

3.2. Multivariate analysis

One of the basic characteristics of the metallic electrodes used for the fabrication of the electronic tongue is that their response shows overlapped sensitivity and a suitable useful technique to obtain conclusions from the large number of experimental measurements in intricate systems such as food degradation is to carry out multivariate analysis. Thus, in order to further investigate the evolution of the response collected signals throughout storage time, PCA studies were carried out. Fig. 2 shows the PCA graphic for three measurements taken each day on the pork loin and also plots the data obtained from the 'standard solution' (vide ante). Data in PCA plot were mean-centered and the two first principal components accounted for 86.57% of the variance. The PC axes are calculated to lie along lines of diminishing levels of variance in the data set.

A separation in two main blocks of data is clearly observed. On the negative part of the PC1 axis lie the measurements obtained from the pork loin, whereas in the positive part of PC1 are the data obtained from the measurements of the 'standard solution'. This

spontaneous clustering was expected taking into account the very different nature of the two samples measured. Data from the 'standard solution' are not exactly coincident but form a cloud. Moreover, it is noticeable that there is no relation between the relative positions of points from the 'standard solution' and their respective measurements of pork meat the same day. Also it is remarkable that whereas calibration measurements are placed quite close to each other, the data obtained from the evolution of the meat of pork are spread in a larger zone in the PCA plot, suggesting that the electrodes are really giving response to the post-mortem evolution of meat. Although there is some dispersion in the calibration measurements, the fact that these do not appear ordered in the PCA plot according to measurement day rules out the presence of significant response drift. Therefore, the main cause of the changes in the electrode potentials with time observed on the PCA plot are changes caused by aging of the pork meat.

The PCA analysis only using the results obtained from the pork meat are plotted in Fig. 3. This three-dimensional graph covers 89.09% of total data variance from the six electrodes. It can be observed that the trials from the same day gather rather together and that three major groups of measures are observed, corresponding to the early days: 1, 2, 3 and 4 (group A); days 7, 8 and 9 (group B); and the day 10 (C).

3.3. Classification with artificial neural networks

The data obtained can also be analysed using artificial neuronal networks. These networks use a training phase with a set of measurements and a further phase for validation.

Therefore, in order to check the power of discrimination of the electronic tongue system, artificial neural networks were used. The aim of this approach is the classification of each sample according to the day the measurement was taken. Two types of artificial neural networks were used in order to compare their response: i.e. MLP and fuzzy ARTMAP types. In both cases, the network had six inputs corresponding to the six electrodes and also eight outputs that corresponded to the ten-day experiences. Moreover, in addition to the artificial neural network with 10 outputs, other network with a smaller number of outputs was also employed. In this process, the measurements were distributed in three groups corresponding to days 1, 2, 3 and 4 (output A), days 7, 8 and 9 (output B) and day 10 (output C). The reason for this classification was derived from the PCA results in which a distribution in these four groups was observed in the score plot (see Fig. 3).

The first artificial neural network used was the MLP. To estimate the rate of success in identifying the samples with each type of classifier, the 'leave-one-out' cross-validation method was applied using the 24 measurements available. At first an MLP was used which had eight output neurons (i.e. eight-category classification). Several training and validation steps were made to determine both the number of neurons in the hidden layer and the

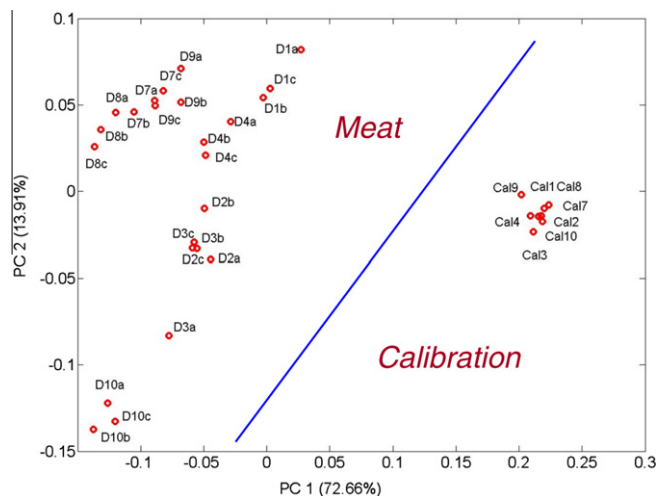


Fig. 2. PCA results of the metallic electrode response using of the combined measurements with meat and the calibration solution.

