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SHORT COMMUNICATION

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Impedance measurements to assess microbial contamination of ready-to-use vegetables

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Abstract An impedometric method for estimating the microbial load of ready-to-use vegetables, either inoculated or not inoculated with antimicrobial-producing lactic acid bacteria, was investigated. The correlation (R^2) between total mesophilic bacteria (log cfu/g) and time to detection was 0.889. A discrepancy was observed for the impedometric measurement of low coliforms counts, particularly in samples inoculated with lactic acid bacteria and stored at 8 °C for 6 days. Time to detection for the two groups of microorganisms was less than 11 h.

Key words Ready-to-use vegetables · Impedometry · Microbial contamination

Introduction

Impedance methodology is successfully used in the food industry to assess the microbiological quality of raw materials and finished products. This technique, compared to the conventional analysis for bacteria enumeration, i.e. standard plate count, generates results more rapidly and reduces the labour time and use of consumables. Impedometry has been shown to be a powerful tool for monitoring several groups of microorganisms, such as total aerobic mesophiles, faecal and total coliforms and for the detection of specific food pathogens in a wide range of foods, particularly in the dairy industry [1]. However, little information is available on the application of this procedure to a fresh vegetable matrix. Hardy et al. [2] rapidly estimated the microbial contamination of frozen vegetables and were able to identify samples containing more than 10^5 organisms per gram. Recently, impedance measurements have been adapted to many new applications, such as the detection of novel antibacterial agents and challenge experiments [1, 3].

This study describes the use of an impedometric method to evaluate the microbial load of ready-to-use vegetables. In addition, the potential benefits of this procedure were investigated with samples of vegetables to which an antimicrobial-producing strain of lactic acid bacteria had been added.

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Materials and methods

Commercial ready-to-use mixed salads consisting of endive, garden rocket, green chicory and carrots, packed in polyethylene trays overlapped with polypropylene film under an ordinary atmosphere, were purchased from local retailers, 1 day after packaging. Usually, these products have a shelf-life of 5 days at refrigeration temperatures. After purchase, the vegetables were transferred on ice to the laboratory and immediately subdivided into two batches: one batch was inoculated with the antimicrobial-producing strain *Lactobacillus casei* IMPC LC34 and the other was the control. The vegetable preparation and the inoculum procedure were performed as described previously [4].

Mixed salad samples were analysed either by standard microbiological counts or by impedometric measurements after their preparation (time 0) and after 6 days of storage at 8 °C. A total of 10 g of vegetables was mixed with 90 ml of 1/4 strength Ringer solution and homogenized for 2 min with a Stomacher Lab-Blender 400 (Seward Medical, London, UK). Appropriate dilutions were plated, in duplicate, on Plate Count Agar (Oxoid) for total mesophilic bacteria and in Violet Red Bile Agar (Oxoid) for total coliforms; plates were incubated at 30 °C for 48 h and 37 °C for 24 h, respectively.

Impedance measurements were performed by a Rapid Automated Bacterial Impedance Technique (RABIT) system (Don Whitley Scientific, Shipley, UK). Whitley Impedance Broth (WIB) was used for total mesophilic bacteria and MacConkey broth (Oxoid) for coliforms. Impedance tubes, containing 9 ml of the appropriate medium, were inoculated, in duplicate, with 1 ml of the first decimal dilution of each sample, vortexed and incubated in the RABIT for 24 h at 30 °C or 37 °C, according to the microbial group. Impedance changes were automatically monitored and graphically represented using the RABIT software package. Conductance values were logged every 3 min and detection criteria were set at three successive reading of 5 μS or more. The time required to reach the threshold level, in hours, is referred to as the time to detection (TTD).

