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

Luis Gil,[a,b] José M. Barat,[c] Diana Baigts,[c] Ramón Martínez-Máñez,[a,d] Juan Soto,[a,d] Eduardo Garcia-Breijo,[a,b] M-Concepción Aristoy,[f] Fidel Toldrá,[f] Eduard Llobet,[e]

[a] Instituto de Reconocimiento Molecular y Desarrollo Tecnológico, Centro Mixto Universidad Politécnica de Valencia – Universidad de Valencia, Spain.

[b] Departamento de Ingeniería Electrónica. Universidad Politécnica de Valencia, Camino de Vera s/n. E-46022 Valencia, Spain.

[c] Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia. Camino de Vera s/n. E-46022 Valencia, Spain.

[d] Departamento de Química. Universidad Politécnica de Valencia, Camino de Vera s/n. E-46022 Valencia, Spain.

[e] MINOS, Departament d'Enginyeria Electrònica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Spain.

[f] Instituto de Agroquímica y Tecnología de Alimentos (CSIC), PO Box 73, 46100, Burjassot- (Valencia), Spain.

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.

Keywords

Electronic tongue, meat freshness, food control, potentiometric electrodes, Fuzzy Artmap

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

49 to bacterial growth and autolysis. Traditionally, there have been two methods to
50 evaluate meat freshness; one consists of a sensory test controlling organoleptic
51 attributes with the help of experts and the other is the chemical or biochemical
52 determination of the concentration of target bio-indicators. The former is rapid but
53 expensive, whereas for the latter a large number of reported studies try to relate
54 freshness and the concentration or presence of certain species. For instance, the
55 determination of biogenic amines, trimethylamine or volatile amines have been
56 proposed as a suitable method to determine meat spoilage and as an index for meat
57 freshness (Edwards, Dainty and Hibbard, 1983; Hurst, 1990; Yano, Yokoyama and
58 Karube, 1996). On the other hand, the determination of degradation products of ATP
59 has also proved to be useful for the determination of meat degradation (Yano,
60 Yokoyama, Tamiya and Karube, 1996). Other methods for evaluating aging rely for
61 instance on the measurement of gaseous components released from food using
62 semiconductor gas sensors (Funazaki et al., 1995), or in the use of electromagnetic
63 sensors, particularly microwave sensors (Clerjon and Damez, 2007). All these analytical
64 techniques currently used to study freshness have proved their validity; however, in
65 general, they are slow and need relatively sophisticated tools. This makes that the
66 application of these techniques in the industry is rather limited, since they are not
67 suitable for rapid monitoring, require highly skilled operators, are relatively expensive
68 and time-consuming and in general are designed for in-laboratory use only. One
69 important consequence is that current methods cannot evaluate correct meat freshness in
70 the market when it is sold. Additionally, from a quality assurance viewpoint it would be
71 advantageous to develop non-destructive sensing methods able to inspect a large
72 number of samples on-line. In fact, the development of simple, undemanding and non-
73 destructive new analytical tools that additionally would be low-cost and that could be

74 applied in a wide range of situations for monitoring food freshness without requiring
75 highly specialized personnel is still an unresolved goal.
76 One of the protocols that fulfil most of these requirements is related with the use of the
77 so-called electronic noses and electronic tongues. These are tools that have been
78 introduced recently, inspired in the mode in which mammalians recognize food through
79 the senses of olfaction and taste (Toko, 2000). These techniques are especially appealing
80 when it is important to characterize complex attributes of the whole sample rather than
81 to know the exact concentration of certain analytes. Electronic tongues and noses do not
82 use specific but unspecific sensors, that are nevertheless able to respond in some way
83 differentially toward a group of related chemical species and whose global response can
84 be related sometimes with certain parameters or characteristics. The unspecific sensors
85 are usually integrated in an array, i.e. the electronic tongue, and their response is
86 commonly analyzed by suitable pattern recognition algorithms.
87 An easy way to build up an electronic tongue is through the use of a set of electrodes
88 using potentiometric or voltammetric (Holmin, Spangeus, Krantz-Rulcker and
89 Winquist, 2001) electrochemical techniques. In fact, several electronic tongues based on
90 electrochemical sensors have been developed (Gallardo, Alegret, del Valle, 2005)**.**
91 Among them, those relying in potentiometric measurements have been the most widely
92 used employing for instance ion-selective electrodes (Mimendia, et al., 2010). A
93 suggestive alternative to avoid the employment of membrane-containing sensors is the
94 use of simple metallic wires as suitable electrodes in electronic tongue devices. (Lvova
95 et al., 2006). Electronic tongues with metallic electrodes are very simple to prepare and
96 easy to use. The response with this kind of electrodes in the potentiometric mode is
97 based on the spontaneous polarization of the metals and other elements in the presence
98 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 Ag_2S) 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.