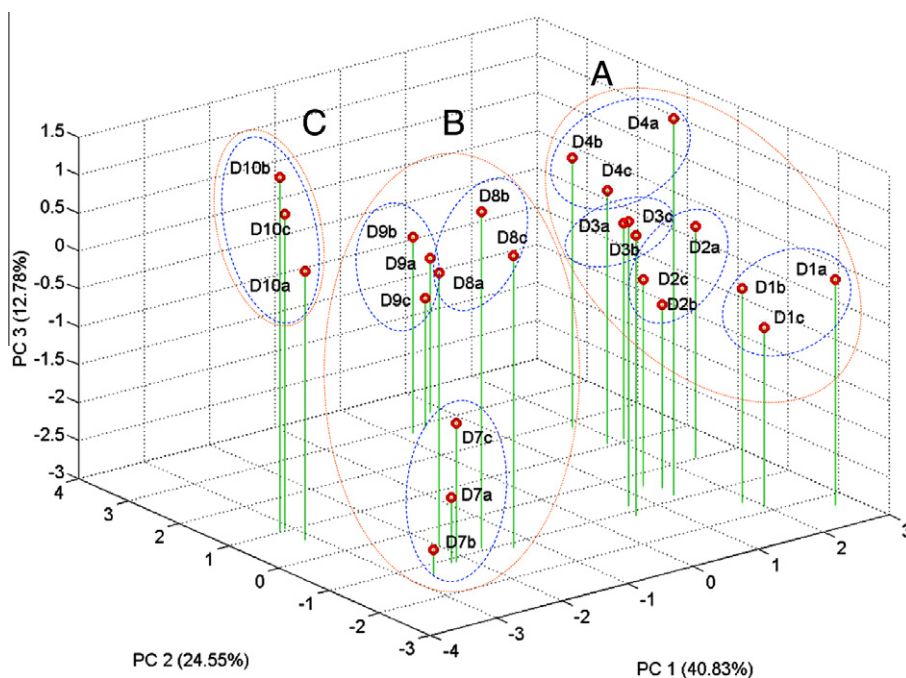


Fig. 3. Three-dimensional (right) PCA plots from the results for the metallic electrode response. Ellipses cluster together measurements carried out the same day (1–10) and dotted lines correspond to groups of consecutive days (A–C).

transfer functions type to be used. The best results were achieved by using 8 neurons in the hidden layer and log-sigmoid transfer functions, which led to an 85.83% success rate in classification. In the second step, an MLP having 3 outputs was used, i.e. three-category classification: A (days 1, 2, 3 and 4), B (days 7 and 8) and C (day 10), which obtained a success rate of 86.23% when 3 hidden neurons and a log-sigmoid transfer function were employed.

The same sample classification strategies we envisaged employing another type of neural network (i.e. fuzzy ARTMAP). In fact from a practical point of view fuzzy ARTMAP works very well when there are very few samples for training (Carpenter, Grossberg, & Reynolds, 1991), so it could be anticipated that this network would outperform the MLP in this particular situation. The algorithm has been implemented in-house using function macros from basic functions of Matlab. Employing the training and validation method described above, a remarkable success rate of ca. 100% was obtained both for the network with eight outputs for the network of three outputs.

3.4. Chemical and biochemical analysis

Above we have detailed that there is a correlation between the overall response of the set of electrodes (the electronic tongue) and the time elapsed since the beginning of the experiments. In this part of the work we are additionally interested in seeing if the changes in potential of the electrodes in the electronic tongue could be related with the evolution versus time of chemical or biochemical parameters determined on the same pork loin where the electronic tongue was used. As it is well-known, once the animal dies a number of degradation reactions begin, which contribute to diminish its freshness; some of those reactions are chemical, mainly the oxidation of organic compounds, and others are of biochemical nature (autolysis). In addition to that, microorganism growth implies an overall decrease in quality and safety.

