THE CORRELATION BETWEEN ELECTRIC IMPEDANCE MEASUREMENTS AND STANDARD PLATE COUNTS IN RAW MEAT

ÇİĞ ETLERDE TOPLAM CANLI SAYIMINDA ELEKTRİK İMPEDANS ÖLÇÜMLERİ İLE STANDART PLAK SAYIMLARI ARASINDAKİ İLİŞKİNİN ARAŞTIRILMASI

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ABSTRACT: A rapid impedimetric determination of total bacterial count in raw meat was tested by using two different inoculation methods in Wilkins-Chalgren Anaerobe Broth (WB) and Brain Heart Infusion Broth (BHI). In the first method the raw meat samples were directly inoculated into WB and BHI broths and then transferred into bactometer modules (WB, BHI). In the second inoculation method the samples which previously diluted in peptone water were inoculated in WB and BHI broths in the modules (PWB, PBHI). Samples diluted in peptone water and subsequently inoculated into WB gave maximum correlation coefficient (0,84), superior signals, good acceleration and sharp slope compared to the samples inoculated directly in the growth media..

ÖZET: Bu araştırmada çiğ et örneklerinde iki ayrı besiyerinde, iki farklı inokülasyon yöntemiyle elde edilen elektrik impedans ölçümleri ile standart plak sayımları arasındaki ilişki araştırılmış ve elde edilen impedans eğrilerinin karakteristikleri karşılaştırılmıştır. Bu denemede birinci inokülasyon yönteminde örnekler Wilkins-Chalgren Anaerobe Broth ve Brain Heart Infusion Broth sıvı besiyerinde direkt seyreltilerek baktometre modüllerine transfer edilmiş (WB ve BHI), ikinci inokülasyon yönteminde ise örnekler peptonlu suda seyreltildikten sonra aynı besiyerlerini içeren modüllere inoküle edilmişlerdir (PWB ve PBHI). Yapılan analiz sonucunda peptonlu suda seyreltildikten sonra WB besiyerine inoküle edilen örneklerde diğer inokülasyon yöntemlerine kıyasla en yüksek korelasyon katsayısının elde edildiği (0,84) ve bu örneklerin impedans eğrilerinde impedans detection time (IDT) çok daha belirgin ve keskin olduğu saptanmıştır.

INTRODUCTION

The impedance method is a rapid automated method for determining bacteriological contamination level. In the last decade the impedance method has been used to estimate the microbial levels in different foods, especially in raw milk (CADY et. al., 1978; O'CONNOR,1979; GNAN and LUEDECKE, 1982; FIRSTENBERG-EDEN, 1984). The impedance method relies on the detection of metabolic activity in a growth medium. Cultures of microorganisms bring about changes in the chemical composition of the growth medium through the enzymatic activity associated with multiplication and metabolism. As microorganisms metabolize they create new end products in the medium. Generally uncharged or weakly charged substrates are transformed into highly charged end products for example proteins are metabolized to amino acids, carbohydrates to lactate and lipids to acetate, which increase conductivity of the solution. These chemical changes alter the impedance of the medium and provide an indirect measure of microbial growth and metabolism. Impedance is the resistance to flow of an alternating current through a conducting material (e.g. growth medium). It is a complex entity composed of a vectorial combination of a conductive element and capacitive element. Changes in impedance (and its components) due to microbial growth can be measured by placing an inoculated growth medium into a module equipped with stainless steel electrodes (FIRSTENBERG-EDEN and EDEN,1985).

The time that is required for an initial concentration of organisms to reach the bactometer threshold level is called the impedance detection time (IDT) which is a function of both the growth kinetics, lag time and concentration of microorganisms in a given sample. The microbial level associated with this change in conductance is called the microbial threshold level.

The impedance method could be used to estimate numbers of microorganisms in any product and always give results much faster than the plate count method. The method relies on the detection of metabolic activity in a growth medium, whereas the plate count methodology depends on the production of visible biomass. In the impedance method, for estimation of the numbers of microorganisms the results must previously be calibrated with the results of standard plate counts.

The aim of this study was to investigate the correlation between the impedimetric measurements obtained by using two different inoculation methods and two different media, and the standard plate counts in raw meat.

MATERIALS AND METHODS

Samples

21 raw meat samples were collected from local supermarkets during a 3 month period at Grimsby town in England. Upon arrival at the laboratory, the samples were analyzed within 2 or 3 hours.