Materials and methods

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 x 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 ºC. Every day, two slices of each loin were cut, one for the potentiometric measurements and one more for the corresponding biochemical analysis (vide infra).

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.

Electrodes

The electronic tongue consists of a set of six electrodes of different materials: i.e. gold, silver, cooper, 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).

147 Signal Conditioning System

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

155 Data Acquisition System

156 The signals from the signal conditioning system are input to the data acquisition system
157 whose mission is to capture the electrical signals coming from different sensors and,
158 once these are suitably adapted, to store them. The data acquisition system additionally
159 enables the display of information in real time. An Adlink PCI-9112 card
160 (www.nudaq.com) was used in the computer that was also equipped with the VEE-Pro
161 software (Agilent Technologies, Santa Clara, CA, www.home.agilent.com) that allows
162 both viewing the data on the computer screen and storing the data for subsequent
163 processing.

164 Multivariate Analysis of data

165 With the response signals from the different electrodes a matrix of data was obtained
166 that was used to perform multivariate analysis. The MATLAB®.(R2007 MathWorks)
167 analysis program was used to apply techniques of pattern recognition that allow to
168 visualize if the data taken from the electrodes were correlated with some parameters (in
169 our case, for instance, the day of measurement, vide infra). One of the techniques most
170 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 was 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 and 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 and Rosen, 1992).

Potentiometric measurement method

The experiments with the electronic tongue consisted of the measurement of the electrode potential during a period of ten days, which usually corresponds to the maximum time of storage for fresh pork meat at a temperature of 4ºC. Measurements

196 were performed every day except days 5 and 6. Each day two slices of each loin were
197 cut (n=3); one of them was used to make potentiometric measurements, whereas
198 biochemical analyses were carried out on the other two.
199 To achieve homogeneity in all measurements, both ends of the loins were removed
200 before sampling and we were careful at making slices of similar size and to puncture in
201 the same area of each slice (centre). The samples belong to the Longissimus dorsi
202 muscle that is a muscle of homogeneous structure
203 One of the main problems when using metallic electrodes is the possible occurrence of
204 response or baseline drift that could lead to obtain irreproducible data or to mistakenly
205 believe that drift variations are changes in meat aging. In order to control these possible
206 drifts, each day the potential of the electrodes, when placed on a 'standard solution' of
207 distilled water buffered at pH 7, was measured.
208 Data gathering in pork meat was carried out by sticking on the meat sample the set of
209 electrodes and the reference electrode. Data was taken for no less than 5 minutes in
210 order to allow the electrodes to reach the equilibrium. The data measured were
211 automatically stored on the computer in an ASCII-file type. The file obtained included
212 one data point for each of the electrodes, the value of which was determined by the
213 arithmetic mean of the potential of the electrode when the steady-state was reached in
214 order to damp possible influences of electrical noise or random variations in the
215 measurements. With data from all the measurements performed, a result matrix was
216 made. The matrix was formed by 6 columns, one for each of the electrodes and by 24
217 rows (3 measurements per day during 8 days).