Therefore in order to somehow validate the potentiometric method outlined above for freshness evaluation of pork meat, it is necessary to compare the changes in the potentiometric data

with some other reported method for the determination of meat freshness. As we have detailed above, there is a quite large number of methods proposed for the evaluation of freshness that can be basically divided into two classes: (i) those measuring the concentration of certain bio-molecules or bio-markers signaling the evolution of meat after the death of the animal and (ii) those based on indirect methods (e.g. changes on the pH or electrical resistance and the use of electronic tongues and noses).

In this study, the evolution of the following parameters has been measured: pH, microbial growth and variation of the concentration of different ATP breakdown compounds.

3.5. Measurement of pH

pH determination showed a decrease of the concentration of protons as a function of time from 5.6 (first day) to 6.27 (day 10th).

3.6. Microbial growth

A healthy and freshly slaughtered animal has its muscle sterile. After a period of time, the duration of which depends mainly on temperature, meat pH and slaughtering management, microorganisms experiment an exponential growth. The activity of microorganisms is the main factor that limits the shelf-life of fresh meat. For the microbiological testing of meat two types of methods are routinely used: those who make a count of the total number of microorganisms present in meat and those based on the count of a particular group. The values of microorganism concentration are shown in Colony-Forming Unit per gram (CFU/g). Unlike other methods, the count of the total number of microorganisms provides no information about the freshness or quality of edible meat but gives an image of the hygienic quality of the animal, including the abuse of temperature during handling and processing.

The microbial growth experienced a significant increase from 3.7×10^3 CFU/g at the beginning of the experiment to values larger than 10^9 CFU/g on day 9.

3.7. Measurement of nucleotides produced by the decomposition of ATP

Products obtained by ATP decomposition are considered to be among the most reliable and useful for a correct meat freshness evaluation. The analysis is based on the concept that after slaughter, the ATP in pork meat decomposes following the sequence: ATP-ADP-AMP-IMP-Ino-Hx. During this process there is a change in the smell and the taste of the meat. Similar autolytic processes occur in all animals but the rate varies greatly among different species. The determination of the concentration of ATP alone cannot be used generally as freshness index because it disappears approximately 24 h *post-mortem* (Karube, Matsuoka, Suzuki, Watanabe, & Toyama, 1984). Usually the same occurs for ADP and AMP. However, the simultaneous evolution of some ATP-degradation compounds has been suggested to be an indication of the state of freshness. From this idea, the concept of the *K*-index was introduced (see Eq. (1)). Fresh meat has a low value of *K*-index, which gradually increases at a rate that depends on the species

$$K\text{-index} = \frac{[\text{Ino}] + [\text{Hx}]}{[\text{IMP}] + [\text{Ino}] + [\text{Hx}]} \times 100 \quad (1)$$

This index was originally proposed to evaluate fish freshness (Battle, Aristoy, & Toldrá, 2001) but also has been applied for the measurement of meat freshness in pork, rabbit or beef (Nakatani et al., 1986). Interestingly, biosensor systems for the determination of *K*-index making use of enzymes have also been reported by Zen, Lai, Yang, and Kumar (2002) and Park and Kim (1999). In our case, in order to calculate the *K*-index during the degradation of the pork meat, the evolution of the concentration of the ATP-related compounds IMP, Ino and Hx was determined using conventional chromatographic procedures. Table 1 shows all the values obtained for chemical and biochemical analysis. The mean value and associated standard deviation for three measurements each day are shown in Table 1. An analysis of variance (one-way ANOVA) was performed on the physicochemical measurements to investigate whether the changes observed in parameter values throughout storage time were statistically significant. ANOVA was performed using the Statgraphics® Plus version 5.1 (Manugistics, Rockville, MD, USA). The *p* values found were 0.0021 for Hx, 0.0282 for Ino, 0.0001 for IMP and 0.007 for *R*. All these values are below 0.05, so the null hypothesis (i.e. the differences observed in the mean values of the parameter throughout storage time are not significant) can be rejected at the 95% significance level.

