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Implications of Electrical Impedance-Based Microbiological Technology in Pork Meat Processing Industry for the Rapid Detection and Quantification of *Salmonella* Spp.

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Abstract: The absence of efficient tools for preventing bacterial contamination in the meat processing industry as well as for detecting *Salmonella* positive samples in real time is a matter of concern. Impedance technology has proved its effectiveness as a bacterial quantification tool for research purposes instead of laborious standard plate count, and as a detection tool to substitute tedious current horizontal method ISO 6579:2002. Calibration curves were carried out for *S. enteritidis* and *S. typhimurium* in raw pork matrix ($R^2 > 0.90$). Calibrations of mixtures of both strains at different ratio were prepared, showing a high efficiency to differentiate bacterial metabolism. Impediometry was also validated against standard plate count in raw pork samples treated by UV-C illumination to inactivate *Salmonella*. Even, damaged but still viable bacteria were recorded. Detection of *Salmonella* by impediometry led to a decrease in false positives, obtaining results within 30 h compared to 72 h in case of conventional method.

Key words: Impediometry, *S. enteritidis*, *S. typhimurium*, quantification, detection, pork meat.

1. Introduction

The high number of outbreaks of food- and water-borne diseases caused by pathogenic bacteria indicates the importance of food and water safety. *Salmonella* is considered to be one of the most common and virulent foodborne pathogen. The ingestion of this microorganism in contaminated food or water may lead to salmonellosis, a zoonotic disease associated with gastroenteritis and fevers. It is responsible for ca. 1.4 million human *Salmonella* infections in the United States [1, 2] and 176,395 reported human cases in the European Union [3]. Two different strains of *Salmonella* have been identified as the main responsible for human illnesses, *S. enteritidis* and *S. typhimurium*.

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Over the past decade, regulations [4, 5] have laid down acceptable microbiological criteria for meat-based products, requiring the absence of *Salmonella* per 25 g of meat. Thus, food safety is becoming a major concern for pork meat since positive samples have been detected up to 18% [3]. The most critical points of microbial contamination correspond to the slaughter and carcass handling together with cross-contamination on surfaces such as conveyor belts in food processing industries [3]. In addition, ready-to-eat (RTE) or processed meat products such as ground meat are more and more demanded by consumers. This trend together with the fact that these products are not fully cooked before their consumption increases the risk of food-borne diseases.

Traditional culture method for *Salmonella* spp. detection in food, according to the current ISO 6579:2002 Standard [6], consists of non-selective

pre-enrichment, selective enrichment, plating on selective agars, and eventually, biochemical and serological confirmation. Despite ISO 6579:2002 Standard is widely used and serves as reference to compare with other techniques, it is labour-intensive and time-consuming, requiring 3-4 days for presumptive results and 5-7 days for confirmation [1, 7]. Therefore, it is impossible to obtain a real time response. When the results are obtained, the product has already been released for sale. This fact demonstrates the lack of proper controls of the presence of *Salmonella* in food. As a result, several strategies are being developed for a better control of foodborne pathogens.

As a strategy, over the past decades, numerous rapid methods have been developed to shorten the assay time and increase its sensitivity [1, 8]. Moreover, since pathogenic distribution is irregular in the final product and a high throughput screening is required for a large number of samples, there may be a tool for fast screening to identify positive samples, allowing the release of negative samples to the market.

To date, the most widely used commercial tests correspond to either immunoassays such as enzyme linked immunosorbent assays (ELISA) or DNA based methods such as polymerase chain reaction (PCR). Although they have notably reduced the assay time and improved the detection limit, they also show some shortcomings including long times of pre- or post-enrichment [7, 9]; cross or inhibited reactions by compounds or background flora from some food matrices; variation of antigens and DNA of the target pathogen [1, 7-10]. Moreover, ELISA shows low suitability in the binding between *Salmonella* and its antibodies, whereas PCR involves far away more costs, labour-intensive tasks [11] and cannot distinguish between death, supposedly non-pathogenic, and viable cells.

Impedance technology is a rapid method which measures changes in electrical impedance in a medium induced by bacterial metabolism due to the release of ionic metabolites from live cells. These metabolites are responsible for the decrease in the impedance of the medium [2, 12-14]. After the important work of Torry Research Station [15] in 1978 and Eden and Eden [16] in 1984, and Owens et al. [17] in 1989 reporting the principle of indirect impedance, several efforts have been made on the detection of *Salmonella* by impedance-based technology in food samples [9, 12, 13, 18-23].

Concerning food hygiene, new emerging non-thermal technologies such as UV-C illumination have started to be developed in recent years [24, 25]. These emerging technologies may prompt to reduce microbial cross contamination in food processing industries, extending the shelf-life of the fresh product, and allowing it to keep its natural appearance, flavour and taste. However, these kinds of studies require intensive experimentation and generation of a large number of data. Impedance would be suitable for carrying out this kind of application, since it distinguishes between dead and viable cells and reduces working time, materials and samples compared to classic microbiology. Moreover, impedance technology may not only reduce the time assay but may also be a potentially powerful and reliable tool to understand microbial growth and other prevention factors [26-28].

Thus, the aim of this work is to validate electrical impedance-based technology for a rapid detection of *Salmonella* in raw pork meat against the conventional current ISO horizontal method. Moreover, the use of electrical impedance-based technology as an alternative to the standard plate count technique for the quantification of *Salmonella* is also addressed especially for food safety research purposes.

2. Material and Methods

2.1 Salmonella Culture, Media and Growth Conditions

Salmonella strains were provided lyophilized by the Spanish Type Culture Collection. Fresh cultures of *Salmonella enteritidis* (CECT 4300, corresponding to

ATCC 13076) and *Salmonella typhimurium* (CECT 4594, ATCC 14028) were grown in buffered peptone water (BPW, Scharlab) at 37 °C under rotary shaking for 24 h. Fresh bacterial cultures of around 10^9 Colony Forming Units (CFU)/mL of stationary concentration were obtained. The cells were pelleted by centrifuging at 3,500 rpm for 25 min and resuspended in sterilized buffered peptone water. Serial dilutions were prepared in sterilized buffered peptone water as well for further applications. *Escherichia coli* K-12 (CECT 4624, ATCC 23631) and *Enterococcus faecalis* (CECT 5143, ATCC 11700) were grown in the same way for further purposes.