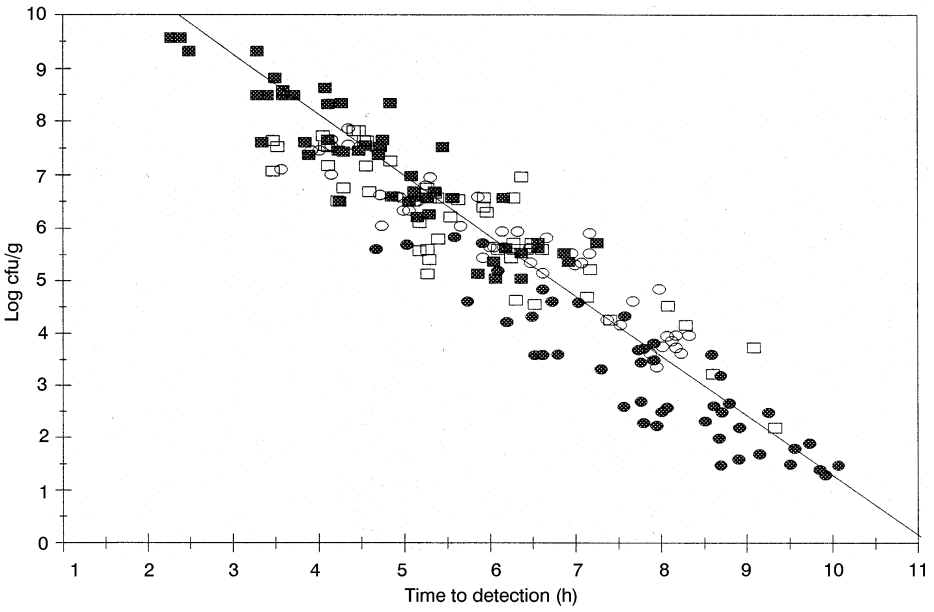

Fig. 1 Linear regression between the total mesophilic bacteria counts (log cfu/g) and the times to detection obtained from 198 vegetable samples either inoculated or not inoculated with *Lactobacillus casei* IMPC LC34. (❑ Control, measurement at time 0, ■ control, measurement after 6 days at 8 °C, ○ samples inoculated with *Lactobacillus casei* IMPC LC34, measurement at time 0, ● samples inoculated with *Lactobacillus casei* IMPC LC34, measurement after 6 days at 8 °C)

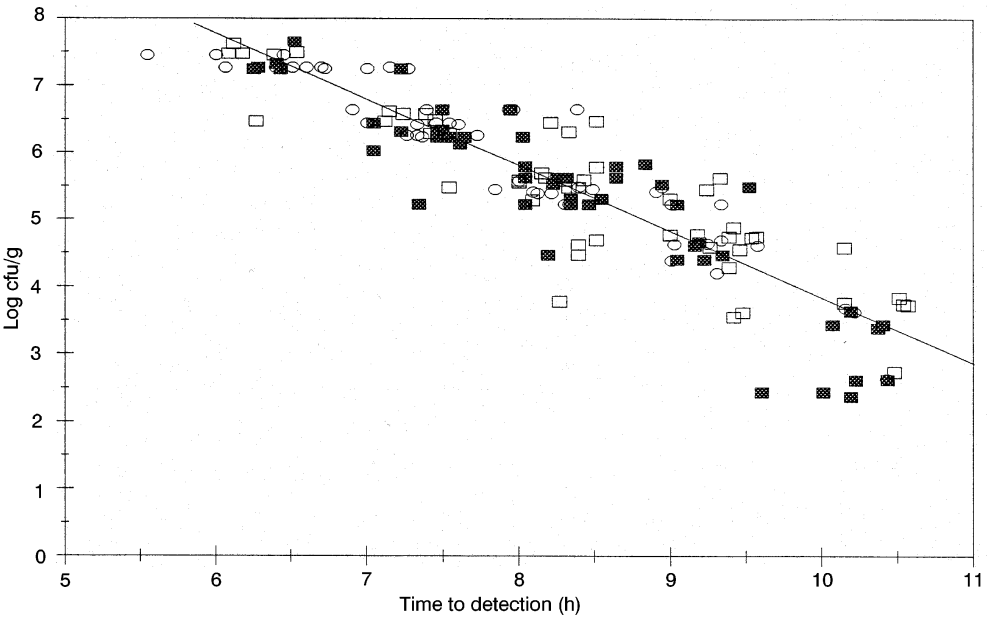

Fig. 2 Linear regression between the total coliform counts (log cfu/g) and the times to detection obtained from 153 vegetable samples either inoculated or not inoculated with *Lactobacillus casei* IMPC LC34. Vegetable samples inoculated with *Lactobacillus casei* IMC LC34 and stored at 8 °C for 6 days were disregarded. (❑ Control, measurement at time 0, ■ control, measurement after 6 days at 8 °C, ○ samples inoculated with *Lactobacillus casei* IMPC LC34, measurement at time 0)

