A FIVE MINUTE TEST FOR TOTAL VIABLE COUNTS IN RAW MEAT

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INTRODUCTION

Microbiology has for many years been associated with the growth of micro-organisms on a suitable growth media e.g. plate count agar. The main problem with this type of method is the time of response which is typically 2-3 days. This is one of the reasons behind the increased interest for a rapid and reliable microbiological method. In the last 10-15 years many of different methods have been 'developed' for rapid microbiological examinations.

One of the most promising techniques is bioluminescence. The mechanism of the bioluminescence (luciferin/luciferase) assay for adenosine triphosphate (ATP) is well documented (DeLuca 1976, Wannlund et al. 1978). ATP is an energy storing substance present in all living cells. The enzyme complex luciferin/luciferase reacts specifically with ATP and emits a light-signal proportional to the amount of ATP present in the sample. A linear relationship between ATP and colony count on plate count agar (PCA) has been shown by several authors (Stannard & Wood 1983). The ATP in the sample originates not only from living microbial cells but also from several other sources e.g. somatic cells. This type of ATP is normally referred to as somatic ATP.

The differentiation between microbial and somatic is essential if the bioluminescence technique is to be used successfully. There are different approaches to this. The two most commonly used are to separate the microorganisms from the sample before extraction of the ATP or to use selective extraction and destruction of the somatic ATP prior to the measurement of the microbial ATP.

The BactoFoss is a unique fully automated instrument which combines the two above mentioned principles in order to remove the 'noise' coming from the sample itself.

MATERIALS AND METHODS

Meat samples

The trial on raw pork meat was performed at a large Danish meat processing plant. The size of the meat lombs ranged from a few grams up to 200-400 g. The beef meat used for the trial was obtained locally from supermarkets, butchers etc. The meat lombs were typically 150-500 g in size. Samples were taken from the surface and the inner part of the meat lombs.

Homogenization

10 g of meat is diluted with 90 g of dilution liquid (8.5 g of NaCl and 1.0 g of peptone in 1 l of destilled water) and homogenized for 30 sec. in a Stomacher (Colworth 400). This suspension is used for the BactoFoss and the reference method, respectively.

BactoFoss method

A small portion of the meat suspension wis centrifuged for 30 sec. at 350 G, in order to remove debris and coarse meat particles. The sample is placed at the BactoFoss (Foss Electric). The instrument automatically takes out the necessary sample volumen and performs the measurement. The different steps in a BactoFoss measurement are illustrated in Fig. 1. After 1 1/2 minute the result appears on the display and is printed out. The instrument will then automatically perform a rinsing cycle (1 1/2 minute) and is ready for a new sample.

A measurement consists of the following operations: intake of sample - filtration - lysing of somatic cells - washing - extraction of microbial ATP - measuring of light - presentation of result - rinsing of instrument.

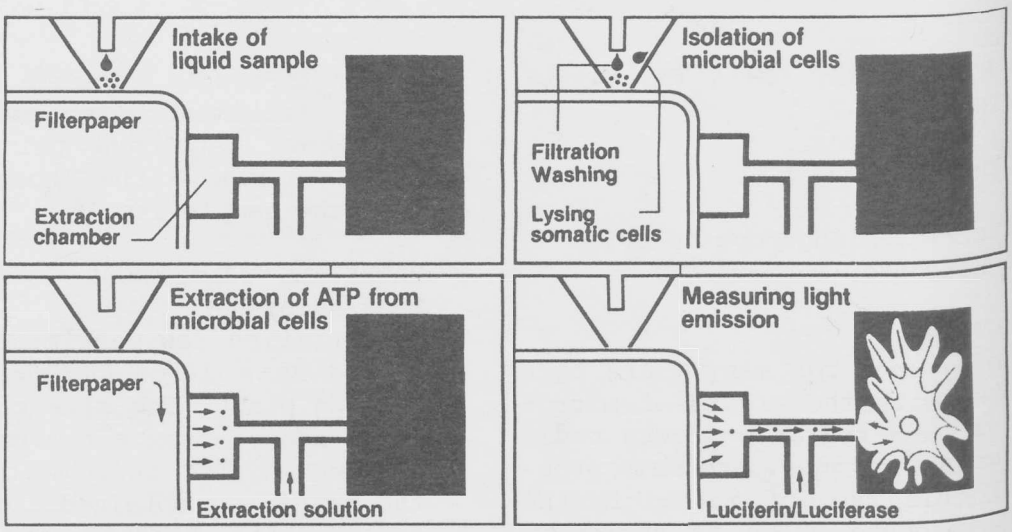

Fig.1. Schematic illustration of samples treatment in the BactoFoss

Reference method
The total viable count (TVC) is determined by the spiral plating technique (Spiral System) on Bacto Plate Count Agar (PCA, Difco). The necessary dilutions are made with the same dilution liquid as used for the homogenization. The plates are incubated at 21°C in 4 days and counted manually.