2.2 Electrical Impedance Measurements

A µ-Trac 4200 (SY-LAB) system was used for recording impedance changes in the growth medium throughout time every 5 min. Modified selenite-cystine medium (Bi205A, SY-LAB) (9.9 mL) was used in this system as selective culture medium for *Salmonella*. In all the cases, the measuring plastic tubes containing the medium were inoculated with 0.1 mL of diluted suspensions of *Salmonella* from an overnight culture. The tubes were tightly closed and placed in the µ-Trac 4200 apparatus and monitored for 24 h as maximum time. The temperature in the incubator was controlled at 37 °C. The impedance relative changes in the medium (% M) were automatically recorded during incubation in the measuring system. The threshold value, 10%, represented the decrease of the initial value of electrical impedance due to the bacterial metabolic activity. The detection time (DT) needed for the M-value of each sample to exceed the threshold value was evaluated since it corresponds to sharp and significant changes in the impedance of the bacterial growth medium.

2.3 Salmonella Quantification Assay

2.3.1 Calibration Curve Preparation

Separate calibration curves were prepared for the quantification of *S. enteritidis* and *S. typhimurium* and analysed by µ-Trac and the standard plate count technique using deoxycholate citrate agar (DCA, Fluka) as selective agar. Raw pieces of pork meat weighting about 25 g in 225 mL buffered peptone water were used as matrix. This fresh raw pork meat was provided by Embutidos La Nuncia S.L., a local supplier in Spain, and kept at 4 °C for a maximum of 4 days. Thus, 15 different batches of raw pork meat were tested in total. To assess the accuracy and reproducibility of impedance data, the relationship between multiple inoculation levels and DT was examined. Moreover, two different ways of preparing the calibration curves were carried out:

(1) Calibration 1: The calibration was performed by preparing serial dilutions (from 10^8 up to 10^{-1} CFU/mL) of each microorganism, *S. enteritidis* and *S. typhimurium*, from the bacterial suspension mentioned in 2.1. whose concentration corresponded to 10^9 CFU/mL. Thus, 0.1 mL of each dilution was inoculated into a plastic tube and measured in the µ-Trac 4200 apparatus.

Additional calibration curves were carried out by mixing both strains. After centrifugation and resuspension up to 5 mL with sterilized BPW for each strain separately, 3 vials containing both strains mixed in a different ratio were prepared. The different ratios tested corresponded to 20:80, 50:50; and 80:20% v/v of *S. typhimurium* and *S. enteritidis*, respectively. Serial dilutions were prepared from each vial and electrical impedance changes of the growth medium were measured as explained above.

(2) Calibration 2: Different concentration of *S. enteritidis* was inoculated onto the surface of 25 g of raw pork meat. The cut pieces of meat were inoculated with a load of *Salmonella* of 10^8 up to 10^2 CFU and spotted with 100 µL taken from the fresh culture previously centrifuged and the serial dilutions prepared from it as mentioned above. They were allowed to dry for about 20 min in a microbiological safety cabinet. Then, the samples were homogenized

in 225 mL of sterile buffered water peptone in a sterile stomacher bag with a stomacher Bag Mixer 400 (Scharlab) for 2 min. One tenth of a milliliter from each sample was added into a plastic tube and measured in the µ-Trac 4200 apparatus.

In both cases, non-inoculated growth medium and matrix, and BPW used as diluent, were used as controls of background values. At the same time, the viable bacterial concentration of *Salmonella* was determined by conventional plating method performed by plating 0.1 mL of appropriate dilutions onto DCA selective agar. Colonies were counted after incubation at 37 °C for 24 h.

The concentration of viable *Salmonella* obtained by standard plate count technique for the calibration 1 was reported in CFU/mL. To be able to include these experimental points in a final calibration in CFU/g, the former values were multiplied by 9 mL/g (considering the dilution 1:9 sample/BPW made in the calibration 2). Eventually, a regression line of logarithmic bacterial concentration against detection time (DT) was calculated to determine the correlation between DT and concentration of viable *Salmonella* (CFU/g).

$$\mathrm{Log_{10}C\ (CFU/g)} = a + b \cdot \mathrm{DT\ (h)}$$

Each calibration was independently repeated for 6 times. In every experiment, each concentration point was repeated in duplicate.

2.3.2 Determination of Sensitivity

Once the µ-Trac 4200 apparatus was calibrated for this type of food sample, data of viable concentration of *S. enteritidis* obtained by impedance measurements were evaluated in comparison with conventional microbiology. Raw pork samples, inoculated with *Salmonella* and illuminated by UV-C as emerging technology to reduce bacterial contamination were used for this purpose.

The UV-C illumination device consisted of three Osram HNS 6W lamps, with an emission peak at 254 nm, and a light flux, determined by a Delta Ohm HD 2102.2 radiometer of 42 W/m^2. The UV-C lamps were enclosed in a wooden box (23.5 × 13.5 × 18 cm) covered with aluminum foil and placed 18 cm above the samples. No noticeable increase in temperature was measured inside the set-up due to the treatment.

Raw pork pieces of meat weighting about 25 g were cut, placed onto sterilised glass Petri plates, inoculated with loads of *S. enteritidis* of 10^6 and 10^4 CFU, and left to dry. After inoculation, meat pieces were exposed to UV-C light treatments for 5, 15 and 30 min. Non-radiated inoculated-pork meat pieces were considered as control of initial concentration of *Salmonella* before the treatment. Non-radiated non-inoculated-pork meat pieces were considered as a control of the bacterial background value. All experiments were conducted in duplicate and quantified in the same day of the UV-C treatment. Samples were homogenized in 225 mL of sterile BPW with a stomacher Bag Mixer 400 for 2 min. Concentration of viable *Salmonella* present in meat was quantified by both, the conventional method, based on dilution, plating on DCA agar and incubation at 37 °C for 24 h; and the impedance technology, based on adding 0.1 mL of the sample into plastic tubes to be measured by µ-Trac 4200. Experiments were repeated in triplicate.

The sensitivity of the impedance-based measurements compared to classic microbiology for quantifying viable concentration of *S. enteritidis* in raw pork meat (CFU/g) was analysed. The potential effect of UV-C illumination to reduce *Salmonella* in meat was evaluated by quantifying the concentration of viable *Salmonella* before and after the illumination.