3.8. PLS analysis

We have seen above that data from the electronic tongue clustered as a function of time that it was possible to obtain a good classification of the data using artificial neural networks. In this section we additionally were interested in analyzing if the measurements taken from the electronic tongue could be used to

predict the values obtained for the pH, the microbial growth, the concentrations of the compounds IMP, Ino, Hx or the value of the *K*-index. In order to achieve this goal, the regression technique Partial Least Square (PLS) has been used. PLS is a multivariate projection method that models the relation between an array of dependent variables (*Y*) and another array of independent variables (*X*). The principle of the technique PLS is to find the components of the matrix of input (*X*) that describe, as much as possible, relevant variations in the input variables, and at the same time get the highest correlation with the objectives (*Y*), giving the minor weight to the variations that are irrelevant or relate to noise (Berruela, Alonso-Salces, & Héberguer, 2007).

For each of the parameters of interest a set training/validation was employed (Vinaixa, Llobet, Brezmes, Vilanova, & Correig, 2005). To perform this task a bootstrap validation strategy was used (Efron & Gong, 1983). This method consists of randomly drawn a group of data from the whole data set. The data that remain are used to conduct training in order to obtain the coefficients PLS and the data extracted is used for validation. This process is repeated a number of times and with the result of all the repetitions a line of adjustment is obtained between the real and predicted values. The use of this method is especially interesting when a relatively small number of measurements are available to create the model. In this way, by repeating many times the algorithm, which results in the random change of training and validation measurements, has similar effects to having carried out the experience with more data.

In our case, to each model built was trained and validated with 20 and 4 measurements, respectively and the bootstrap process was repeated 24 times, thus a line of adjustment was obtained with 96 data points. Data from the models were centered with respect to the average value of responses from each of the 6 sensors. For each model built the optimal number of factors (latent variables) was determined by cross-validation employing training measurements only.

PLS prediction models for pH, bacteria concentration, IMP, Ino, Hx and *K*-index were created with the potentiometric experimental data obtained from the metallic electrodes. Fig. 4 shows the PLS graphic in which measured vs. predicted values of the *K*-index are plotted. Hence measured values represent the known *K*-index of the meat, meanwhile predicted values are the calculated values according with the PLS algorithm. Measured and predicted values are plotted together in order to evaluate both the accuracy and precision of the created prediction models. A preliminary evaluation can be done just by a simple visual inspection of the difference between measured and predicted values. However, a more rigorous analysis is achieved by a linear fitting of the experimental points (predicted). Here, by using a simple linear model namely $y = p1 * x + p2$, a fitting line and also the adjusting parameters ($p1$, $p2$ and regression coefficient) are obtained. The parameters $p1$ (slope of the fitting line) and $p2$ (intercept with *y* axis) represents the accuracy in prediction; meanwhile the regression coefficient can be related with the precision of the PLS model. Ide-

Table 1
Results of chemical and biochemical analysis as function of time in pork meat.

Time (days)	pH	Microb. (CFU/g)	Hx	Ino	IMP	<i>K</i> -index
1	5.6 ± 0.09	3.7 × 10 ³ ± 22	0.184 ± 0.012	2.088 ± 0.218	4.662 ± 0.401	13.212 ± 1.324
2	5.64 ± 0.086	1.2 × 10 ⁴ ± 25	0.190 ± 0.024	1.985 ± 0.232	4.541 ± 0.282	14.330 ± 2.412
3	5.7 ± 0.089	1.4 × 10 ⁴ ± 30	0.202 ± 0.007	2.130 ± 0.005	4.685 ± 0.077	15.553 ± 2.235
6	5.7 ± 0.091	7.2 × 10 ⁵ ± 38	0.256 ± 0.004	2.349 ± 0.048	4.305 ± 0.376	16.986 ± 0.758
7	5.83 ± 0.085	3 × 10 ⁷ ± 53	0.305 ± 0.016	2.559 ± 0.116	4.153 ± 0.313	18.597 ± 0.613
8	6.1 ± 0.081	8.1 × 10 ⁸ ± 77	0.341 ± 0.054	2.648 ± 0.130	3.857 ± 0.090	18.991 ± 0.704
9	6.2 ± 0.077	≥ 10 ⁹	0.438 ± 0.031	2.567 ± 0.326	3.376 ± 0.268	19.331 ± 1.363
10	6.27 ± 0.085	≥ 10 ⁹	0.661 ± 0.247	2.438 ± 0.515	2.650 ± 0.950	21.969 ± 1.903

