5 - 10

ATP-BIOLUMINESCENCE : A RAPID METHOD FOR THE ESTIMATION OF MICROBIAL CONTAMINATION OF MEAT AND MEAT PRODUCTS

H. Labots, M.Sc. and F.K. Stekelenburg

TNO Netherlands Centre for Meat Technology, Zeist, The Netherlands

1. Introduction

The bioluminescence phenomenon has already been observed and described many centuries ago. The study of luminous bacteria and other luminescent organisms is, however, of a relatively recent date. Different light emitting reactions appear to occur in luminescent organisms. Since the discovery in 1947 by McElroy of the requirement for ATP (adenosine triphosphate) in the bioluminescent system of the firefly Photinus spiralis, the luciferin-luciferase complex of the firefly has been used in many sensitive analyses based on luminescence (8,10).
The reaction can be summarized as follows:

$$\text{ATP} + \text{luciferin} + O_2 \xrightarrow[Mg^{2+}]{\text{luciferase}} \text{AMP} + \text{pyrophosphate} + CO_2 + \text{oxyluciferin} + \text{light}$$

If the concentrations of luciferin, luciferase, Mg^{2+} and O_2 are kept constant, the system will emit light of an intensity proportional to added low levels of ATP. A more or less stable light output (falling by a few % each minute) is apparent about 1 sec after mixing. For the assay of ATP, the amount of light (photons) produced over a fixed period of time (generally 10 sec, beginning 2 sec after mixing) is measured (Figure 1).

Application of ATP bioluminescence measurements aiming at the specific estimation of bacteria presents a serious problem with regard to selectivity, in that many types of samples contain ATP derived from biological tissues other than bacterial cells (7). Therefore, before the assay, the sample should be subjected to a selective treatment to remove non-bacterial ATP.

A simple filtration might be effective, but sometimes complex treatments including cation exchange resin or centrifugation are needed, which impedes a more general use of this method (1,5,6,9). However, a very simple technique was developed by Lumac®, with two different detergent reagents. The Lumac®/3M system has been tested on milk (2,11) and meat (3).

2. Materials and methods

39 samples of raw minced meat - beef as well as beef/pork mixtures - were bought in local shops, and 20 samples of different raw meat materials for the production of cooked cured meat products were obtained from a meat plant.
The raw meat samples were homogenized with dilution fluid in a Stomacher® for 1 minute. This homogenate was used to estimate the Standard Plate Count (Plate Count Agar, 3 days at 30 °C) and the ATP content.
18 samples of vacuum packed cooked cured meat products were obtained from meat plants; they were swabbed with cotton swabs (100 cm^2), shaken in 10 ml dilution fluid with 0.1 % Tween 80 and glass beads. With this fluid Standard Plate Counts and ATP-estimations were carried out.

The tests were carried out with the Lumac®/3M IMC test kit, according to the scheme in Figure 2. The original Lumac® reagents: NRS®, SOmase™, NRB®, Lumit®-PM, Lumit®-buffer, as well as an ATP standard were used. With the NRS reagent the ATP from the somatic cells is extracted selectively and immediately inactivated with Somase, an ATP-ase. Then the ATP from the bacterial cells is extracted with NRB. The thus released ATP reacts with the luciferin-luciferase complex added; the light emitted is measured with a photometer (Biocounter®) (Figure 3) and expressed as Relative Light Units (RLU).

3. Results and discussion

The results for minced meats, raw meat materials and cooked cured meat products respectively are summarized in Figures 4, 5 and 6. These results became available within one hour after homogenizing. Good correlations were calculated for the raw meats as well as the swab samples of the cooked cured meat products. The regression lines for the different products were not identical, possibly owing to the limited number of samples, the different microflora or varying degrees of quenching or light loss in the different samples.
Nevertheless, the Lumac/3M method appears to be rapid, reliable and sensitive and is also promising for on-line monitoring of bacteriological qualities.