2.4 Salmonella Detection Assay

The validation of this impedance-based technology for the detection of *Salmonella* was carried out against the traditional method (ISO 6579:2002 Standard) for 15 samples of raw pork meat. Despite 20 samples are required to validate a detection method according to ISO 16140:2003 Standard [29], this value of samples would be acceptable for studying the potential of

impedance-based technology in this application. Food samples of ca. 25 g were homogenised in 225 mL of buffered peptone water and pre-enriched under rotary shaking for 6-8 h at 37 °C. Following this, 0.1 mL of the pre-enrichment culture was transferred to 9.9 mL of the growth medium to be measured by impediometry at 37 °C for 24 h as maximum time. To compare the result to the conventional method, the pre-enrichment step was conducted up to 24 h, followed by a selective enrichment of 0.1 mL in Rappaport Vassiliadis (RVS, Scharlab) and 1 mL in Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn, Panreac), being the final volume of 10 mL, for 24 h at 42 °C and 37 °C respectively. The selective enrichment media were streaked on solid selective media, xylose-lysine-deoxycholate agar (XLD, Scharlab) and DCA, for 24 h at 37 °C to isolate presumptive positive *Salmonella* colonies. Presumptive *Salmonella* colonies isolated on plating media were incubated in Tryptic Soy Agar (TSA, Scharlab) for 24 h at 37 °C followed by biochemical confirmation with Enteropluri-Test®. This test permitted the confirmation of these presumptive *Salmonella* colonies. Positive results obtained by impediometry were also confirmed by this test. Enteropluri-Test® is based on the inoculation of the microorganism to be identified in 15 special culture media, allowing the execution of 15 biochemical reactions. Microorganisms were identified evaluating the colour change of the different culture media after 18-24 h of incubation at 37 °C. The combination of positive and negative reactions allowed building up a code number to identify bacteria by using a Codebook supplied by Enteropluri-Test®.

The sensitivity of impediometry for the detection of *Salmonella* in raw pork meat was checked by carrying out the conventional and alternative method by inoculating food samples, previously to their pre-enrichment, with a load of *S. enteritidis* and *S. typhimurium* corresponding to 10^4 CFU. Its specificity was also confirmed by inoculating a load of *E. coli* and *E. faecalis* of 10^6 CFU onto two food samples respectively.

3. Results and Discussion

3.1 Salmonella Quantification Performance in Raw Pork Meat Samples by Impedance Measurements

3.1.1 Correlation between Detection Time and Concentration of *Salmonella*

Fig. 1 shows typical impedance growth curves for different initial concentration of *S. enteritidis* ranging from 10^8 to 10^1 CFU/mL. Microbial metabolism produces lower molecular, charged decomposition products, leading to a decrease in the electrical impedance of the growth medium. These relative electrical impedance changes (% M) are recorded throughout the incubation time.

Regarding the shape of the impedance growth curves, three regions are observed according to other authors [2, 20, 22, 27, 30]: (1) an initial region where the impedance change is hardly affected. Bacteria are adapting their metabolism to the nutrients present in the growth medium, so bacterial population is not high enough or their growth rates are not fast enough to produce detectable changes in impedance; (2) the second region where the impedance change sharply increases, since microorganisms have reached a concentration of ca. 10^6-10^7 CFU/mL, starting to proliferate, rising linearly; (3) and the third region, where the impedance change remains practically parallel to the time axis again, since microorganisms cease their metabolism as all the resources in the medium have been metabolized to an end product, and there is also a depletion of nutrients. It clearly matches a typical shape of a bacterial growth curve, which includes the lag phase where bacteria are metabolizing but not multiplying, the exponential growth phase where bacteria multiply exponentially, and the stationary phase where the bacterial cell number remains relatively constant.

To consider a positive detection, a threshold value of 10%, as shown in Fig. 1, has to be exceeded by the

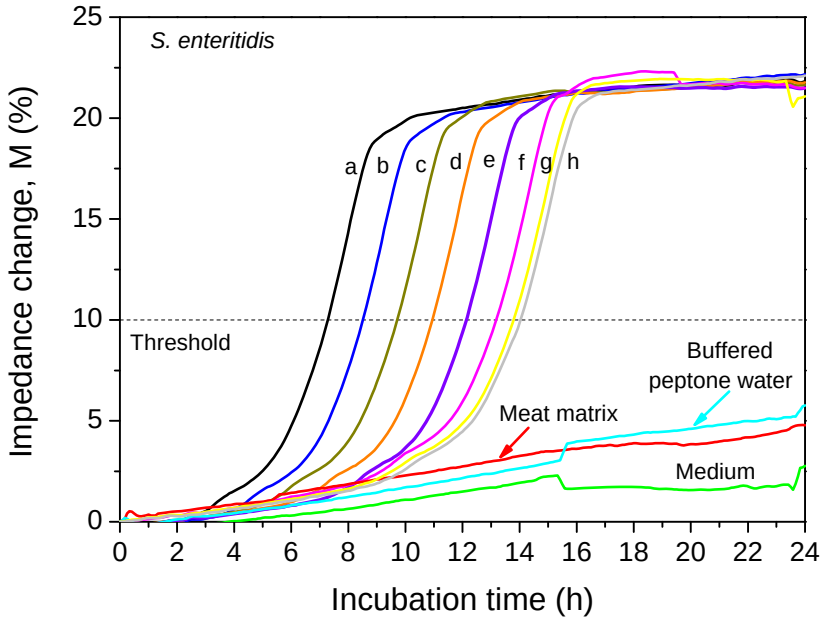

Fig. 1 Typical impedance growth curves obtained from samples containing different initial concentration of *S. enteritidis* according to the 1st method of calibration detailed in 2.3.1. Curve (a) 1.1×10^8; (b) 1.2×10^7; (c) 1.3×10^6, (d) 1.4×10^5; (e) 1.1×10^4; (f) 9.6×10^2, (g) 1.2×10^2; (h) 6.1×10^1 CFU/mL diluted from a stock solution (1×10^9 CFU/mL).

impedance curve. The incubation time required to reach the threshold value is called detection time (DT). This point of inflection of the curve and also the earlier exponential phase has the most relevance for this measuring technology. It indicates a sharp decrease in the initial value of the impedance in the growth medium.

The control samples, which contain no bacteria, such as the medium, raw pork meat matrix and BPW, show no change in impedance change, indicating that there is no ionic release resulted from bacterial metabolism in the medium throughout the incubation time. All the samples containing *S. enteritidis* reach a point where the rate of change in impedance starts to change, corresponding to the DT. It is also seen that the detection time is related to the initial bacterial concentration in the medium. Thus, the higher the initial concentration of *Salmonella* is, the shorter the detection time, and the earlier the results will be obtained [2, 26, 27, 30]. Therefore, according to Fig. 1, results are available within only a few hours (6 h) for the most contaminated sample (1.1×10^8 CFU/mL). Moreover, the delay in detection time for serial dilutions of the initial sample allows predicting an approximated value of bacterial concentration present in the sample.

