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THERMAL DENATURATION OF BACTERIAL CELLS EXAMINED BY DIFFERENTIAL SCANNING CALORIMETRY*

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Abstract

Thermal stability of vegetative cells of *Listeria monocytogenes, Escherichia coli* and *Lactobacillus plantarum* was studied by counting viable fractions and determining DSC curves of their suspensions. DSC curves in the 5–99°C range showed a series of endothermic transitions between 50 and 60°C, where the heat destruction of cells occurred. Heat denaturation of DNA required a higher temperature than cell killing. Thermal death was strongly influenced by the pH, composition and NaCl content of the suspending buffer. A mathematical model developed by us enabled comparison of DSC peak temperatures and temperatures required for loss of viability.

Keywords: DSC, thermal denaturation, vegetative bacteria

Introduction

Heat inactivation of microorganisms is of eminent importance to many food processing technologies. The thermal death of cells is usually considered being related to heat denaturation of some crucially important biopolymers. Irreversible thermal denaturation of membranes and/or nucleic acids and certain enzymes are thought as the primary process responsible for thermal death. Previous studies on the differential scanning calorimetry (DSC) of bacterial cells revealed that the DSC-technique may help better understanding of this process.

Materials and methods

Test organisms and bacteriological evaluation

The thermal stability of vegetative cells of some food-borne bacteria *(Listeria monocytogenes* 4ab No.10, OHKI, Budapest; *Escherichia coli* No. 9270, Univ. of

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Reading, U.K.; *Lactobacillus plantarum* B 01149, NCAIM, Budapest) was studied comparatively both by counting viable fractions and by determining DSC curves of their suspensions. Preparation of bacterial suspensions for heat treatments and loss of viability as a function of heat treatments have been performed according to our previous publication [1].

DSC-measurements

DSC profiles of stationary phase, 0.9 cm^3 dense suspensions (~10^{11} cells cm^{-3}) were determined in a 'SETARAM Micro-DSC-II' microcalorimeter by scanning from 5 to 99°C, using cell-free buffer as the reference. After heating, the samples were cooled down and reheated as before.

Mathematical modeling

For mathematical modeling the STATGRAPHICS 5.1 program package (Statistical Graphics Corporation, USA) was used.

Results and discussion

Our investigations are illustrated mainly with the results obtained with *Listeria monocytogenes*. DSC curves of vegetative bacteria taken from 5 to 99°C temperature range showed an exothermic heat flow below the maximum temperature of growth and a series of endothermic transitions began to appear between 50 to 60°C (Fig. 1), in the temperature range, where the heat destruction of cells occurred (Fig. 2). Unfortunately, the onset temperature of denaturation could not be determined because no realistic base line estimation was possible, due to the preceeding exotherm process.

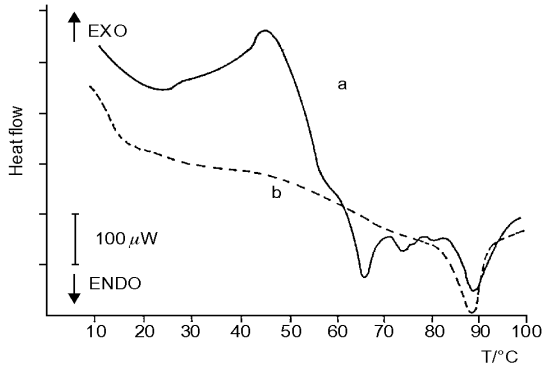

Fig. 1 DSC curves of *Listeria monocytogenes* cells suspended in pH 6.8 phosphate buffer. a – first heating; b – second heating. Scanning rate: 0.5°C min^{-1} [1]

When the cell suspensions heat treated by DSC-microcalorimetry were cooled down and a second run was performed, the curves obtained by the second heating showed that the exothermic and endothermic processes of the first heating were irreversible transitions, except the highest-temperature endotherm, which appeared

