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APPLICATION OF BIOLUMINESCENCE AS A RAPID METHOD FOR ASSESSMENT OF THE MICROBIAL STATUS OF MEAT

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Introduction

One of the main drawbacks of classical microbiological procedures for assessment of the microbial status of a food is the time lag between the actual analysis and the time at which the results are available. Over the years attempts to develop rapid methods have therefore been made on several occasions.

The procedures for rapid microbiological methods are based on various principles, such as electrical impedance(see e.g. Hardy et al, 1977, Cady et al,1978, Wood & Gibbs,1982) radiometry (see e.g.Rowley et al, 1978), or reductase tests using dyes such as resazurin (Holley et al,1977), the direct epi-fluorescent filter technique, DEFT (Pettipher & Rodriques, 1982) or in combination with immuno - technique for enumeration of Salmonella (Thomason, 1971) pH-measurements (se e.g. Rogers & McClestey 1961) and other physical determinations such as microcalometry (se e.g.Cliffe et al, 1973)have also been used with varying success.

In the present study the determination of adenosine triphosphate (ATP) as a measure of the bacterial status of fresh meat has been investigated. The object of the use of such rapid methods as this has been to see, whether it would be possible to use the procedure in a public surveillance programme for assessment of freshness of meat and meat products at the point of sale at retail level. There are several limitations in introducing the ATP method in a public control programme. First of all, an ATP determination can only indicate the bacterial level, where exact bacterial numbers really are what is normally required. Secondly, ATP-determinations do not indicate anything regarding any presence of potentially pathogenic bacteria. However, since ATP in a sample can be determined within an hour, the speed with which the information can be obtained is such that a high ATP content in a sample can be reacted on much faster than if the same sample had been analysed by means of conventional methods. A low bacterial total count using conventional methods is of course no proof that pathogenic bacteria are absent, but on the other hand, neither is the presence of high bacterial counts proof of the presence of pathogens. Provided though that there is a fairly satisfactory correlation between the ATP-content in a sample and the total bacterial counts, this has to effect that it will be possible very rapidly to screen a large number of samples, and it is then possible to concentrate further work on 10 to 20 per cent of the samples, thus saving much work and material resources; but, at the same time it will be possible quickly to inspect retail outlets from where samples with dubious results have been obtained.

Materials and Methods

All samples have been collected from retail outlets, i.e. stores, public catering units, restaurants etc. by a public analyst laboratory. Thus, they constitute typical specimens from a public surveillance programme. Most of the samples reported here are ground beef, some of which have been packed in modified atmosphere, but some samples stem from raw, coarsely ground pork sausage, others from cured sliced meats intended for use on open sandwiches.

Quantification of bioluminescence as a means of assessing the bacterial status of a food is based on the principle that the concentration of ATP in a sample can be measured by meansof the luciferin-luciferase enzyme system, since luciferin under suitable conditions reacts with free ATP and emits light which can be measured quantitatively. By means of an internal standard this is converted to measurement of the total content of ATP, which then can be correlated to the total bacterial numbers of the sample. Some of the ATP in foods stems from the food itself - the so-called somatic ATP - and the rest from the microflora, the bacterial ATP. ATP stemming from these two sources can be separated and thus measured individually by means of different extraction solvents.

The procedure employed for an ATP determination may be divided in four operations: homogenizing, cleaning-out, extraction, and measurement. For analysis, a ground sample is added a diluent (sterile water containing 0.9% NaCl and 0.1% peptone). The mixture is homogenized in a Stomacher for one minute. 2 aliquots of 7 ml. each of the homogenate are centrifuged for 5 minutes at 1300g, and 1.5 ml. supernatants are drawn for extraction. Total ATP is then determined after extraction for 10 minutes with dimethylsulfoxide(DMSO)/trichloracetic acid(TCA), whereas somatic ATP is measured after extraction for 10 minutes with Triton X-100. This procedure is usable for ATP determinations from fresh meats only. At the moment new experiments are carried out in order to optimize the procedures for cured meats and especially heat treated meats.

Instruments for measuring ATP are produced by several suppliers. In the investigation described here is used a luminometer, type LKB-Wallac, model 1251 equipped with a matrix printer and dispensers for automatic admixture of enzyme and internal ATP standard. In addition, the instrument is connected to a key-board and a video screen.

For correlation purposes, the total bacterial numbers were also determined on plate count agar(PCA) according to normal procedures. Samples were incubated both at 30°C and 17°C for 72 and 96 hours respectively.

Results and discussion

In figure 1 is shown the bacterial content of ATP versus the bacterial numbers enumerated on PCA and incubated at 30°C. The results comprise 42 samples of ground beef. As will be seen, the correlation coefficient is only 0.55. However, as shown in figure 2, if the ATP content of the samples is compared with the total bacterial numbers on PCA, but incubated at 17°C, the correlation coefficient obtained for the same 42 samples is 0.72. From both figures it seems obvious that the scatter is higher at low values. It is also a contributing factor to the scatter that some of the samples come from ground beef which has been retail packed in modified atmosphere and stored under those conditions for up to 5 to 7 days.