4. References

1. Baumgart, J., K. Fricke und C. Huy. Schnellnachweis des Oberflächenkeimgehaltes von Frischfleisch
   durch Bestimmung von Adenosintriphosphat mit einem Biolumineszenz-Verfahren
   Fleischwirtschaft 60 (1980) 266
2. Bossuyt, R. A 5-minute ATP-platform test for judging the bacteriological quality of raw milk
   Neth. Milk Dairy J. 36 (1982) 355
3. Carlier, V. et al. Etude de l'application de la bioluminescence au dénombrement de la flore bactérienne superficielle des carcasses à l'aide du Biocounter M 2010
   Revue technique des véterinaires hygienistes de l'alimentation 21 (1982) (184) 13
4. Catsaras, M. et A. Lacheretz. Flore aérobie mésophile et mesure de l'ATP dans la viande hachée
   Proc. 8th Int. Symp. W.A.V.F.H., Dublin, 1981
5. Patel, P.D. and J.M. Wood. Separation of micro-organisms from raw meats and their rapid estimation by measurement of microbial ATP
   Leatherhead Food R.A. Res. Rep. no. 420 (1983)
6. Pikelis, S., K. Littel and A. Spurgash. Rapid bioluminescence screening method for estimating total bacteria in fresh beef
   Abstr. 4th Int. Symp. Rapid Methods and Automation in Microbiology and Immunology, Berlin (1984)
7. Sharpe, A.N. et al. Adenosinetriphosphate (ATP) levels in foods contaminated by bacteria
   J. Appl. Bacteriol. 33 (1970) 758
8. Stanley, Ph.E. Instrumentation for luminescence methods of analysis
   Trends Anal. Chem. 2 (1983) 248
9. Stannard, C.J. and J.M. Wood. The rapid estimation of microbial contamination of raw meat by measurements of ATP
   J. Appl. Bacteriol. 55 (1983) 429
10. Thore, A. Technical aspects of bioluminescent firefly luciferase assay of ATP
    Sci. Tools 26 (1979) 30
11. Waes, G. et al. A rapid method for the detection of non-sterile UHT-milk by the determination of bacterial ATP
    Milchwissenschaft 39 (1984) 707

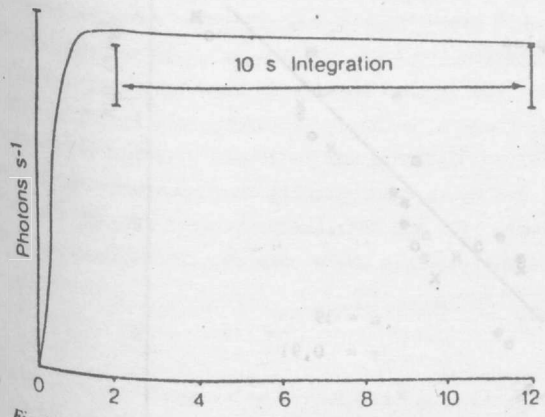


Fig. 1. Kinetic profile for light produced in the reaction of ATP with a modern firefly luciferase reagent prepared for analytical applications. Reagents were mixed at 0 s.

Figure 2 - Schematic of rapid bacteria counting technique using firefly luciferase

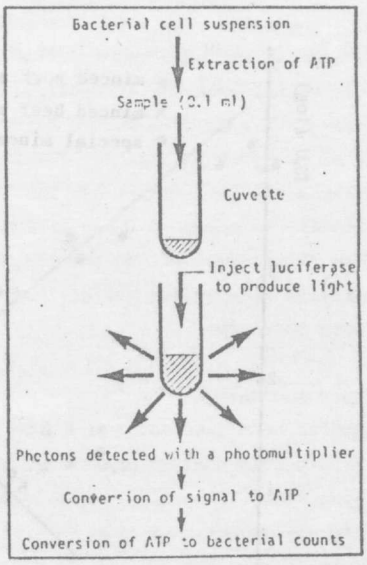
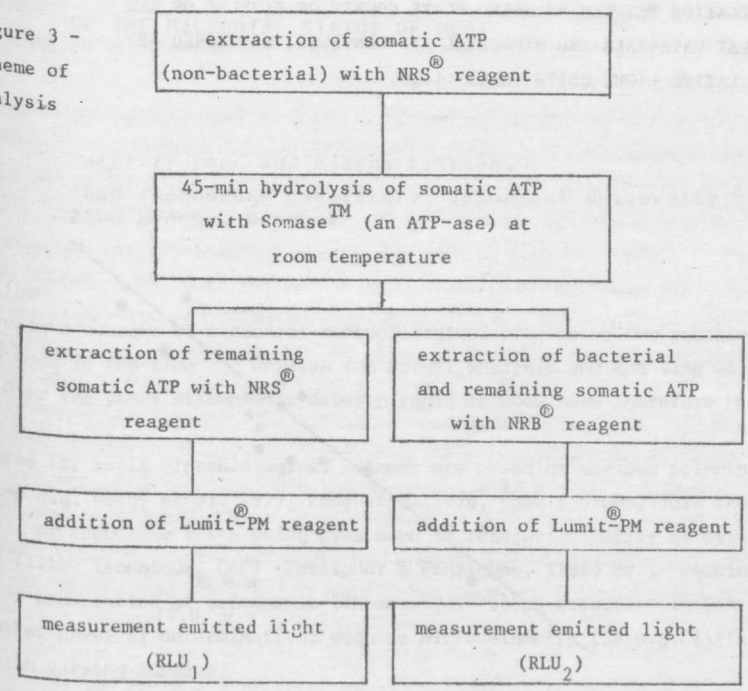