Impedance Procedure

Instrumentation: Impedance measurements were carried out on a Vitek Microbial Monitoring System Bactometer Model B-64 (Vitek, Missouri).

The Bactometer Processing Unit was adjusted to 30°C and the total impedance was monitored at this temperature.

Media and inoculations of wells: Wilkins-Chalgren Anaerobe Broth (WB, Oxoid CM 643) and Brain Hearth Infusion Broth (BHI, LabM Bury, Lancs, BL 9 6AU,Lab 49) were used for the impedimetric measurements. In the first inoculation method, 10 g. of raw meat samples were aseptically homogenized for 60 seconds in a stomacher model 400 (Seward Medical, London, UK) with 90 ml of WB and BHI broth. The caps removed from the wells and 1 ml of the homogenates were aseptically transferred into three wells of bactometer modules.

In the second inoculation method 10 g. of raw meat samples were aseptically homogenized for 60 seconds in a stomacher with 90 ml of peptone water (PW, Oxoid CM 9). The caps were removed from the wells and 0,5 ml of the homogenates (10-1 dilutions of each sample) were transferred into each of three 0,5 ml media (WB and BHI) filled module wells. The caps were aseptically placed on the modules, and the impedance was monitored at 30°C for 20 hours. Module wells filled with the same media were used for testing samples as the negative control (The amount of the media was equal to the total standard volume, sample and medium, placed in a well).

During testing, the impedance detection times (IDT's) of the samples were automatically determined by the instrument and the impedance curves (% change curves) obtained were visually examined.

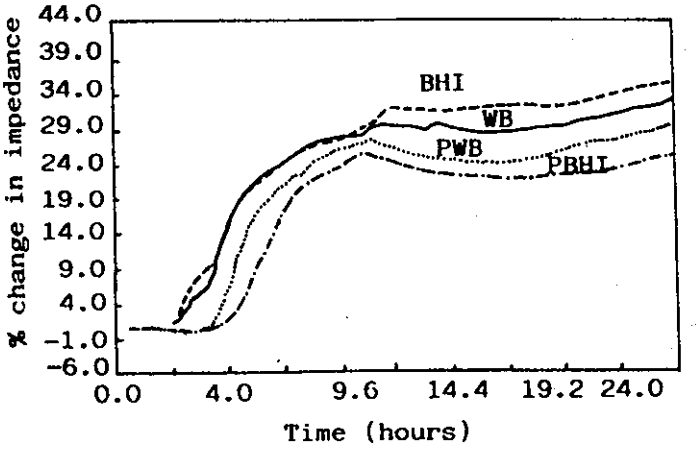

Figure 1. Representative impedance (% change) cures of a raw meat sample. (*WB:* samples directly stomached in WB, *BHI:* samples directly stomached in BHI, *PWB:* samples diluted in peptone water and then inoculated in WB, *PBHI:* samples diluted in peptone water and then inoculated in BHI)

Standard Plate Count Method

10 g. of each raw meat sample were aseptically homogenized for 60

seconds in a stomacher with 90 ml of PW. Other serial decimal dilutions, using the same diluent, were prepared from the first dilution. The count was determined by the spread plate method using Plate Count Agar (PCA, Oxoid CM 325). Incubation was at 30°C for 48 hours.

Statistical Method

Linear regression analysis was used to determine the relationships between the log plate counts as the dependent variable and the mean of triplicate detection times as the independent variable.

RESULTS AND DISCUSSION

Representative impedance curves obtained from samples directly stomached into WB and BHI, and samples stomached into PW (PWB and PBHI) are presented in Figure 1. The results of statistical analysis indicated that the relationships between the impedimetric results obtained in two different media by using two different inoculation methods and the standard plate counts were significantly different ($p<0.05$). Samples stomached in peptone water (PWB and PBHI) produced minimal drift, good acceleration, sharp slope and detection, but the maximum change in impedance and ambiguous earlier detection times were obtained in WB and BHI broth. Similar patterns were observed for the 21 raw meat samples tested. These results indicate that the impedance curves obtained from samples stomached into peptone water and then inoculated in WB and BHI (PWB and PBHI) were superior than the curves obtained from samples stomached directly into WB and BHI.