Heat treated products seem to present special problems. In a small trial ground beef heated for 1,2, or 4 minutes at 85°C was examined, and an increasing concentration of ATP as a function of the heat treatment was recovered. The same trend was found if the experiment was carried out using sterile samples. The preliminary explanation for this phenomenon is that heating deliberates somatic ATP which normally is bound to protein. Free ATP in this amount is not found and measured in unheated samples.

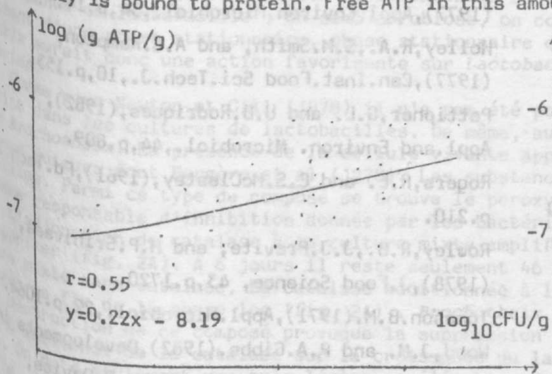

Figure 1. Relation between ATP concentration and $\log_{10}$ bacterial numbers of ground beef. Incubation at 30°C.

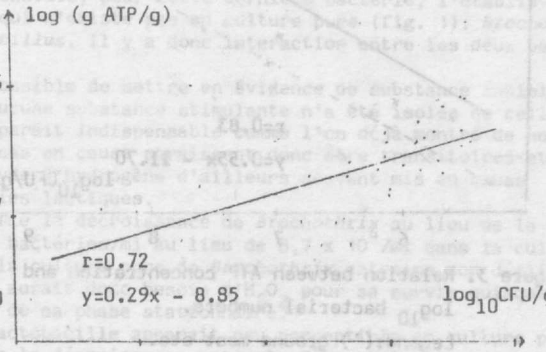

Figure 2. Relation between ATP concentration and $\log_{10}$ bacterial numbers of ground beef Incubation at 17°C.

A number of other sources may contribute to inaccuracies when comparing ATP content with total microbial counts. The yeast content may e.g. be unusually high. In yeast the ATP content per cell is typically a hundredfold higher than the content of an average bacterial cell. However, it has often been observed in food microbiology that microbial deterioation can take place in a food with a yeast content which numerically is very low compared with spoilage caused by bacteria. This means though that the recorded ATP content can be considered a true measure of the microbial activity of the sample, i.e. a measure which is superior to a total bacterial count.

A number of cured meat products have also been investigated. Besides the fact that many of these are heat treated (pasteurized) during processing they contain sodium chloride, which is known to be inhibitory to the light emission which is the basis for the bioluminescence method. However, this difficulty can largely be overcome through dilution, since the decrease in inhibition is exponential, while decreasing light emission is linear. If, therefore, the supernatant after centrifugation is diluted tenfold, it will be possible to use exactly the same procedure as described above.

Figure 3 shows the results of a comparison between ATP concentration and the total bacterial numbers of samples enumerated on PCA and incubated at 17°C. The samples comprise various meat products, such as raw, ground beef, pork and a mixture of veal and pork, uncooked pork sausage, minced meat mix, Bologna type sliced sausage, meat roulade, head cheese, and sliced cooked and smoked ham. The correlation coefficient has been calculated to 0.87 for ground meat 0.83 for meat products. It will be seen that the lower the bacterial numbers are, the poorer is the correlation, i.e. for bacterial numbers above 10^6/g the results are usually very reliable.

In conclusion, the purpose of this work has been to develop a rapid microbiological method for screening the bacterial status of a number of perishable foods in a public surveillance programme. Provided the method is used for screening it seems possible, quickly to divide samples in such a way using this method that the more traditional method need only be used for further examination on a fraction (e.g. 20 per cent) of the incoming samples.

Work is in progress at the moment with the purpose of removing some of the causes of deviations between expected and obtained results. It seems that one of the main obstacles for improvements is the concentration of free ATP which is sometimes present in some types of samples, but free ATP may possibly be removed through filtration or enzymatic inactivation. This could be included in the routine procedure.

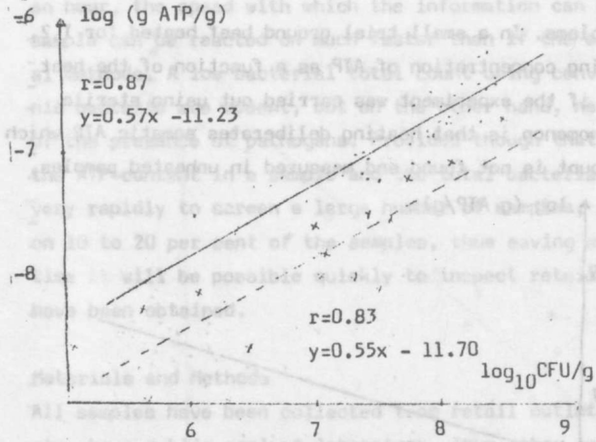

Figure 3. Relation between ATP concentration and log_{10} bacterial numbers.
Legend:(·) ground meat etc.
(x) Bologna type sausage etc.

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