218 *Analytical determinations*

219 Measurement of pH

220 pH was determined in samples throughout the storage time by using a puncture
221 pHmeter (micro pH 2000, Crison). pH was assayed in different points of the same
222 section with no variation.
223
224 Microbiological analysis
225 Tenfold dilutions in 0.1% peptone water were prepared from each sample obtained
226 from every container at every measurement day (n = 3) and 1 ml aliquots were plated in
227 duplicate. Aerobic counts were determined by using Plate Count Agar (Merck).
228 Duplicate pour plates were prepared per dilution and incubated at 28^{o}C for 48 h.
229
230 ATP breakdown compounds.
231 The ATP-degradation compounds, consisting of IMP, Ino and Hx, were determined by
232 HPLC. The extraction procedure was similar to that described by Burns and Kee (1985).
233 Five grams of each sample were homogenized with 20 ml of 0.6 M of cold perchloric
234 acid for 4 min at 4^{o}C by using a masticator (IUL Masticator, Barcelona, Spain). The
235 obtained extract was centrifuged at 10,000 g under cold conditions (4^{o}C) for 20 min.
236 The supernatant was filtered through glass wool and neutralized by adding solid
237 potassium carbonate. The neutralized extract was kept in ice for 5 min and then
238 centrifuged at 12,000 g in a refrigerated microcentrifuge for 10 min. The supernatant
239 was stored at -28^{o}C prior to analysis.
240 An HPLC, model 1100, equipped with a diode array detector was used (Agilent
241 Technologies, Palo Alto, CA). Nucleotides were separated in a LiCrospher 100 RP-18
242 column (150 x 4 mm) (Agilent Technologies) by using a program gradient between two
243 solvents; 0.05M dipotassium hydrogen phosphate buffer, pH 7.0 (A) and methanol. (B).
244 Thus, after 8 min of isocratic elution with 100% solvent A, a 7 min gradient to 30%

245 solvent B followed by a 10 min washing step with 50% solvent B was achieved. Before
246 a new analysis, initial conditions (100% solvent A) were maintained for 15 min. Flow
247 rate was 0.9 ml/min and separation was achieved at 30ºC. Injection volume was 10 µl.
248 ATP-related compounds were monitored at 254 nm and their spectral signal in the range
249 190–350 nm was obtained to assure peak identification.

250 **Results and discussion**

251 Potentiometric measurements

252 The values of the potential of each electrode versus time (eight days of storage, from
253 day 1 to day 10) are shown in Figure 1. It can be seen that the range of variation of the
254 potential depends on the type of electrode. The potential of silver and gold electrodes
255 varies between 0 and -200 mV. Copper electrode has a fairly continuous behavior, in the
256 -200 mV and -300 mV range, lead electrode behaves similarly to copper but within the
257 range -450 mV and -550 mV, whereas the zinc electrode shows responses between -1 V
258 and -1.1 V. Finally, graphite (*C*) is the only electrode with positive potential and moves
259 in the 180 mV to 250 mV range. A direct observation of the data in Figure 1 gives no
260 conclusive information about the evolution of sensor response with the increase in
261 storage time of meat and, therefore, a more detailed study using multivariate analysis is
262 required.

263

264 Insert here Figure 1

265

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

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. Figure 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.

Insert here Figure 2

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

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

292 are placed quite close to each other, the data obtained from the evolution of the meat of
293 pork are spread in a larger zone in the PCA plot, suggesting that the electrodes are really
294 giving response to the post-mortem evolution of meat. Although there is some
295 dispersion in the calibration measurements, the fact that these do not appear ordered in
296 the PCA plot according to measurement day rules out the presence of significant
297 response drift. Therefore, the main cause of the changes in the electrode potentials with
298 time observed on the PCA plot are changes caused by aging of the pork meat.
299 The PCA analysis only using the results obtained from the pork meat are plotted on
300 Figure 3. This three-dimensional graph covers 89.09% of total data variance from the
301 six electrodes. It can be observed that the trials from the same day gather rather together
302 and that three major groups of measures are observed, corresponding to the early days:
303 1, 2, 3 and 4 (group A); days 7, 8 and 9 (group B); and the day 10 (C).
304
305 Insert here Figure 3
306
307 **Fig. 3. Three-dimensional (right) PCA plots from the results for the metallic electrode**
308 **response. Ellipses cluster together measurements carried out the same day (1 to 10) and**
309 **dotted lines correspond to groups of consecutive days (A to C).**
310
311 *Classification with Artificial Neural Networks*
312 The data obtained can also be analyzed using artificial neuronal networks. These
313 networks use a training phase with a set of measurements and a further phase for
314 validation.
315 Therefore, in order to check the power of discrimination of the electronic tongue
316 system, artificial neural networks were used. The aim of this approach is the