Contradictory to these findings, FIRSTENBERG-EDEN (1983) reported that samples directly stomached into growth media gave superior signals compared to those stomached into the diluent and inoculated into the growth medium. On the other hand superior signals and higher changes in impedance were obtained in PWB than in PBHI. This is probably due to the low conductivity of BHI medium as explained by FIRSTENBERG-EDEN (1986).

Figure 2 and 3 presents the scattergrams relating impedance detection times to total plate counts for raw meat samples. The scattergram shows the highest correlation (0.84) obtained in PWB. The high correlation which existed between total plate counts and IDT's at 30°C indicates that the impedance measurement

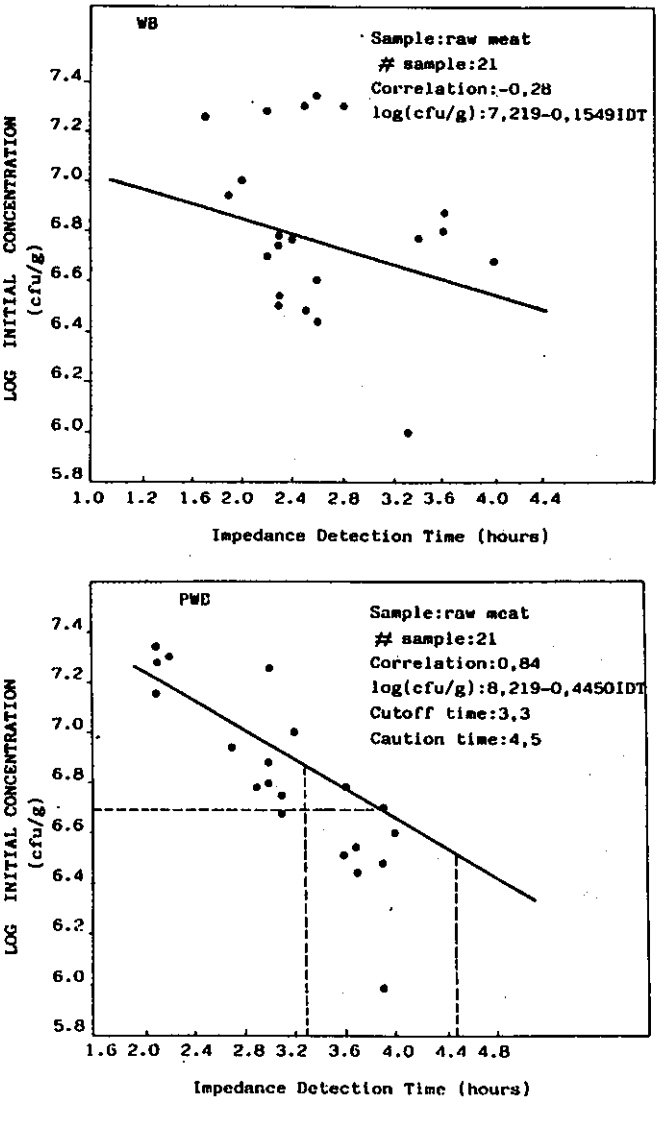

Figure 2. Scattergrams relating impedance detection times obtained in WB and PWB to total plate counts for raw meat samples (WB and PWB explained in Fig 1)

may be a useful alternative to the standard plate count method for raw meat samples. The resulting scattergram relating colony counts to IDT's was used to divide the raw meat samples into three categories depending on their relative contamination levels. If $5x10^6$ colony forming units/g. is chosen as an example of the acceptable cutoff level for raw meat, then the corresponding detection time would be 3.9 hours. If one standard deviation is added to each side of this value (dashed line in Fig. 3), a grey zone is created in which the bacterial levels are close to $5x10^6$. Samples which were detected in minimum 3.3 h (cutoff time) or below contained unacceptable levels of organisms, while samples detected in maximum 4.5 h (caution time) or greater contained acceptable levels of organisms.