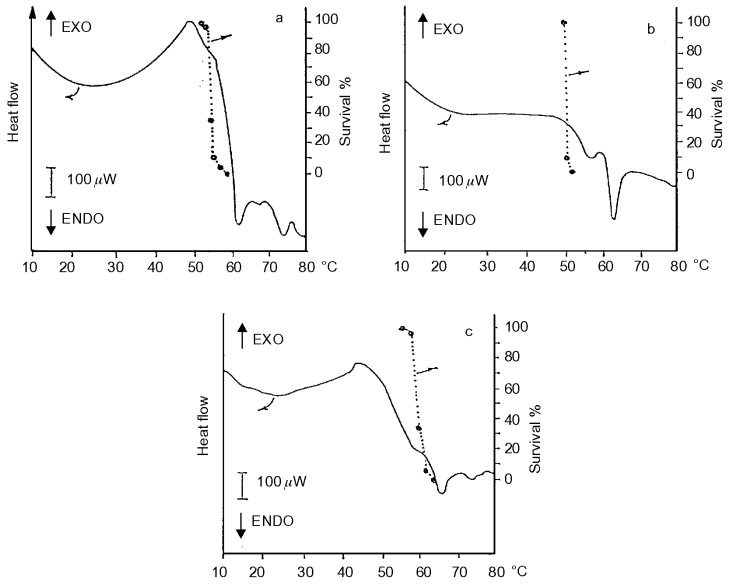

Fig. 2 Comparison of the lowest temperature range of heat inactivation of vegetative bacteria with their DSC transitions at pH 6.8 of the suspending medium. A – *Escherichia coli*; B – *Lactobacillus plantarum*; C – *Listeria monocytogenes* 4ab [1]

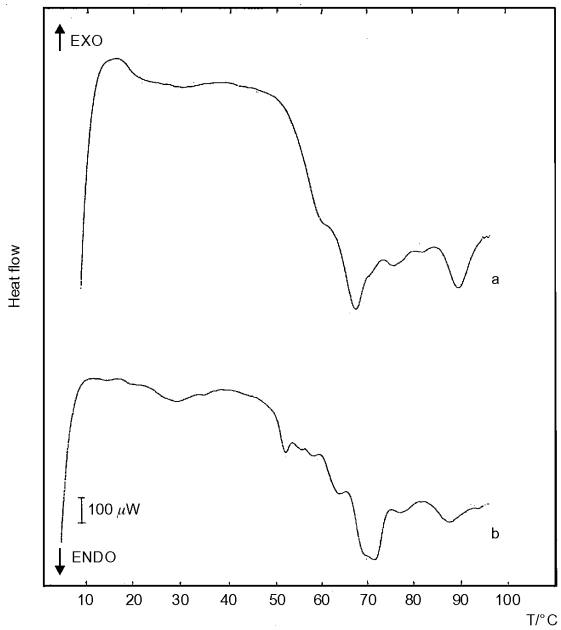

Fig. 3 DSC curves of *Listeria monocytogenes* 4ab. A – suspending medium phosphate buffer; B – suspending medium Tris-buffer. Scanning rate: 1.2°C min^{-1}

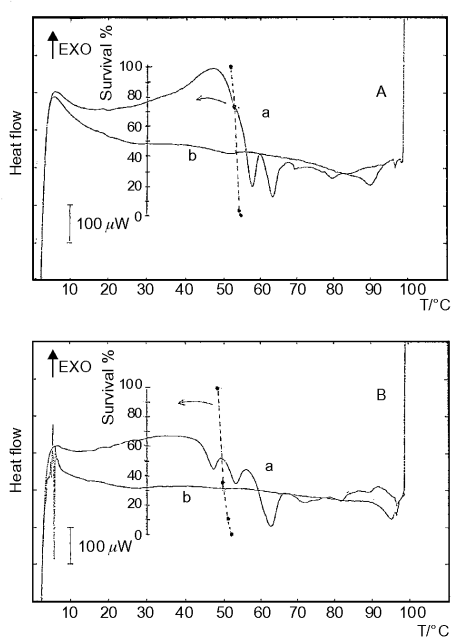

Fig. 4 Effect of NaCl content of the suspending medium on survival and DSC curves of *Listeria monocytogenes* 4ab. Suspending medium: A – Tris-buffer; B – 9.38% NaCl-containing Tris-buffer. a – first heating; b – second heating. Scanning rate: 0.5°C min^{-1}