317 classification of each sample according to the day the measurement was taken. Two
318 types of artificial neural networks were used in order to compare their response; i.e.
319 MLP and fuzzy ARTMAP types. In both cases, the network had 6 inputs corresponding
320 to the six electrodes and also 8 outputs that corresponded to the ten-day experiences.
321 Moreover, in addition to the artificial neural network with 10 outputs, other network
322 with a smaller number of outputs was also employed. In this process, the measurements
323 were distributed in three groups corresponding to days 1, 2, 3 and 4 (output A), days 7,
324 8 y 9 (output B) and day 10 (output C). The reason for this classification was derived
325 from the PCA results in which a distribution in these four groups was observed in the
326 score plot (see Figure 3).
327 The first artificial neural network used was the MLP. To estimate the rate of success in
328 identifying the samples with each type of classifier, the 'leave-one-out' cross-validation
329 method was applied using the 24 measurements available. At first an MLP was used
330 which had 8 output neurons (i.e. eight-category classification). Several training and
331 validation steps were made to determine both the number of neurons in the hidden layer
332 and the transfer functions type to be used. The best results were achieved by using 8
333 neurons in the hidden layer and log-sigmoid transfer functions, which led to an 85.83 %
334 success rate in classification. In the second step, an MLP having 3 outputs was used, i.e.
335 three-category classification: A (days 1, 2, 3 and 4), B (days 7 and 8) and C (day 10),
336 which obtained a success rate of 86.23% when 3 hidden neurons and a log-sigmoid
337 transfer function were employed.
338 The same sample classification strategies we envisaged employing another type of
339 neural network (i.e. fuzzy ARTMAP). In fact from a practical point of view fuzzy
340 ARTMAP works very well when there are very few samples for training (Carpenter,
341 Grossberg and Reynolds, 1991), so it could be anticipated that this network would

342 outperform the MLP in this particular situation. The algorithm has been implemented
343 in-house using function macros from basic functions of Matlab. Employing the training
344 and validation method described above, a remarkable success rate of ca. 100% was
345 obtained both for the network with 8 outputs for the network of 3 outputs.

346 *Chemical and Biochemical analysis*

347 Above we have detailed that there is a correlation between the overall response of the
348 set of electrodes (the electronic tongue) and the time elapsed since the beginning of the
349 experiments. In this part of the work we are additionally interested in seeing if the
350 changes in potential of the electrodes in the electronic tongue could be related with the
351 evolution versus time of chemical or biochemical parameters determined on the same
352 pork loin where the electronic tongue was used. As it is well-known, once the animal
353 dies a number of degradation reactions begin, which contribute to diminish its
354 freshness; some of those reactions are chemical, mainly the oxidation of organic
355 compounds, and others are of biochemical nature (autolysis). In addition to that,
356 microorganism growth implies an overall decrease in quality and safety.
357 Therefore in order to somehow validate the potentiometric method outlined above for
358 freshness evaluation of pork meat, it is necessary to compare the changes in the
359 potentiometric data with some other reported method for the determination of meat
360 freshness. As we have detailed above, there is a quite large number of methods
361 proposed for the evaluation of freshness that can be basically divided in two classes; (i)
362 those measuring the concentration of certain bio-molecules or bio-markers signaling the
363 evolution of meat after the death of the animal and (ii) those based on indirect methods
364 (e.g. changes on the pH or electrical resistance and the use of electronic tongues and
365 noses).

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

369 Measurement of pH

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

372 Microbial growth

373 A healthy and freshly slaughtered animal has its muscle sterile. After a period of time,
374 the duration of which depends mainly on temperature, meat pH and slaughtering
375 management, microorganisms experiment an exponential growth. The activity of
376 microorganisms is the main factor that limits the shelf-life of fresh meat. For the
377 microbiological testing of meat two types of methods are routinely used: those who
378 make a count of the total number of microorganisms present in meat and those based on
379 the count of a particular group. The values of microorganism concentration are shown in
380 Colony-Forming Unit per gram (CFU/g). Unlike other methods, the count of the total
381 number of microorganisms provides no information about the freshness or quality of
382 edible meat but gives an image of the hygienic quality of the animal, including the
383 abuse of temperature during handling and processing.
384 The microbial growth experienced a significant increase from $3.7x10^3$ CFU/g at the
385 beginning of the experiment to values larger than 10^9 CFU/g on day 9^{th}.
386

387 Measurement of nucleotides produced by the decomposition of ATP

388 Products obtained by ATP decomposition are considered to be among the most reliable
389 and useful for a correct meat freshness evaluation. The analysis is based on the concept