Several investigators have attempted to develop impedimetric methods for the estimation of total, psychrotrophic, Enterobacteriaceae, Salmonella and coliform counts in different foods, and found that there were generally good agreement between the conventional methods and impedimetric measurements (FIRSTENBERG-EDEN and KLEIN,1983; FIRSTENBERG-EDEN and TRICARICO,1983; FIRSTENBERG-EDEN et al 1984; FRYER and FORDE,1989; IRVING et al 1989; KYRIAKIDES and THURSTON, 1989; PETITT,1989; PRENTICE et.al 1989; PUGH and ARNOTT, 1989). FIRSTENBERG-EDEN and TRICARICO (1983) studied the impedimetric determination for total, mesophilic and psychrotrophic counts in raw milk and found that the correlations between impedance detection times and standard plate counts were very high; -0.96, -0.95 and -0.96; respectively. Similarly FRYER and FORDE (1989) reported an acceptable correlation coefficient of 0.89 for total viable counts on cornflour using the bactometer. But the correlation coefficients for coliform counts obtained on skimmilk powder and fat-filled powder did not correlate well to the impedimetric measurements and reported to be unacceptable (0.58 and 0.56, respectively) by the same investigators. Contradictory to the findings of FRYER and FORDE (1989), the impedance detection times in CM (a medium developed for the impedimetric detection of coliforms) and confirmed plate counts on Violet Red Bile Agar for the raw and pasteurized milk, heavy cream, and ice cream mix. The results of a collaborative study carried out to establish the reproducibility of the impedance method in predicting counts of raw milk suggested that the impedimetric procedure yielded more reproducible results than the standard plate count (FIRSTENBERG-EDEN 1984).

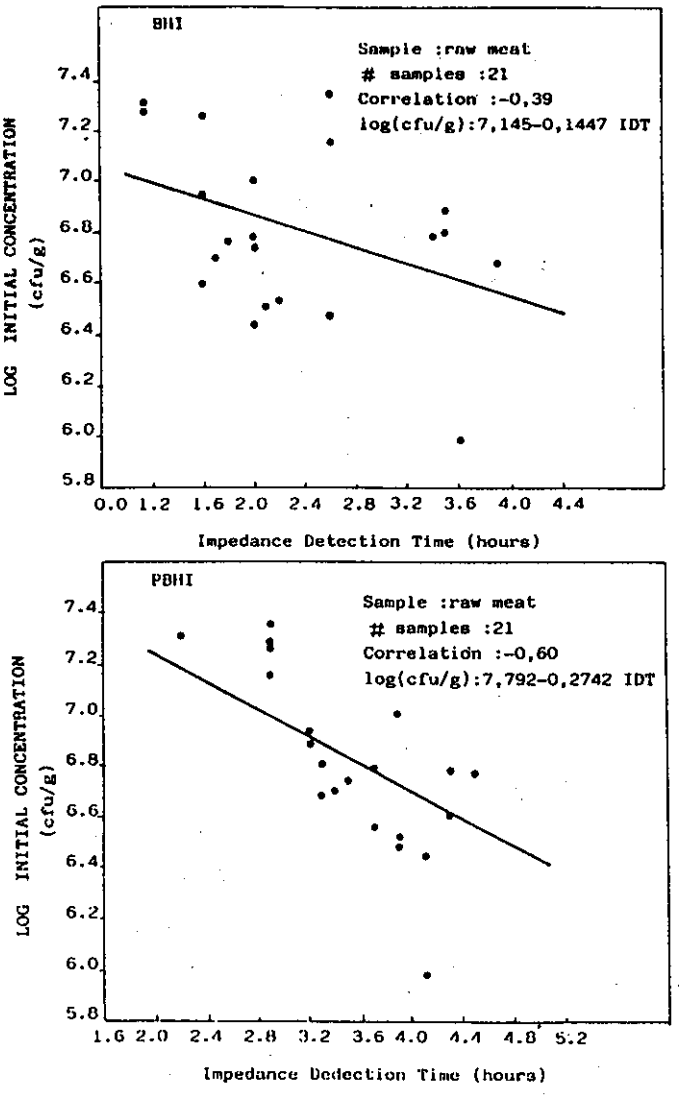

Figure 3. Scattergrams relating impedance detection times obtained in BHI and PBHI to total plate counts for raw meat samples (BHI and PBHI explained in Fig 1)

When comparisons were attempted with the findings for raw meat, the information in the literature was found to be rather scanty and the results presented very limited. FIRSTENBERG-EDEN (1983) reported that there was a high correlation (0,97) between IDT's and CFU for raw meat samples. The results obtained in this study showed that Wilkins-Chalgren Anaerobe Broth proposed by Vitek Bactometer firm gave maximum correlation coefficient (0,84), superior signals, good acceleration and sharp slope compared to the Brain Heart Infusion Broth. These results also indicate the importance of selection of a proper media for successful impedance monitoring. A medium which results in a smooth impedance curve and a single acceleration is very important to obtain reliable impedance results.

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