Figure 3 - Scheme of analysis



$RLU_2 - RLU_1$ = RLU correlated with bacterial cells

FIGURE 4 - RELATION BETWEEN THE STANDARD PLATE COUNTS OF MINCED MEATS AND MICROBIAL ATP-CONTENTS, EXPRESSED AS RELATIVE LIGHT UNITS (RLU) (log)

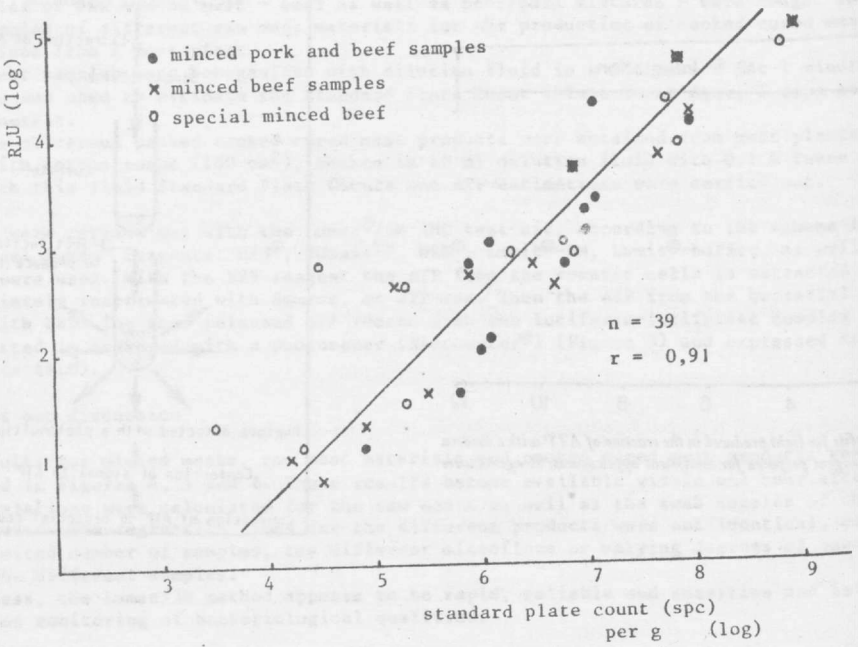


FIGURE 5 - RELATION BETWEEN STANDARD PLATE COUNTS OF SAMPLES OF RAW MEAT MATERIALS AND MICROBIAL ATP-CONTENTS, EXPRESSED AS RELATIVE LIGHT UNITS (RLU) (log)

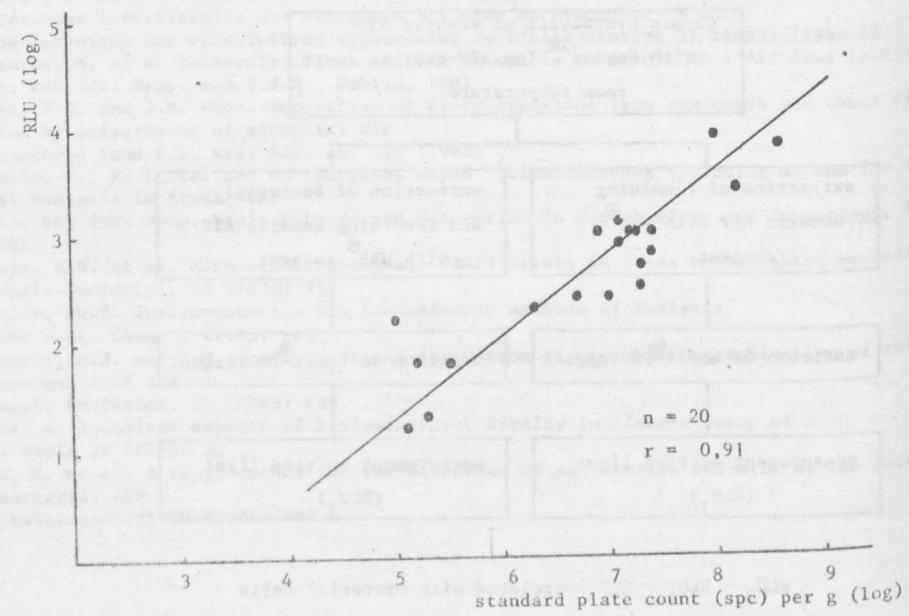


FIGURE 6 - RELATION BETWEEN STANDARD PLATE COUNTS OF SAMPLES OF THE SURFACE OF COOKED CURED MEAT PRODUCTS AND MICROBIAL ATP CONTENTS EXPRESSED AS RELATIVE LIGHT UNITS (RLU) (log)