390 that after slaughter, the ATP in pork meat decomposes following the sequence: ATP -
391 ADP - AMP - IMP - Ino - Hx. During this process there is a change in the smell and the
392 taste of the meat. Similar autolytic processes occur in all animals but the rate varies
393 greatly among different species. The determination of the concentration of ATP alone
394 cannot be used generally as freshness index because it disappears approximately 24 H
395 *post-mortem* (Karube, Matsuoka, Suzuki, Watanabe and Toyama, 1984). Usually the
396 same occurs for ADP and AMP. However, the simultaneous evolution of some ATP-
397 degradation compounds has been suggested to be an indication of the state of freshness.
398 From this idea, the concept of the K'-index was introduced (see equation 1). Fresh meat
399 has a low value of –K´, which gradually increases at a rate that depends on the species.

400 $$K' = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \times 100 \qquad (1)$$

401 This index was originally proposed to evaluate fish freshness (Batlle, Aristoy and
402 Toldrá, 2001) but also has been applied for the measurement of meat freshness in pork,
403 rabbit or beef (Nakatani, Fujita, Sawa, Otani, Hori and Takagahara, 1986).
404 Interestingly, biosensor systems for the determination of K'-index making use of
405 enzymes have also been reported by Zen, Lai, Yang and Kumar (2002); Park and Kim,
406 (1999) and Park, Cho and Kim (2000). In our case, in order to calculate the K' index
407 during the degradation of the pork meat, the evolution of the concentration of the ATP-
408 related compounds IMP, Ino and Hx was determined using conventional
409 chromatographic procedures. Table 1 shows all the values obtained for chemical and
410 biochemical analysis. The mean value and associated standard deviation for three
411 measurements each day are shown in Table 1. An analysis of variance (one-way
412 ANOVA) was performed on the physicochemical measurements to investigate whether
413 the changes observed in parameter values throughout storage time were statistically
414 significant. ANOVA was performed using the Statgraphics® Plus version 5.1

415 (Manugistics, Rockville, M.D. USA). The p values found were 0.0021 for Hx, 0.0282
416 for Ino, 0.0001 for IMP and 0.007 for R. All these values are below 0.05, so the null
417 hypothesis (i.e. the differences observed in the mean values of the parameter throughout
418 storage time are not significant) can be rejected at the 95% significance level.
419

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

421 **Insert here Table 1**

422

423 *PLS analysis*

424 We have seen above that data from the electronic tongue clustered as a function of time
425 that it was possible to obtain a good classification of the data using artificial neural
426 networks. In this section we additionally were interested in analyzing if the
427 measurements taken from the electronic tongue could be used to predict the values
428 obtained for the pH, the microbial growth, the concentrations of the compounds IMP,
429 Ino, Hx or the value of the K-index. In order to achieve this goal, the regression
430 technique Partial Least Square (PLS) has been used. PLS is a multivariate projection
431 method that models the relation between an array of dependent variables (Y) and
432 another array of independent variables (X). The principle of the technique PLS is to find
433 the components of the matrix of input (X) that describe, as much as possible, relevant
434 variations in the input variables, and at the same time get the highest correlation with
435 the objectives (Y), giving the minor weight to the variations that are irrelevant or relate
436 to noise (Berruela, Alonso-Salces and Héberguer, 2007).
437 For each of the parameters of interest a set training/validation was employed (Vinaixa,
438 Llobet, Brezmes, Vilanova and Correig, 2005). To perform this task a bootstrap

439 validation strategy was used (Efron and Gong, 1983). This method consists of randomly
440 drawn a group of data from the whole data set. The data that remain are used to conduct
441 training in order to obtain the coefficients PLS and the data extracted is used for
442 validation. This process is repeated a number of times and with the result of all the
443 repetitions a line of adjustment is obtained between the real and predicted values. The
444 use of this method is especially interesting when a relatively small number of
445 measurements are available to create the model. In this way, by repeating many times
446 the algorithm, which results in the random change of training and validation
447 measurements, has similar effects to having carried out the experience with more data.
448 In our case, to each model built was trained and validated with 20 and 4 measurements,
449 respectively and the bootstrap process was repeated 24 times, thus a line of adjustment
450 was obtained with 96 data points. Data from the models were centered with respect to
451 the average value of responses from each of the 6 sensors. For each model built the
452 optimal number of factors (latent variables) was determined by cross-validation
453 employing training measurements only.
454 PLS prediction models for pH, bacteria concentration, IMP, Ino, Hx and K-index were
455 created with the potentiometric experimental data obtained from the metallic electrodes.
456 Figure 4 shows the PLS graphic in which measured vs. predicted values of the K-index
457 are plotted. Hence measured values represent the known K-index of the meat,
458 meanwhile predicted values are the calculated values according with the PLS algorithm.
459 Measured and predicted values are plotted together in order to evaluate both the
460 accuracy and precision of the created prediction models. A preliminary evaluation can
461 be done just by a simple visual inspection of the difference between measured and
462 predicted values. However, a more rigorous analysis is achieved by a linear fitting of the
463 experimental points (predicted). Here, by using a simple linear model namely