Unlike the standard plate count technique, impedance technology allows the detection of live cells in a dynamic way. It not only avoids time-consuming plate counts, but also monitors the bacterial growth and metabolism in real time, which allows determining the metabolic capacities of proliferating microorganisms, their level of activity and their pathogenic potential on food from the beginning of the analysis.

Fig. 2 shows the calibration curve obtained for *S. enteritidis* in raw pork meat by plotting the logarithmic values of initial concentration of *Salmonella* against the detection time. It must be noticed that the overlapped points from both calibrations follow the same linear trend. It agrees with similar values of slope and intercept obtained from each method of calibration considering a linear regression model.

The detection time is inversely proportional to the initial cell number as observed in the impedance growth

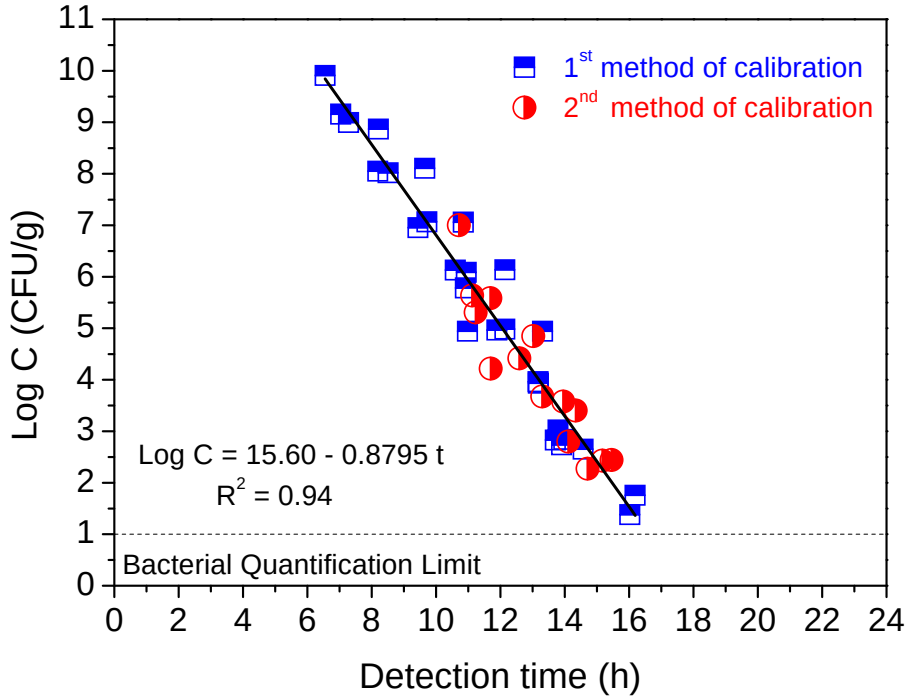

Fig. 2 Effect of calibration method: Logarithmic values of initial concentration of *S. enteritidis* ranging from 10^{10} to 10^{1} CFU/g as a function of detection time obtained from the impedance growth curves. Regression equations obtained for each calibration method corresponded to: Log C = 15.70–0.8884 t (R^2 = 0.95) (1st method) and Log C = 15.07–0.8380 t (R^2 = 0.86) (2nd method).

curves. A linear relation between the detection times and the initial cell number in terms of logarithmic values, determined by plate count, are obtained, as was pinpointed by Fehlhaber and Krüger [26], Ruan et al. [11], Yang et al. [30], Yang and Bashir [2], and Johnson et al. [27]. A value of $R^2 > 0.9$ is achieved after fitting a regression line by the method of least-squares considering all the experimental data, indicating a strong linear correlation between initial *Salmonella* concentration and DT. Moreover, the impedance method does not become more erratic for meat samples containing lower concentration of *Salmonella* (< 2 $\log_{10}$ CFU/g) unlike what was pointed out by Russell [14]. Thus, the detection time of the impedance method for the quantification of *S. enteritidis* in raw pork meat ranges from about 6.05 h to 16.05 h for initial bacterial concentration of 10^{10} CFU/g to 10^{1} CFU/g respectively.

The quantification and detection limits of *Salmonella* correspond to 10^{1} CFU/g and 1 CFU/g respectively. Therefore, a low detection limit is achieved by impedance technology in *Salmonella* quantification assays. This fact may reduce pre-enrichment period of meat in *Salmonella* detection assays compared to the traditional method, together with an improvement in efficiency of the method since the demand for detection limit in raw pork meat is less than 1 cell per 25 g of food.

3.1.2 Effect of the Kind of Bacterial Strain on the Detection Time. Comparison of Bacterial Growth Rates

Fig. 3 shows that linear responses have also been found between detection times obtained from impedance bacterial growth curves and initial concentration of *S. typhimurium* in the range of 10^{1} CFU/g to 10^{10} CFU/g. Thus, this calibration can be used for quantification of bacterial cells.

It is worth noting that the detection time is approximately 11 h and 21.5 h for 10^{10} CFU/g and 10^{1} CFU/g of viable *S. typhimurium* respectively. So, a longer

