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THE POTENTIALITY OF MICROCALORIMETRY AS A RAPID METHOD FOR MONITORING THE MICROBIOLOGICAL QUALITY OF RAW MEAT AND FISH

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ABSTRACT

The exothermic heat production rates (HPRs) of ground meat and cod suspended in culture media were measured at different operating temperatures using a LKB BioActivity Monitor and correlated to bacterial numbers determined by plate colony counts at varying time/temperature incubation conditions. Significant correlation between peak HPRs and colony counts were obtained with all experimental conditions. Bacterial levels in the range of 10^4-10^8 g^{-1} could be estimated in <24h. Peak HPR-times were also related to sensory quality scores (0-10) for cod samples. By using a cut-off time of 11h it was possible to differentiate between fish of high (scores > 6) and inferior quality.

INTRODUCTION

The objectives of food microbiology are threefold: (i) safeguarding of the wholesomeness of finished products (ii) surveillance of good manufacturing practices securing against microbial contamination and (iii) assessment of the keeping qualities. Current methods are almost exclusively based on counting of visible bacterial colonies growing in non-selective media (total counts) or in media selective and/or indicative for specific groups or organisms: pathogens, indicator bacteria. The major drawbacks of traditional methods are that incubation periods of 2 to 10 d are required. Consequently, the operational value of microbiological quality assurance schemes are seriously restricted.

During recent years, several indirect indices of microbiological food quality have been studied which allow assessment of microbial levels within 1 - 24 h. Several of these rapid methods are based on the recording of physical or biochemical changes resulting from microbial activity in the foods.

Physical rapid methods in food microbiology include: conductance/impedance measurements (1,2), microcalorimetry (3) and radiometry (4). Gram-negative bacteria can be estimated by measuring en-

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dotoxin using the Limulus test (5) and the total load of viable microorganisms has been determined by measuring ATP-levels (6).

The application of microcalorimetry for monitoring microbial contamination of foods was first suggested by Insalata et al. (7). Studies by Cliffe et al. (8) have revealed that the heat output from samples of raw milk correlated closely with the bacterial counts. The sensitivity of microcalorimetry for estimation of bacterial levels in milk was studied by Berridge et al. (9). Detectable heat effects were produced by $< 5 \times 10^5$ bacteria ml^{-1}. Lampi et al. (10) found that the minimum HPR detectable with their system required the presence of 10^4 bacteria ml^{-1}. They also demonstrated a linear relationship between initial bacterial numbers and the times until maximum HPRs were attained.

In the present report, the potentiality of microcalorimetry as a rapid method for measuring bacterial quality of raw ground meat and fresh fish is evaluated.

MATERIALS AND METHODS

Food samples

Twenty-two samples of ground beef and pork were purchased in retail stores. Three lots of freshly caught codfish each consisting of 30 were examined. The fish was stored on ice ($0^{o}C$) for 14 d. On days 1, 4, 7, 9 and 14, 10-20 g of the bellies of 6 fish were excised and pooled.

Plate counting procedures

Primary 10^{-1} suspensions of meat samples were made by homogenizing 5 g with 45 ml nutrient broth (NB) using a Colworth Stomacher 400. Further decimal dillutions were made in saline +0,1% peptone (PS). One ml of appropriate dilutions were pour plated induplo in tryptone glucose extract agar (TGE). One set of plates were incubated at $30^{o}C$ for 3 d (mesophilic counts) and the other one was preincubated at $17^{o}C$/17 h followed by incubation at $7^{o}C$/72 h (psychrotrophic counts).

Bacterial counts of fish samples were made in the same way with the following modifications: brain heart infusion broth (BHI) was used for the primary suspension and plates for psychrotrophic counts were incubated at $7^{o}C$/10 d.

Sensory quality assessment of fish

On each day of examination fillets of 6 fish were poached and the odour and flavour evaluated by a panel of 6 persons according to a scoring scale of 0-10. Scores of 10-6 indicate fresh fish (grade 1), 6-4 indicate slight off-odours/-flavours (grade 2). Scores <4 are used to indicate fish of rejectable sensory quality.