464 *y=p1*x+p2*, a fitting line and also the adjusting parameters (p1, p2 and regression
465 coefficient) are obtained. The parameters p1 (slope of the fitting line) and p2 (intercept
466 with y axis) represents the accuracy in prediction; meanwhile the regression coefficient
467 can be related with the precision of the PLS model. Ideally, the predicted values should
468 lie along the diagonal line that would indicate that the predicted and actual values are
469 the same.
470 PLS prediction models including the values of p1, p2, the regression coefficient and
471 number of latent variables are shown in Table 2 for pH, the logarithm of the bacteria
472 concentration, concentrations of IMP, Ino, Hx and the K-index. As it can be seen, a
473 good correlation exists for most of the parameters, being the better results those for pH
474 and K-index. Despite these remarkable correlations it has to be pointed out that the
475 potentiometric changes are not due to variations on pH or the nucleotide concentrations,
476 yet the measurement of the electrode potential correlated well with these variations. The
477 results strongly suggest the feasibility of this system for easy rapid and effective meat
478 freshness assessment.
479
480 Insert here Figure 4
481
482 **Fig. 4. Predicted versus actual values of K-index using data from the potentiometric**
483 **electronic tongue**
484
485 **Table 2**. Prediction results of some quality parameters for meat
486
487 Insert here Table 2

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 is 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 rage 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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Figure 1