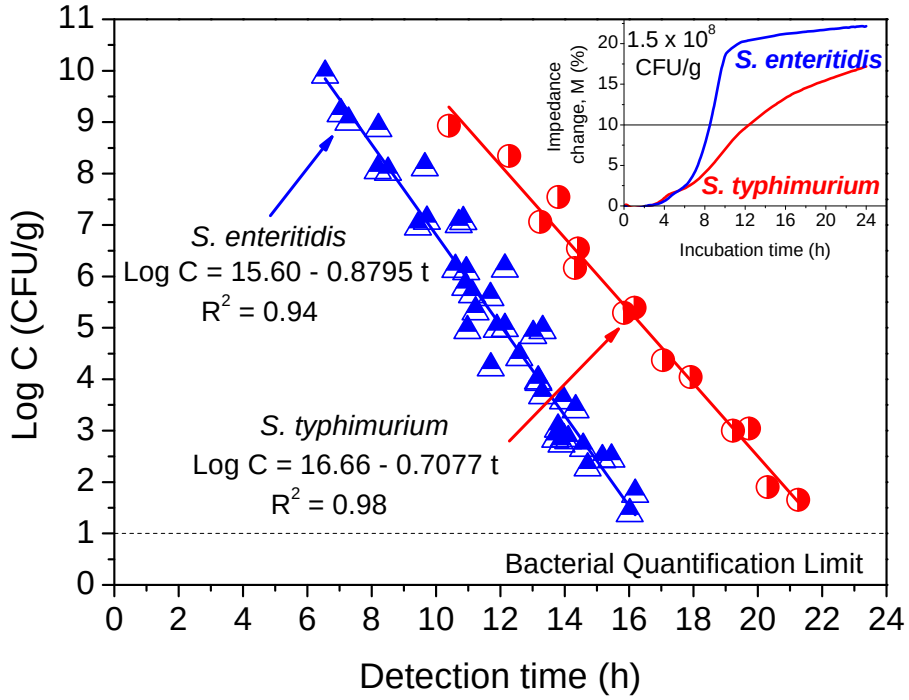

Fig. 3 Comparison of linear response between detection times and logarithmic values of initial concentration of two different strains of *Salmonella*: *S. enteritidis* and *S. typhimurium*. Upper inset: Impedance bacterial growth curves for both strains of *Salmonella* which correspond to the same value of initial concentration (1.5×10^8 CFU/g).

incubation time is required for quantification of viable *S. typhimurium* compared to that of *S. enteritidis*. It agrees with the flat nature of the impedance growth curve of *S. typhimurium* shown by Fig. 3, upper inset, indicating a delay for a significant change in impedance, the DT, to takes place. Therefore, DT not only depends on the initial concentration of bacteria but also on the bacterial growth rate, being specific for each strain. This fact agrees with Johnson et al. [27], who stated that the slope of the DT line taken over multiple inoculation levels gives a direct measurement of the bacterial growth rate. It is also confirmed by the slope value obtained from the calibration curves for each strain. *S. enteritidis* reveals a higher value of slope in comparison with *S. typhimurium*, which would indicate a faster bacterial growth rate for the former. This fact obliges to carry out a calibration for each strain with quantification purposes. As a result, since bacteria may be stressed by compounds present in either growth medium or matrix, a new calibration would be required for each matrix and growth medium to be tested [22].

3.1.3 Effect of the Mixture of Bacterial Strainson the Detection Time. Comparison of Bacterial Growth Rates

Fig. 4 depicts how the presence of two strains of *Salmonella*, with different growth rate, mixed in different ratio influences the overall bacterial growth rate and DT. Three different levels of initial concentration are shown in Fig. 4.

As the initial concentration of *Salmonella* increases, a lower difference among impedance growth curves is observed. In addition, the DT given by the impedance growth curves becomes more similar to that of pure *S. enteritidis* when both strains are mixed, independently of the ratio. It seems that impediometry is detecting impedance changes in growth medium for the bacteria with a faster metabolism and growth rate. In fact, when all the inoculation levels tested are plotted against detection time (Fig. 5), a decrease in DT is seen for all the mixtures of bacteria. As a result, overall time of analysis is reduced. It is also confirmed

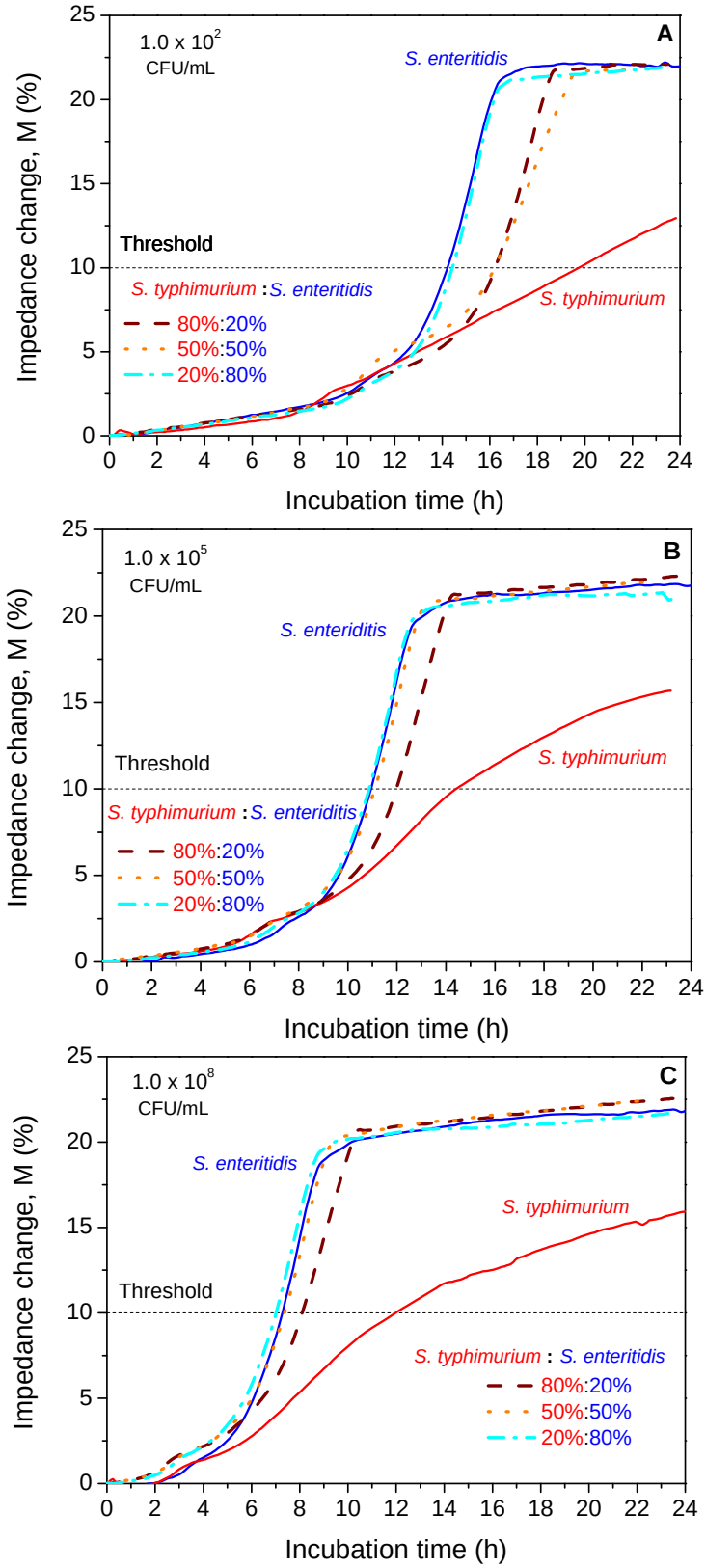

Fig. 4 Impedance growth curves for pure *S. enteritidis* and *S. typhimurium* (solid lines, blue and red, respectively), and mixtures of both strains in a different proportion obtained by diluting from 2 stock solutions from pure *Salmonella* (1×10^9 CFU/mL) at 3 levels of initial concentration: (a) 1.0×10^2 CFU/mL; (b) 1.0×10^5 CFU/mL; (c) 1.0×10^8 CFU/mL.