Results and discussion

In total, 102 samples of commercial ready-to-use vegetables, half of them inoculated with lactic acid bacteria, were analysed for total mesophilic and coliform bacteria at time 0 and after 6 days of storage at 8 °C. Standard microbiological counts and the impedometric method by RABIT system were compared. TTD for the mixed salad samples were plotted against the logarithm of the number of mesophilic and coliform bacteria as determined by pour plates (Figs. 1 and 2). The level of initial microbial contamination in vegetable samples was high, ranging from 4.56 to 8.77 log cfu/g for total mesophilic bacteria and from 3.75 to 7.63 log cfu/g for total coliforms. During storage at 8 °C for 6 days, there was an increase of the population in the uninoculated salad samples, while a strong reduction was observed in the samples to which *Lactobacillus casei* IMPC LC34 had been added. This confirms the data already reported in relation to similar products concerning the validity of the use of lactic acid bacteria as an inhibitor of spoilage and pathogenic microorganisms [4, 5]. Linear relationships were found between the TTD values and the logarithm of the microbial content of each bacterial group at different times. The relative equations are reported in Tables 1 and 2. TTD determined by direct impedance correlated well (R^2 = 0.889) with the

Table 1 Relationship between the total mesophilic bacteria count (log cfu/g) and the time to detection in vegetable samples either inoculated or not inoculated with *Lactobacillus casei* IMPC LC34

Vegetable sample	No. of samples	Regression equation	R^2	SE[a]
Control	102	$y = 11.499–0.948\ x$	0.822	0.609
Inoculated with *Lb. casei* IMPC LC34	96	$y = 11.528–1.023\ x$	0.884	0.619
Total	198	$y = 11.862–1.048\ x$	0.889	0.639

[a] Standard error of *y* estimated

Table 2 Relationship between the total coliforms count (log cfu/g) and the time to detection in vegetable samples either inoculated or not inoculated with *Lactobacillus casei* IMPC LC34

Vegetable sample	No. of samples	Regression equation	R^2	SE[a]
Control (A)	102	$y = 13.391–0.952\ x$	0.798	0.573
Inoculated with *Lb. casei* IMPC LC34, measurement at time 0 (B)	51	$y = 13.304–0.923\ x$	0.869	0.409
Inoculated with *Lb. casei* IMPC LC34, measurement after 6 days at 8 °C (C)	42	$y = 8.013–0.729\ x$	0.685	0.536
Control and inoculated, measurement at time 0 (A+B)	153	$y = 13.456–0.955x$	0.824	0.526

[a] Standard error of *y* estimated

total mesophilic bacteria number obtained by conventional counts.

A discrepancy was observed in the coliform measurements for samples with a low level of contamination (1–3.5 log cfu/g), particularly in those inoculated with lactic acid bacteria culture and stored for 6 days at 8 °C; this could be attributed to the growth of non-coliform bacteria in the MacConkey medium used in RABIT. Previous trials with pure culture of *Escherichia coli* showed that there is a linear regression ($R^2 > 0.9$) between the number of inoculum cells and the TTD obtained in MacConkey broth at 30 °C in a range from 10^2 to 10^8 log cfu/g (unpublished results). Sorrells [6] reported similar observations using the Bactometer system to detect the bacterial content in cereal grain products and in pure culture of coliforms; this author suggested that the impedometric procedure for coliforms could be valid only for samples containing more than 100 coliforms/g. Deeper studies are needed to better understand the cause of such an interference.

This work confirms the possibility of detecting, by impedometry, the levels of contaminating microorganisms in foods in a shorter time (less than 11 h) compared to the normally required 24–48 h and demonstrates its potential for analysis of ready-to-use vegetables inoculated with antimicrobial-producing lactic acid bacteria.

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