Microcalorimetric measurements

HPRs of meat samples were measured using an LKB 2277 BioActivity Monitor (LKB-Produkter AB, Bromma, Sweden). For the codfish samples a prototype 44 model of the LKB instrument was used.

One ml of the primary 10^{-1} suspensions were enclosed in disposable 3 ml glass ampoules. Sterile distilled water was used as reference. The loaded ampoules were temperature equilibrated for 30 min before insertion into the thermopile zone. Eleven meat samples were examined at an operating temperature of 21°C and 11 samples at 30°C. All fish samples were run at 25°C. Recordings of HPRs were continued until the maximum heat effects were obtained.

RESULTS

Ground meat samples

HPR curves for meat suspensions in NB showed an initial lag phase followed by a rather steep increase of heat output. The peak HPR was usually rather distinct, depending on the initial colony counts (Fig. 1). At 21°C the maximum HPRs were in the range of 350-515 µW and at 30°C the range was 505-815 µW.

In the two series of experiments with 11 meat samples in each, peak HRPs at 21° and 30°C, respectively were related to initial mesophilic and psychrotrophic counts. The levels of the 2 groups of bacteria were similar. At identical initial bacterial concentrations, maximum HPRs were obtained in shorter time at 30°C as compared to 21°C. The difference was about 6 h (Fig. 2). Even with extremely low bacterial counts at 10^4 g^{-1} maximum HPRs were obtained within 24 h. At high bacterial levels of 10^7-10^8 g^{-1} HPR reached a maximum after 10-12 h. The correlation between bacterial counts and "peak times" was best at 21°C. Correlation coefficients were -0.98 and -0.97, respectively, for mesophilic and psychrotrophic counts. At 30°C the corresponding figures were -0.81 and -0.88.

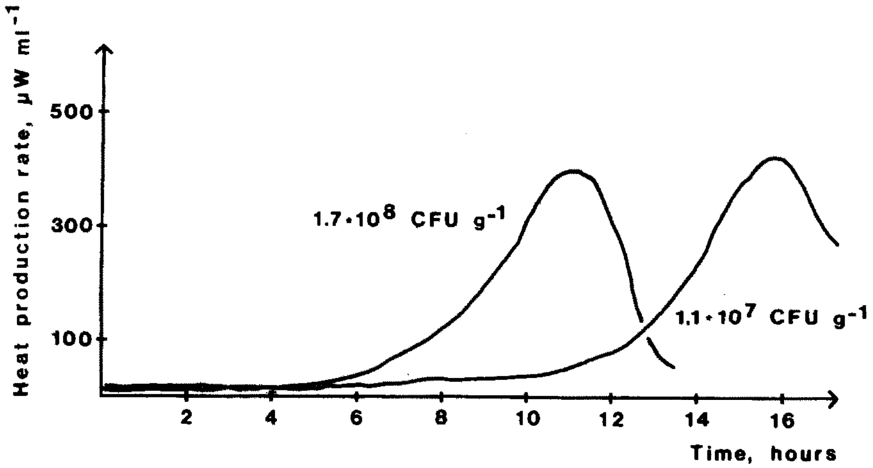

Fig. 1. Heat production rate curves at 21°C for two 10^{-1} suspensions of ground meat in NB.