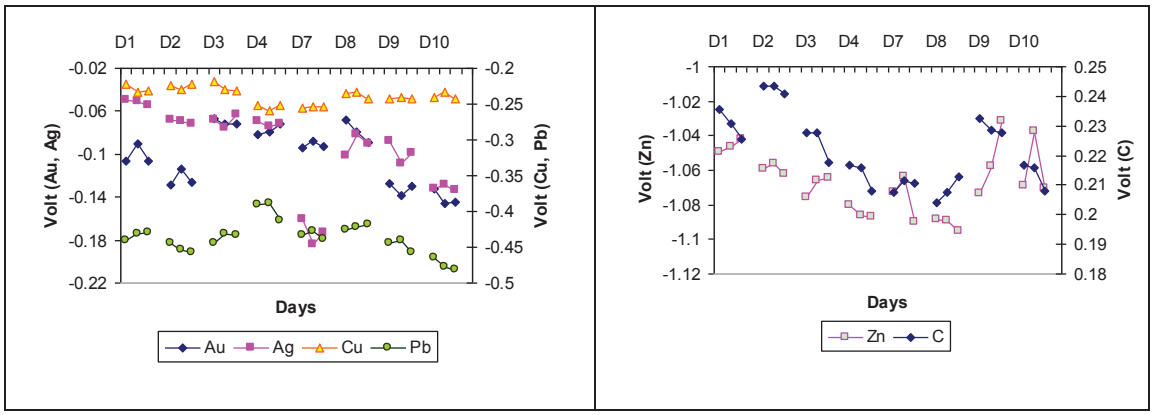

Figure 1

Figure 2

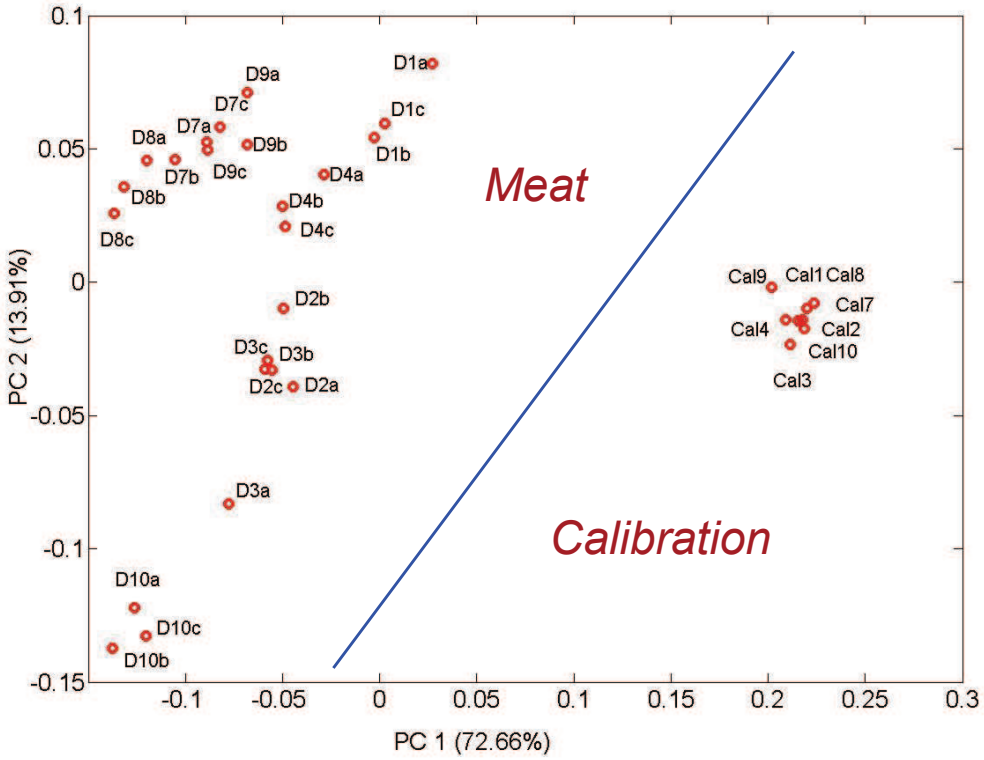

Figure 2

Figure 3

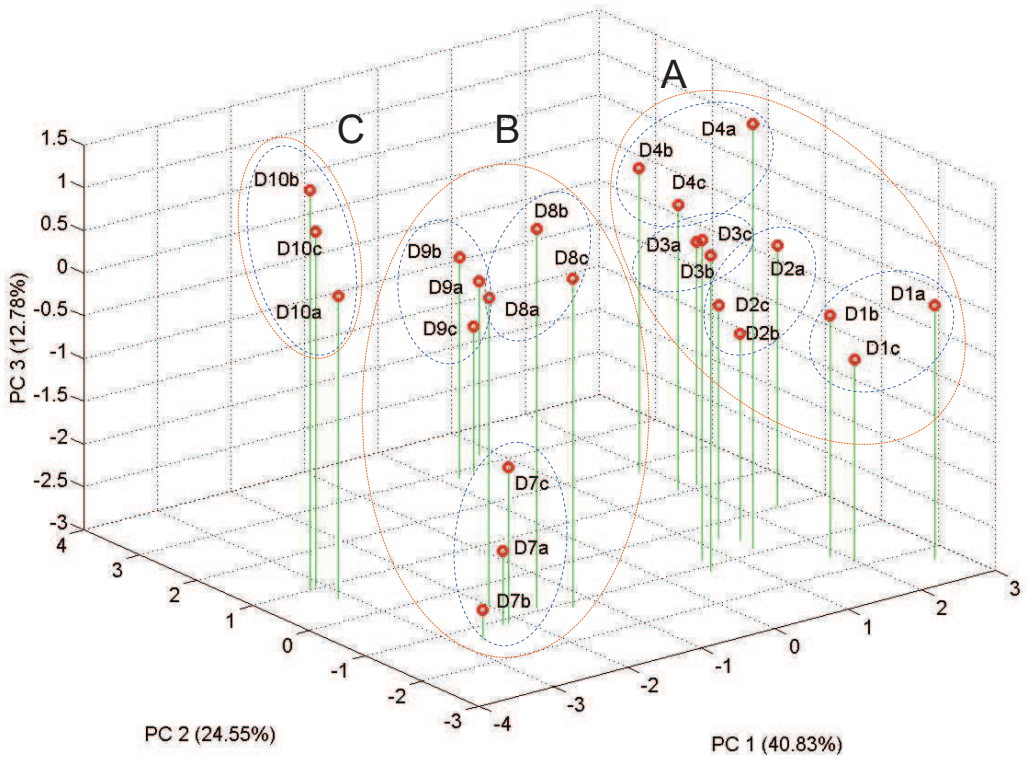

Figure 3

Figure 4

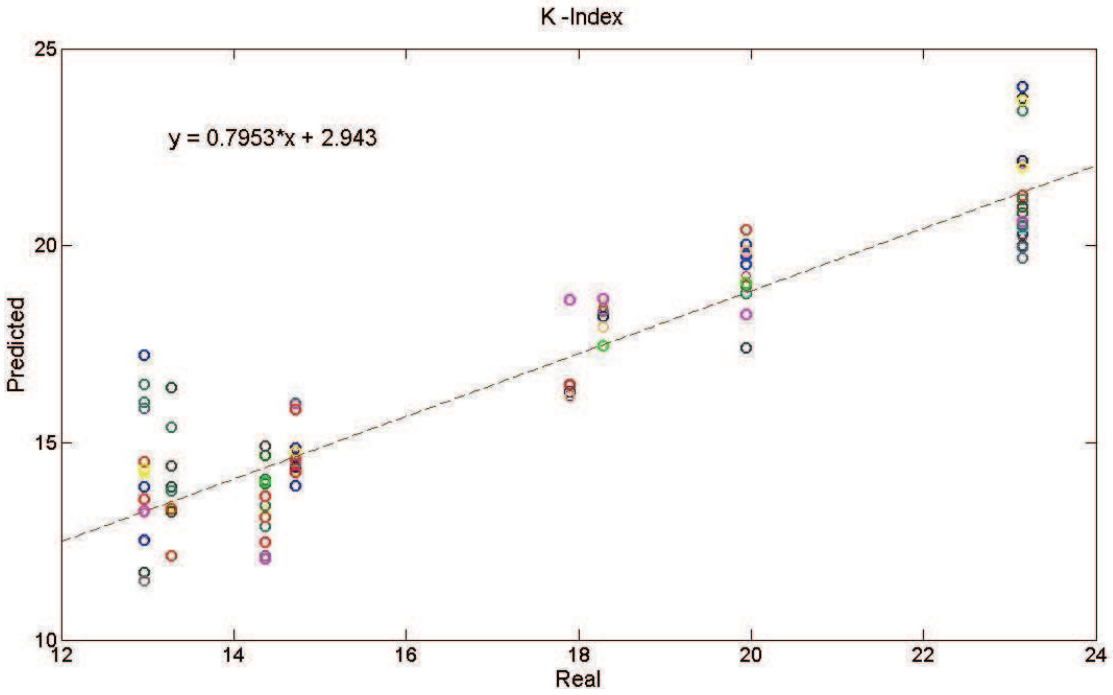

Figure 4

Table 1

Table 1

Time (days)	pH	Microb. (CFU/g)	Hx	Ino	IMP	K'
1	5.6 ± 0.09	$3.7x10^3$ ± 22	0.184 ± 0.012	2.088 ± 0.218	4.662 ± 0.401	13.212 ± 1.324
2	5.64 ± 0.086	$1.2x10^4$ ± 25	0.190 ± 0.024	1.985 ± 0.232	4.541 ± 0.282	14.330 ± 2.412
3	5.7 ± 0.089	$1.4x10^4$ ± 30	0.202 ± 0.007	2.130 ± 0.005	4.685 ± 0.077	15.553 ± 2.235
6	5.7 ± 0.091	$7.2x10^5$ ± 38	0.256 ± 0.004	2.349 ± 0.048	4.305 ± 0.376	16.986 ± 0.758
7	5.83 ± 0.085	$3x10^7$ ± 53	0.305 ± 0.016	2.559 ± 0.116	4.153 ± 0.313	18.597 ± 0.613
8	6.1 ± 0.081	$8.1x10^8$ ± 77	0.341 ± 0.054	2.648 ± 0.130	3.857 ± 0.090	18.991 ± 0.704
9	6.2 ± 0.077	$\geq 10^9$	0.438 ± 0.031	2.567 ± 0.326	3.376 ± 0.268	19.331 ± 1.363
10	6.27 ± 0.085	$\geq 10^9$	0.661 ± 0.247	2.438 ± 0.515	2.650 ± 0.950	21.969 ± 1.903

Table 2

	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
K-index	9	0.9269	0.7953	2.943