A RAPID IMPEDIMETRIC PROCEDURE FOR ENUMERATING *Escherichia coli* AND GRAM NEGATIVE BACTERIA IN MEAT

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Background

E.U. Directive 93/43 requires that all food processing plants develop and implement a system of preventive controls, taken at each step in the food production process, designed to improve the safety of food products and to reduce the risk of foodborne illness. This system requires rapid microbiologic controls for hygiene indicators and pathogenic microorganisms (e.g. Gram negative bacteria, *E. coli*) of raw materials and end products. Traditional methods used to enumerate bacteria, such as most probable number (MPN) or plate counts, are labor intensive, expensive on a cost per test basis, and require normally from 1 to 5 days. Inexpensive and rapid methods would allow plants to keep control of hygienic conditions of processing lines before products are released from the plant.

Microorganisms growing in culture media metabolize substrates with low conductivity properties into higher conductivity compounds decreasing, thereby, the impedance of the media (Jay, 1996). Impedance measurements of broth cultures give curves reproducible for species and mixed cultures can be identified by use of selective media or specific growth inhibitors. Various methods for enumerating bacteria using electrical measurements have been developed. Impedance protocols have been applied to psychrotrophic, coliform, mesophilic and total count in raw milk (Senyk et al., 1988), for total count in crustaceans and cephalopods (Pellegrino et al., 1998), for coliforms in meat (Firstenberg and Klein, 1983, Martins and Selby, 1980) and for *E. coli* in broiler carcasses (Edmiston and Russel, 1998, Russel et al, 1995).

Objective

The aim of this study was to evaluate a rapid impedimetric procedure for enumerating *E. coli* and Gram negative bacteria in meat. The impedimetric performance was compared to standard plate count for determining if it could provide more rapid results and could be a less complex analytic method.

Methods

More than 300 samples of fresh pork and a similar amount of semiprocessed pork products were analyzed both with conventional counts and with impedimetric procedures for the enumeration of *E. coli* and Gram negative bacteria as required by the internal HACCP control plan.

Standard plate counts were carried out using a chromogenic selective medium Coli ID (bioMérieux) for the detection of coliforms and *E. coli* βD-glucuronidase-positive, the selective medium Gram Negative PMK Agar MUG (PMK) (Biolife) was used for the detection of Gram negative bacteria and *E. coli* βD-glucuronidase-positive. 1 ml aliquots of each decimal diluition were transferred to petri dishes and incubated at 37°C for 24 h (PMK) or 48 h (Coli ID).

The impedance-monitoring instrument used in this study was the Bactometer M 128 (bioMérieux). Both for *E. coli* and for Gram negative bacteria protocols, 0.5 ml of the first decimal diluition of sample were added to module wells that had been prefilled with 1 ml of CM broth (bioMérieux) and EM broth (bioMérieux), respectively. Modules were afterwards placed in the Bactometer processing Unit at 44°C for *E. coli* count and 37°C for Gram negative count. Detection time (DT) is defined as the time at which the impedance change becomes detectable. Impedance Detection Times (IDT) were automatically determined by the instrument. Such parameter is related to several factors: number of cells, generation time, production of low conducttivity substrates. Visual examination of impedance curves was used to verify IDT.

Results and Discussion

The results are shown in Fig. 1 and 2, respectively for the samples analysed for the enumeration of *E. coli* and the samples for Gram negative bacteria. The UFC/g (plate count) and IDT (impedimetric method) were highly related: -0.91 for *E. coli* and -0.85 for Gram negative bacteria (linear regression).

The relationship between initial number of microrganisms and IDT or plate count allows a rapid evaluation of CFU/g of the sample, on condition that there is an algorithm obtained from regression analysis of *selected* data. The correlation between the two methods improves if the less reliable results (e.g. population with bimodal IDT, low repeatability of plate count, storing condition, etc.) are deleted from the analysis (not selected data).

Figure 3 shows an exemple of impedimetric response curves for Gram negative bacteria. The region from 1 to 2 on this plot is called the baseline. Drift was defined as the average slope of the baseline expressed as the percentage change of impedance per hour. The IDT (marked as point 3) is the onset of acceleration in the impedance curve due to bacterial metabolism. The region from 3 to 4 is defined as the slope during the first hour after detection and is expressed as a percentage of the impedance per hour. Impedance curves with low drift values and high slopes are the best because the instrument can more easily determine the acceleration point.

Conclusions

The repeatability of the results allow the use of impedimetric method in place of the conventional plate count. Compared with the standard plate count method available for estimating *E. coli* and Gram negative bacteria in meat, the impedimetric procedure is simple to execute (needs only one diluition of food sample), requires only 12-15 h to conduct (24-48h for standard analysis), is relatively inexpensive, and the results are recorded automatically. The cost of the instrument can be rapidly paid off if moderate to high levels of testing are performed. (Edmiston and Russel, 1998). For these reasons this procedure would be a useful tool to assist plants in the hygienic control of processing lines.