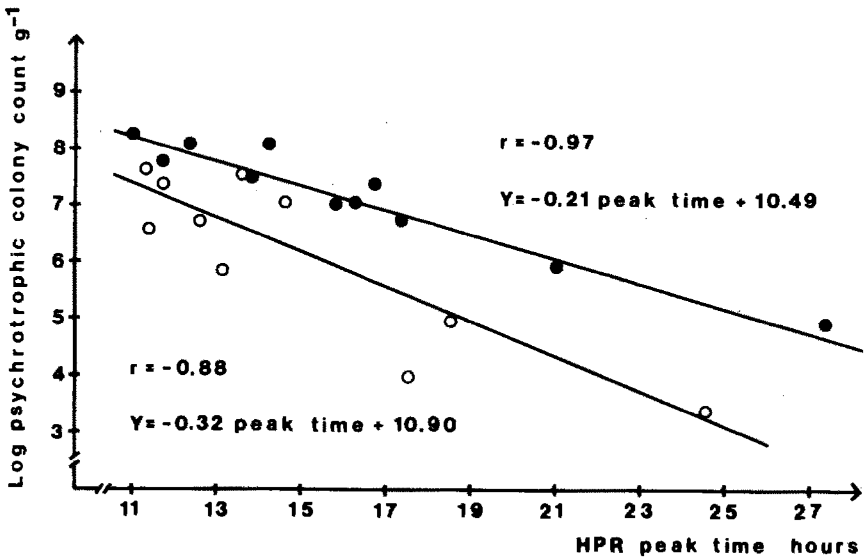

Fig. 2. Relationship between HPR peak times and psychrotrophic colony counts of ground meat at microcalorimeter operating temperatures of 21°C (●——●) and 30°C (○——○).

Codfish samples

During storage at $0^{o}C$ log_{10} colony counts g^{-1} increased from about 6.00 to 9.50. A linear relationship was observed between colony counts and HPR peak times which varied between 8.4 and 16.0 h (Fig. 3). The correlation coefficient was -0.83.

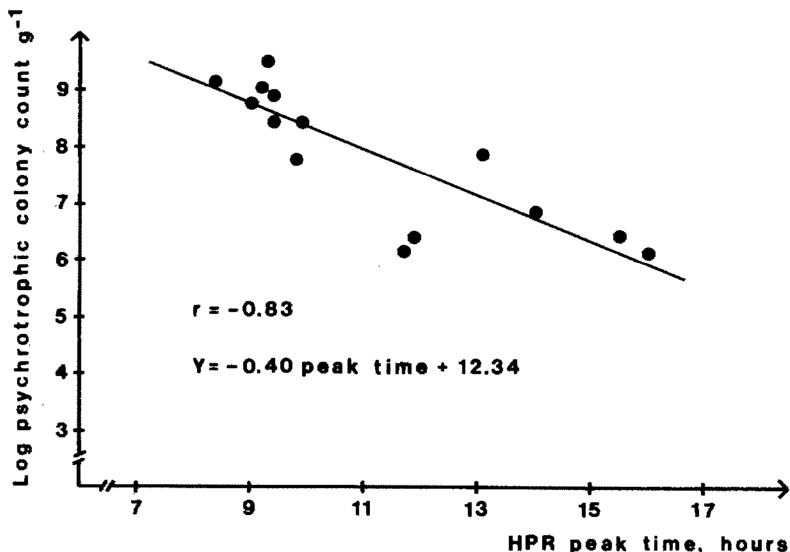

Fig. 3. Relationship between HPR peak times and psychrotrophic colony counts of codfish. Instrument operating temperature $25^{o}C$.

In the food industry there is a demand for methods of rapid grading of the quality of raw materials. Deterioration of raw fish is primarily due to microbial activity resulting in off-odours and off-flavours. Fig. 4 shows the relationship between sensory quality and HPR peak times. Selecting 11 h as a cut-off time, raw fish can be classified as grade 1 ($\sim$ scores $>$ 6) or poorer quality.

As pointed out already prediction of the keeping quality (shelf-life) of foods is an essential demand in food microbiology. In the sensory quality system applied a score ≤ 4 indicates spoilage. In this study shelf-life of codfish was defined as No. of days until scores ≤ 4 were reached. A positive correlation between HPR peak times and shelf-life was demonstrated. The standard deviation of the regression line, however, was rather high. More extensive studies are needed to assess the potentiality of microcalorimetry as a rapid method for shelf-life prediction.