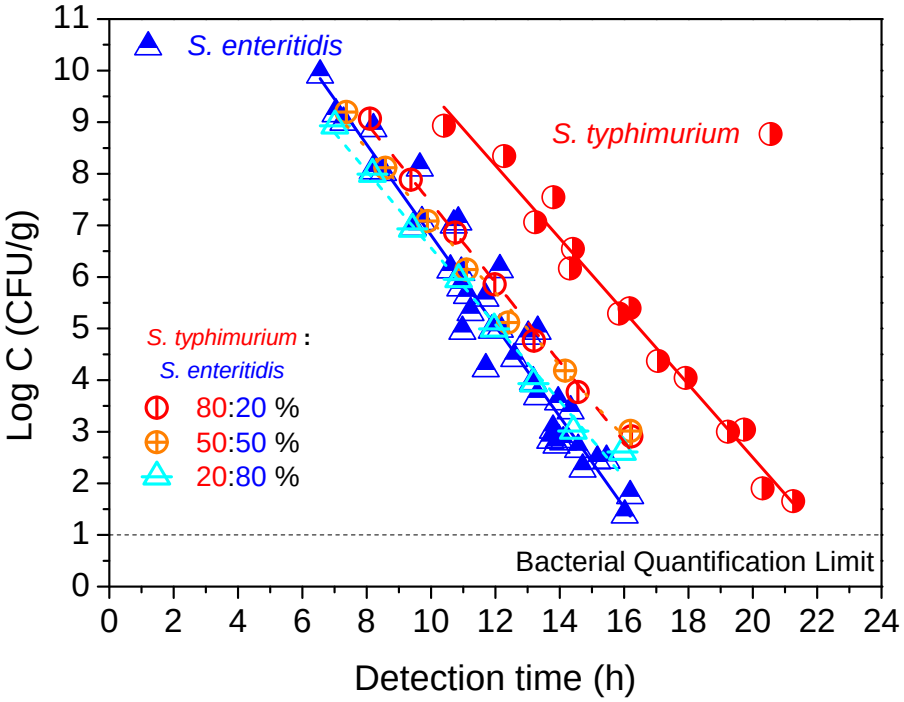

Fig. 5 Logarithmic values of initial concentration of pure *S. enteritidis* and *S. typhimurium*, and mixtures of both strains in a different proportion as a function of detection times obtained from the impedance growth curves ranging from 10^{10} CFU/g to 10^{1} CFU/g.

Table 1 Values of intercept, slope and correlation coefficient obtained from the linear equation after fitting a regression line by the method of least-squares to the experimental calibrations shown in Fig. 5.

Strain	Intercept (CFU/g)	Slope (CFU/g·h)	R^2
S. typhimurium (A)	16.66	-0.7077	0.98
80% A:20% B	15.15	-0.7713	0.99
50% A:50% B	14.09	-0.6995	0.99
20% A:80% B	14.00	-0.7433	0.99
S. enteritidis (B)	15.60	-0.8795	0.94

by the increase in the values of slope (Table 1), resulting from the linear equation acquired from the calibration curves illustrated in Fig. 5, which provide information of bacterial growth rate too.

Moreover, as viewed in Fig. 5, there are overlapped points from regression lines between the calibration curves of all the mixtures of both *Salmonella* strains with pure *S. enteritidis* for concentrations higher than 10^4 CFU/g. It suggests that, despite the delay in *S. typhimurium* growth, when this strain starts its exponential stage of growth, both *Salmonella* strains seem to grow at the same pace.

Again, values of correlation coefficient higher than 0.9 are obtained as seen in Table 1, which suggest a strong linear correlation between initial *Salmonella* concentration and DT for all the cases. Fluctuations are not observed for low concentrations of *Salmonella* (< 2 $\log_{10}$, CFU/g) either. Therefore, the application of impediometry seems to be a successful tool for rapid quantification of mixtures of different bacterial strains as well.

3.1.4 Validation of Quantification of *Salmonella* in Raw Pork Meat by Impedance-Based Technology against Standard Plate Count

To validate impedance-based method for quantification of *Salmonella* in meat, raw pork meat samples were inoculated with *S. enteritidis* at two values of bacterial load corresponding to 10^4 CFU and

10^6 CFU and illuminated throughout time with UV-C light. UV-C illumination may be considered as a promising emerging technology which has been reported to be able to reduce the amount of bacteria in food, extending its shelf-life, reducing the probabilities of foodborne illnesses, and therefore, enabling a longer-distance trade [24, 25].

Fig. 6 displays the correlation between the values of concentration of *S. enteritidis* (CFU/g) in untreated and treated meat samples of initially unknown concentration determined by standard plate count and those predicted by the DT provided by µ-Trac apparatus. On the one hand, the coefficient of variance (CV) was calculated from at least two independent experiments for bacterial levels ranging between 2 and 5 $\log_{10}$, CFU/g. Values are lower than 15% for all the levels of bacterial concentrations analysed and for both methods, standard plate count and impediometry. Thus, the degree of variability is considered acceptable since CV values are lower than 20% [27]. On the other hand, very good agreement in $\log_{10}$ bacterial concentration between count in plates and impedance-based measurements is reached ($R^2 = 0.91$). Only differences between results given by plate and impediometry appear for high levels of bacteria since the slope of the regression line becomes higher than 1. This fact may indicate a higher sensitivity of impediometry (112% ± 2%) against standard count plate (100%) which is in agreement with Donaghy and Madden [18], Bolton and Gibson [13], Quinn et al. [21], Wawerla et al. [22], Hoorfar [8] and Russell [14]. Moreover, higher errors are attained by standard plate count (horizontal error bars) compared to those of impedance-based method (vertical error bars) which reduces the reproducibility of the quantification assay when using classic microbiology [14, 28]. A more negative effect on plate count accuracy may be due to the presence of natural flora in meat which may hinder the growth of the target microorganism, together with an increase in competence between *Salmonella* and background flora by nutrients. In contrast, since impedance technique is based on metabolic capacity of the target microorganism, the presence of natural flora may not be such a crucial factor in the accuracy of the