Pertinent literature

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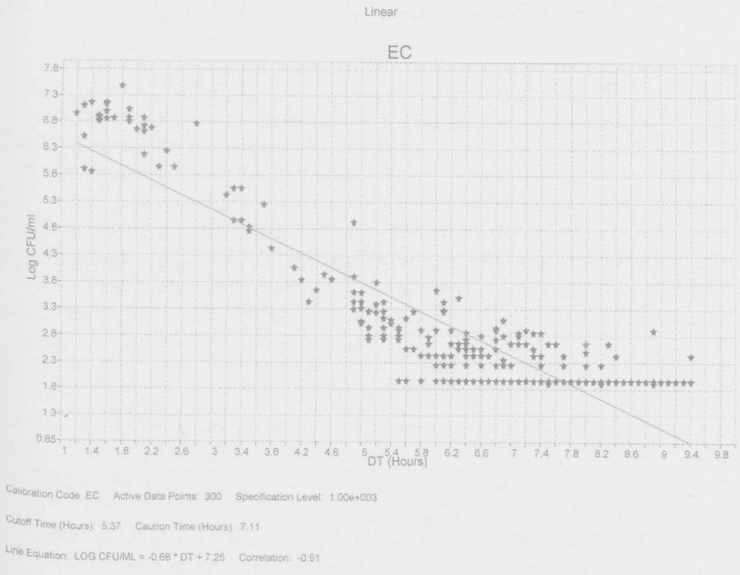

Fig. 1. Linear regression of *E. coli*. Plotted data were obtained from 300 samples of meat

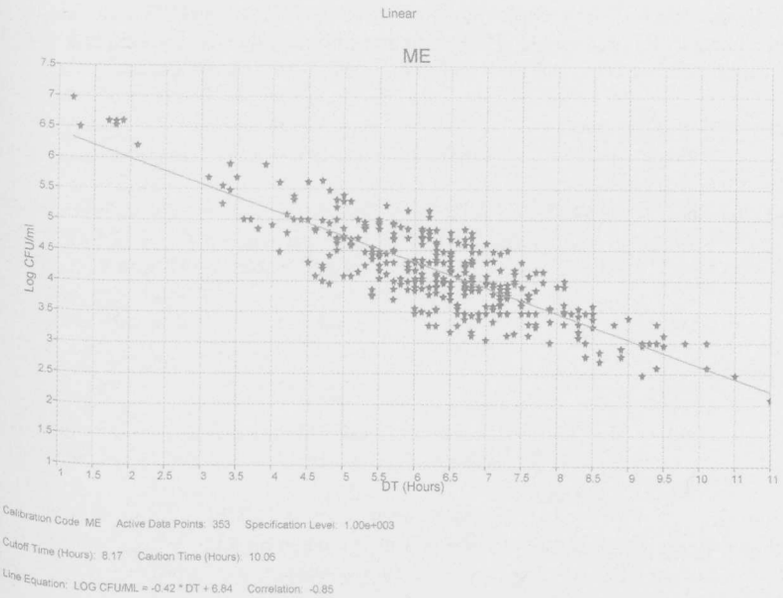

Fig. 2. Linear regression of Gram negative bacteria. Plotted data were obtained from 353 samples of meat.

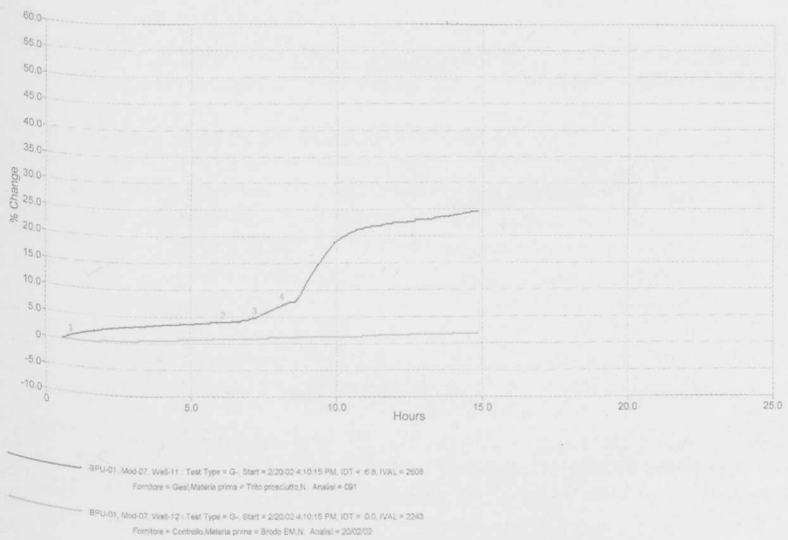

Fig. 3. Impedance changes due to the growth of Gram negative bacteria in meat. The region from 1 to 2 is the impedance detection time. The region from 3 to 4 is the slope after detection.