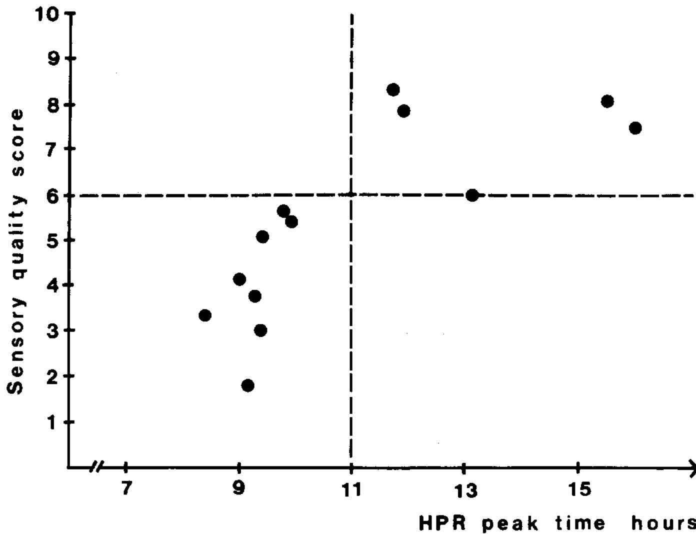

Fig. 4. Classification of the sensory quality of codfish based on microcalorimetric measurements.

DISCUSSION

The data presented indicate that HPR peak times correlate significantly with the initial bacterial counts in suspensions of raw meat and fish. Thus microcalorimetry of food products represents an indirect assessment of bacterial levels in these food products. The lowest number of bacteria in a sample used in this study was c. 10^4 g^{-1}. Due to the 1:10 suspension in NB, the initial cell number in the sample that was inserted into the microcalorimetric vessel was 10^3. With this inoculum the HPR reached a maximum at 30°C after 24 h. Heavily contaminated samples (10^8-10^9 g^{-1}) produced peak HPRs after 7-11 h.

Results of microcalorimetric measurements of bacterial suspensions can be recorded either as the minimum HPR detection time or as in the present study as peak HPR times. Detectable HPRs usually occur 4-6 h earlier than peak HPRs. In our experience, however, the precise time at which significant deflection of the power-time curve from the base line occurred was difficult to establish. In contrast, peaks of the p-t curves were regularly quite distinct.

The minimum detectable HPR with the LKB instrument is 0.15 µW$\sim$ 0.0358 µcal sec^{-1}. At 37°C a pure culture of Staphylococcus aureus produces 0.028 x 10^{-5} µcal sec^{-1}. It might therefore be expected that samples with bacterial concentrations $>10^5$ would produce de-

tectable heat immediately after insertion into the instrument. However, a lag phase of 4-6 h was observed (Fig. 1). Several explanations are possible: (i) the metabolic activity of the bacteria is lower at 21-30°C, (ii) bacteria in foods are frequently sublethally injured for which reason a resuscitation period is necessary to restore enzymatic activity, (iii) the temperature equilibration period at 30 min was not sufficient to prevent heat absorption. Such an endothermic upset has been reported previously (8, 10).

The bacterial flora of raw food products is composed of psychrotrophic and mesophilic organisms. The optimum growth temperatures for these two groups are 20-25°C and 30-37°C, respectively. By selecting the operating temperature of the microcalorimeter either one of these groups are favoured. In our study, psychrotrophic colony counts were used as the reference. The better correlation obtained for meat samples at 21°C as compared to 30°C reflects that the former operating temperature is favourable for psychrotrophs. The significance of the instrument operating temperature for correct estimation of bacterial counts in milk was reported by Berridge et al. (9). Spoilage of non-processed food products is mainly due to the metabolic activity of psychrotrophs which can grow at 0-30°C. Consequently, an appropriate operating temperature for microbiological quality of raw foods will be within the range of 20-25°C.

In our judgement, microcalorimetry constitutes a relatively simple and rapid screening procedure for assessment of the microbiological and sensory quality of non-processed food. At present, however, available instruments have too low capacity and are too costly for routine use in food microbiology laboratories.

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