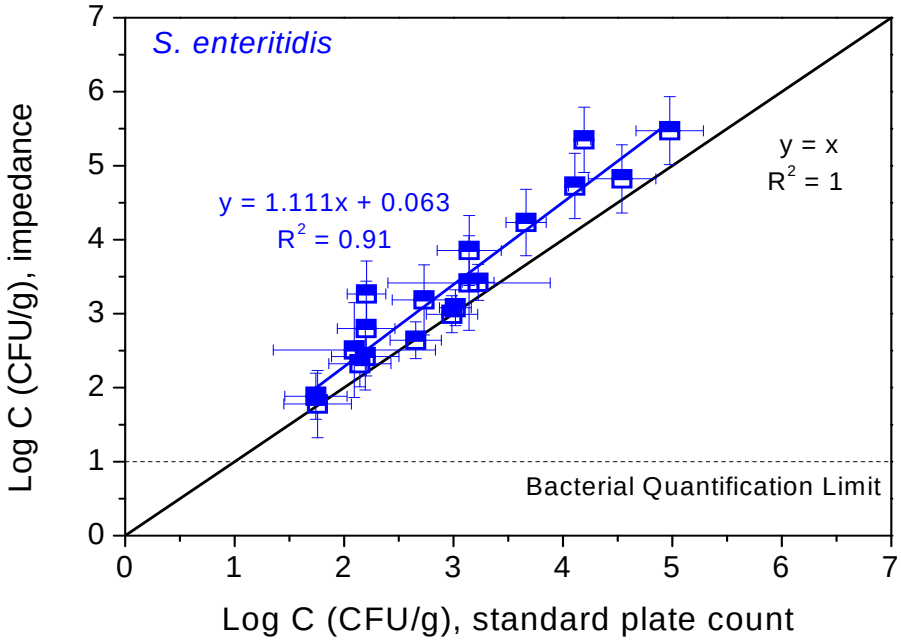

Fig. 6 Concentration of *S. enteritidis* (CFU/g) predicted by the linear equation obtained from the calibration curve given by impedance measurements (Fig. 2) and standard plate count for raw pork meat samples of unknown concentration. Graph function y = x involves a correlation of 100% in terms of bacterial concentration reported by both methods. Independent experiments have been repeated in triplicate. Experimental points have been collected in duplicate per experiment. Error bars have been calculated from all these repetitions.

enumeration method.

Since differences in quantification results between both methods have only been reached at high concentrations, it would not be a problem in food research since those levels of bacteria are not commonly found in food processing industries. It must be pointed out that impedance technique not only exhibits much less error and higher sensitivity for quantifying high levels of bacteria in comparison with plate count, but also, even possible sublethally damaged bacteria by UV-C treatment seem to have been successfully quantified. This observation is opposite to that stated by Wawerla et al. [22], whereas agrees with Russell [14]. This author asserted that despite bacteria may be injured, they are able to produce metabolites as a consequence of their metabolism.

The concentration of *Salmonella* in logarithmic units present in the treated samples after 5, 15, and 30 min of UV-C illumination have been determined by both methods, standard plate count and impedance changes. The results of the treatment in terms of *Salmonella* inactivation are depicted in Fig. 7. Similar values in *Salmonella* concentration acquired by impedance-based technology and standard plate count are achieved. It indicates that quantification of *Salmonella* in food safety research, e.g. testing the ability of UV-C illumination to inactivate bacteria onto meat samples, can successfully be carried out by impediometry. In addition, apart from reducing time assay compared to that of standard plate count, impedance results can provide much more data in real time, concerning to not only bacterial concentration, but also to the level of activity which is useful in order to know the pathogenic potential of the sample, bacterial kinetics, etc. Those can be useful not only for experimental research but also for predictive microbial modeling.

It must be mentioned that a noticeable reduction in viable concentration of *Salmonella*, corresponding to 3 orders of magnitude, after 15 min of illumination for 10^5 CFU/g and 10^3 CFU/g is accomplished. This emerging technology allows reducing the presence of bacteria in food together with the possibility of getting positive samples of *Salmonella*, fast enough to avoid

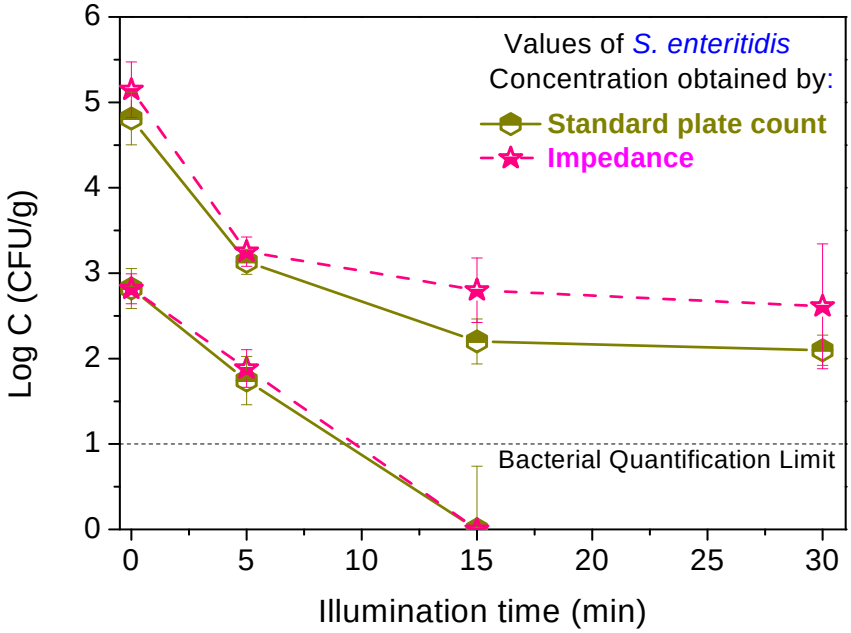

Fig. 7 Log_{10} reductions in concentration of *S. enteritidis* in raw pork meat treated with UV-C light. Load of *Salmonella* previously inoculated onto raw pork meat before UV-C illumination: ca. 10^4 CFU and 10^6 CFU. Error bars represent the standard deviation. Independent experiments were repeated in triplicate.