RESULTS
Pork Meat
The new method (BactoFoss) was tested at a large Danish meat processing plant. 70 samples of pork meat were examined on the BactoFoss and PCA.

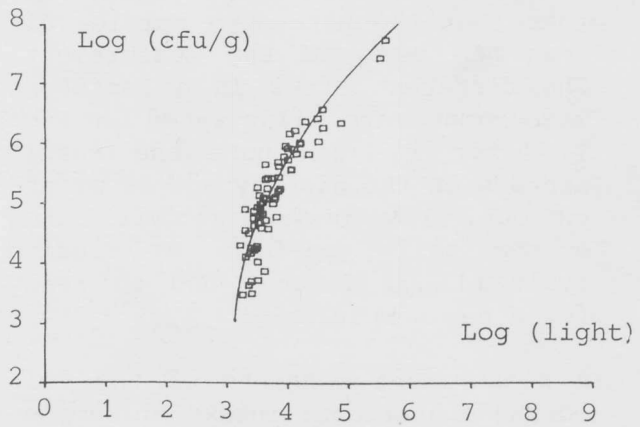

Fig.2. Pork meat (70 samples). Bio-Luminescence (raw data) versus TVC

The level of contamination ranged from 3,000 cfu/g to 50,000,000 cfu/g. The correlation between the two methods is shown in Fig.2. The line drawn in Fig.2. is the calibration line which is used by the instrument to estimate the TVC according to the amount of light measured.

The standard deviation of estimate is 0.39 log cycles. If the calibration mode is used and the BactoFoss estimates are correlated with the TVC the data shown in Fig.3. appears. A linear regression in the range of 10^5 - 5 x 10^7 cfu/g gives a correlation coefficient of 0.93 and the standard deviation of estimate is 0.23 log cycles.

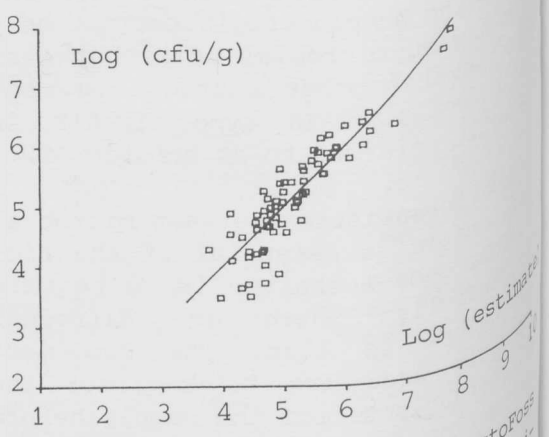

Fig.3. Correlation between BactoFoss (pork meat calibration) estimates and TVC for 70 samples of pork meat.

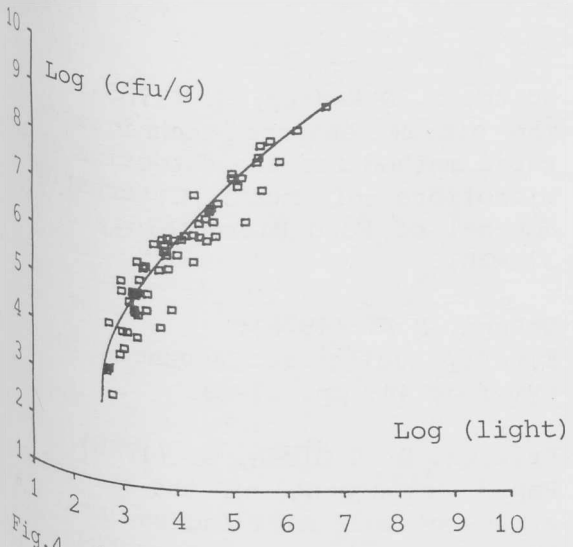

Fig.4. 65 samples of beef meat measured on BactoFoss (raw data) correlated with TVC.