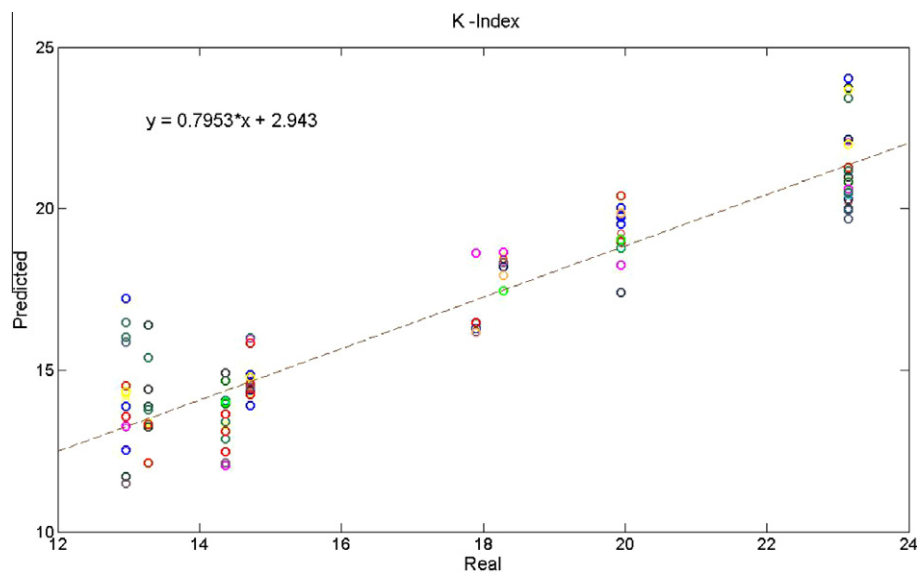


Fig. 4. Predicted versus actual values of *K*-index using data from the potentiometric electronic tongue.

Table 2

Prediction results of some quality parameters for meat.

	No. latent var.	Regression coef.	Slope	Intercept
pH	4	0.9446	0.9237	0.5165
Microbial analysis	5	0.8878	1.229	0.5517
Hypoxanthine	9	0.8782	0.8412	0.007
Inosine	7	0.8762	0.7479	0.6145
IMP	8	0.8934	1.343	1.611
<i>K</i> -index	9	0.9269	0.7953	2.943

ally, the predicted values should lie along the diagonal line that would indicate that the predicted and actual values are the same.

PLS prediction models including the values of p_1 , p_2 , the regression coefficient and number of latent variables are shown in Table 2 for pH, the logarithm of the bacteria concentration, concentrations of IMP, Ino, Hx and the *K*-index. As it can be seen, a good correlation exists for most of the parameters, being the better results those for pH and *K*-index. Despite these remarkable correlations it has to be pointed out that the potentiometric changes are not due to variations on pH or the nucleotide concentrations, yet the measurement of the electrode potential correlated well with these variations. The results strongly suggest the feasibility of this system for easy rapid and effective meat freshness assessment.

4. Conclusions

The potentiometric data of certain metallic (Au, Ag, Cu, Pb and Zn) and graphite electrodes have demonstrated to show a variation versus time when in contact with a piece of pork loin. Additionally, PCA and neural network analyses showed that it was possible to determine the post-mortem time elapsed by using these simple potentiometric measures and rather conventional electrodes suggesting that such an easy device could be employed for the determination of freshness in meat. Data from the electronic tongue were additionally compared with other well-known methods for the evaluation of freshness based on the determination of the concentration of certain bio-molecules as a function of time after death. A relatively good correlation was found between the response of the electronic tongue and certain degradation indexes such as the pH, microbial count and nucleoside concentrations. The better correlations were observed for pH and the so-called *K*

index that is related with the evolution of the concentration of certain nucleosides with time. The method we have reported is fast, low-cost and non-destructive and it might be a suitable mode to evaluate meat freshness in a wide range of situations. Further studies in relation to repeatability and accuracy in pork and other meat products using different electrodes and storage conditions are being carried out.

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