Table 2 Raw pork meat samples analysed by horizontal method (ISO 6579:2002 Standard) and impedance-based method, for the detection of *Salmonella.*

Sample	ISO 6579:2002 Standard			Impedance method		
	Plating media detection (+/-)[g]	Biochemical confirmation (+/-)	Total detection time (h)	Electrical impedance, DT (h)	Biochemical confirmation (+/-)	Total detection time (h)
1	—	—	72	—[c]	—	30
2	—	—	72	—[c]	—	30
3	+	—	120	21.3 (+)	—	51.3
4	+	—	120	—[c]	—	30
5	—	—	72	—[c]	—	30
6	—	—	72	—[c]	—	30
7	—	—	72	—[c]	—	30
8	+	—	120	—[c]	—	30
9	+	—	120	—[c]	—	30
10	+	—	120	21.9 (+)	—	51.9
11	—	—	72	—[c]	—	30
12[a]	+	+	120	7.4 (+)	+	37.4
13[b]	+	+	120	6.3 (+)	+	36.3
14[d]	—	—	72	—[c]	—	30
15[e]	—	—[f]	72	—[c]	—[f]	30
False positives (FP)	5/15 (33.3 %)			2/15 (13.3%)		
False negatives (FN)	0/15			0/15		
True positives (TP)	2/2			2/2		
True negatives (TP)	2/2			2/2		
Positives	2/15			2/15		
SensitivityTP/(TP+FN)	100%			100%		
Specificity TN/(TN+FP)	100%			100%		
Accuracy (TP+TN)/ (TN+TP+FP+FN)	4/9 (44.4%)			4/6 (66.7%)		

[a] *S. enteritidis* ($1 \cdot 10^4$ CFU) was previously inoculated in a 25 g meat sample, positive control.
[b] *S. typhimurium* ($1 \cdot 10^4$ CFU) was previously inoculated in a 25 g meat sample, positive control.
[c] No signal after 24 h.
[d] *E. coli* ($1 \cdot 10^6$ CFU) was previously inoculated in a 25 g meat sample, negative control.
[e] *E. faecalis* ($1 \cdot 10^6$ CFU) was previously inoculated in a 25 g meat sample, negative control.
[f] Confirmation test has not carried out since *E. faecalis* is a gram positive bacterium.
[g] Absence (-) and presence (+) of presumptive *Salmonella* colonies isolated on plating media.
TP: positive test result when *Salmonella* is present.
TN: negative test result when *Salmonella* is not present.
FP: positive test result when *Salmonella* is not present.
FN: negative test result when *Salmonella* is present.

damage in the external appearance of food, even though high levels of bacteria exist.

3.2 Salmonella Detection Performance in Raw Pork Meat Samples by Impedance Measurements against Traditional Method (ISO 6579:2002 Standard)

The evaluation of impedance technology in comparison with current horizontal method (ISO 6579:2002 Standard) for the detection of *Salmonella* in samples of raw pork meat appears in Table 2.

AOAC (Association of Official Analytical Chemists) and other validation agencies do not specify acceptance values for sensitivity and specificity but they should be as high as possible [1]. Some authors have reported values of 59.3%-96.2% and 92.3%-97.9% respectively for other rapid methods such as

enzyme-immunological and nucleic acid-based assays [7]. Statistical parameters such as sensitivity, also called the true positive rate (proportion of actual positive samples correctly identified by the method as positives); specificity, also called the true negative rate (proportion of actual negative samples correctly detected by the method as negative) [31]; and accuracy (closeness of a measurement to the true value) have been determined.

Both methods provide 100% sensitivity (when 2 strains of *Salmonella* were previously inoculated in two samples) and 100% specificity (when 2 strains different from *Salmonella*, *E. coli* and *E. faecalis,* were inoculated). This indicates that the alternative method is adequate detecting true positive and true negative samples. It must be worth noticing that any of the methods report false negative results. However, the impedance-based method exhibits a higher accuracy (66.7%) compared to the standard method (44.4%) as well as a lower rate of false positive out of 15, corresponding to 13.3% (2/15) in comparison with 33.3% (5/15) obtained for the conventional method. In general, a higher number of false negatives and positives are identified for the conventional method according to Quinn et al. [21], and Wawerla et al. [22]. It may be due to natural flora [1] present in meat sample which may either hinder the growth of the target microorganism or lead to misleading results as happens for *Proteus* spp. Actually, *Proteus mirabilis* and *P. vulgaris* together with *Citrobacter freundi* were identified in the biochemical confirmation test for presumptive *Salmonella*-positive samples. Therefore, accuracy and efficacy may be lower than that of rapid detection methods [7].

In addition, the impedance method noticeably reduces the total time of the *Salmonella* detection assay since a negative result will be obtained between 30 h (6 h of pre-enrichment following the impedance measure for 24 h) up to 54 h in case a biochemical confirmation is required. Positive samples can be detected between 8 and less than 30 h, before carrying out the biochemical test.

Impedance method studied in the present work seems to be very suitable as a screening test for the pork meat processing industry. Using the impedance method, the total time for detection of *Salmonella* in negative meat samples can be decreased from 42 up to 66 h, in comparison with the standard method. In view of the time of analysis, this method may be even superior to the enzyme-immunological and nucleic acid-based tests whose detection times range between 38 to 72 h due to the necessity of longer pre-enrichment periods.

4. Conclusions

High values of correlative coefficients obtained from calibration curves of different strains of *Salmonella* in raw pork meat together with good correlation between impediometry and standard plate counting to compare the reduction of microbial load throughout UV-C illumination of pork meat samples show that impedance technology represents an approach to quantitative microbiology. Although a calibration curve is required per bacteria and matrix prior to performing routine food assays, it would not be a shortcoming for food safety research as a high percentage of similar samples are used. This fact along with the possibility of monitoring impedance bacterial growth curves in real time gives rise to different applications to be developed in food safety research. As novelty confirmed by experimentation shown in this work, not only low bacterial detection and quantification limits have been reached, but also damaged but still viable bacteria can successfully be detected. Both facts are significantly important in experimentation concerning to emerging technologies as they exert mechanisms of stress for inactivating bacteria.

Efficiency of impedance technology used as tool for detection of *Salmonella* has also been proven as the unnecessarily rejection of batches is avoided due to its ability to distinguish between live and dead cells. In

addition, impediometry yields a negative result within 30 h compared to 72 h required for conventional method, enabling to release negative batches in a faster and safer way.

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