Beef Meat

The examined beef meat had a level of contamination from $7^2 \times 10^9$ to 10^9 cfu/g. Fig.4. illustrates the correlation between the raw data from the BactoFoss and the TVC. The standard deviation of estimate is 0.47 log cycles.

If a linear regression on the calibrated data is performed it gives a correlation coefficient of 0.94 (Fig.5).

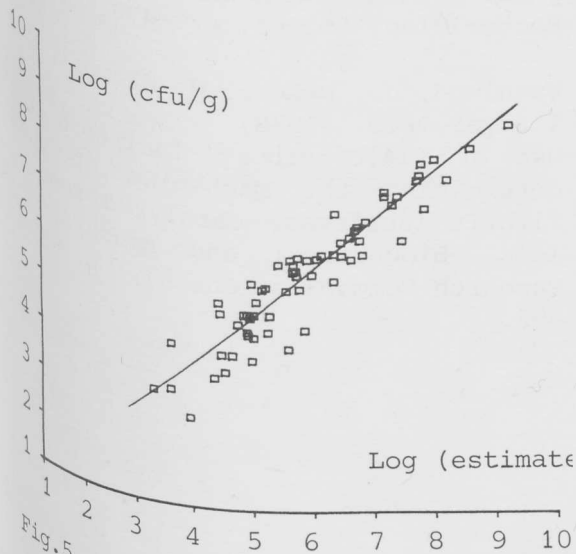

Fig.5. 65 samples of beef meat measured on BactoFoss (meat calibration) and correlated with the corresponding TVC.

DISCUSSION

The correlation between the new rapid method (BactoFoss, bioluminescence) and the traditional method (plate count agar) is linear down to 10^5 cfu/g, Fig.2 and 4. Below 10^5 cfu/g the relationship is no longer linear. This is probably due to residual somatic ATP which has not been removed prior to the measurement of the microbial ATP. The interference from somatic ATP has been shown by several other investigations (Baumgart et al. 1980, Bülte & Reuter 1985, Kennedy & Oblinger 1985).

To take this deviation from the linear correlation into account the BactoFoss uses a curve fit for its calibration programs.

The pork meat with TVC below 10^4 cfu/g is estimated by the BactoFoss to a level which is approximately ten times higher than the TVC from PCA. This is probably due to the fact that somatic ATP is being measured as microbial ATP.

The data obtained on beef (Fig.4 and 5) resembles the pork meat data. The correlation is linear down to 10^5 cfu/g and deviates below. Three data points in Fig.5. are marked with the letter 'A'. These samples are overestimated according to the corresponding TVC with approximately one log cycle.

There are at least two explanations for this 'overestimation'. The first possibility is that the level of contamination is correctly estimated on PCA. It is very important to note that a TVC coming from a petri-dish is not necessarily correct. Various factors i.e. temperature, composition of the agar and incubation (aerobe/anaerobe) will always influence on the number of bacteria capable of growing. Although PCA is, by definition, designed for giving optimal growth conditions for bacteria there are some bacteria which will show little or no growth at all on PCA. The other possibility is that the TVC from the PCA is correct

and the BactoFoss count is too high. An overestimation with the bioluminescence technique can be caused by somatic ATP which has not been removed prior to the extraction of microbial ATP. Alternatively, the microbial population could consist of bacteria with a unusual high content of ATP per cell.

CONCLUSION

The general impression of the results obtained on meat with the new instrument BactoFoss, shows that it has a great potential as a rapid and reliable microbiological method.

The results obtained on various kinds of beef and pork meat have confirmed the possibility of getting a count within 5 minutes with this new method. The BactoFoss is capable of giving a figure if the samples has a level of contamination above 10^5 cfu/g. For samples with TVC below 10^5 cfu/g the instrument can confirm this.

Investigations on raw bulk milk have shown a linear correlation in the range 10^4 - 10^8 cfu/ml with a standard deviation of estimate at 0.27 log cycles (Eriksen & Olsen 1988). This indicates that the BactoFOss is a versatile instrument for the industry.

Rapid microbiology close to the processing line has become a realistic possibility with the aid of this new instrument. The simplicity of operating a BactoFoss will certainly be appreciated by the user.

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