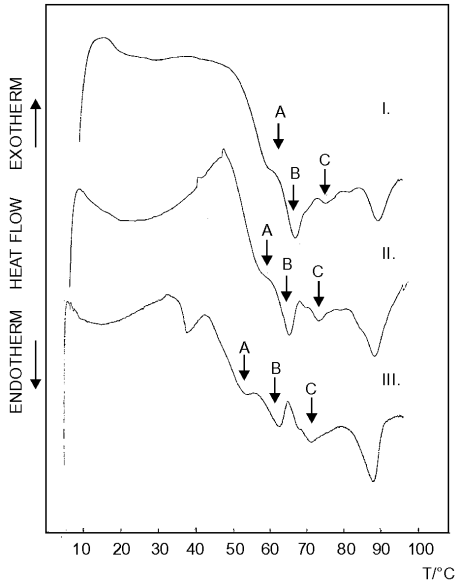

Fig. 5 Effect of heating rate on the DSC curves of *Listeria monocytogenes* 4ab. I: scanning rate 1.2°C min^{-1}; II: scanning rate 0.5°C min^{-1}; III: scanning rate 0.1°C min^{-1} (A, B and C peaks are indicated in the text)

more or less pronounced during the second run, too. According to previous literature [2–4], this reversible, or partially reversible transition was the 'melting' process of the intracellular DNA, which is probably combined with denaturation of a component of the cell wall [5]. Heat denaturation of DNA required higher temperature than cell killing, and did not correlate with thermal stability of the bacteria.

The process responsible for thermal death is strongly influenced by the pH [1], composition (Fig. 3) and NaCl content (Fig. 4) of the suspending buffer. Since the citrate/phosphate buffers are Mg^{2+}-chelating, and the Tris-buffer is not [6], the observed effect of the used phosphate buffer may be due to destabilization of ribosomes via Mg^{2+}-loss. The high ionic strength at the 9.38 % NaCl content showed to cause an increased heat sensitivity resulting in a shift of the positions of the denaturation endotherms towards lower temperatures.

DSC curves obtained with different scanning rates (Fig. 5) showed a temperature shift in the major transition peaks (A, B, C) as a function of the heating rate. Therefore a mathematical model was developed [7] to extrapolate peak of denaturation endotherms obtained by different heating conditions for a 'zero heating rate' in order to enable their better comparison with the microbiological observations. Because the heating rate of our instrument can be set only between 0.1–1.2°C min^{-1}, data from available literature for heating rate 10°C min^{-1} [8] was also involved to extend the validity of the model.

Table 1 Comparison of the 50 % heat-inactivation temperature of vegetative bacteria with the 0-heating rate temperatures of B-peaks of their DSC-curves

	T_{pB}/°C	T_o/°C	Heat-inactivation temperature/°C
Listeria monocytogenes	65.7	59.2	59
Escherichia coli	62.7	54.1	55
Lactobacillus plantarum	56.4	47.8	50

The mathematical model describing the relationship between the heating rate and the DSC peaks:

$$T_p = T_o + b\nu^a$$

where T_p – peak temperature of the denaturation endotherm at a given heating rate (°C); T_o – peak temperature of the denaturation endotherm at 0°C min^{-1} heating rate (°C); ν – heating rate (°C min^{-1}); *a*, *b* – constants.

The results of the model-fittings were as follows:

A-peak $T_p=47.51+12.0\nu^{0.25}$ $R^2=0.996$

B-peak $T_p=57.06+10.2\nu^{0.25}$ $R^2=0.998$

C-peak $T_p=66.72+8.4\nu^{0.25}$ $R^2=0.996$

Comparing the temperatures at which 50% of populations became inactivated and the peak temperatures extrapolated to '0 heating rates' can be seen in Table 1, illustrating that the temperatures of the loss of viability are close to the extrapolated temperatures of the B-peaks. Thus, the first major endothermic peak seems to represent a transition of primary importance in heat-inactivation of vegetative bacterial cells. This may be associated with some critical event of crucial protein denaturation in the complex process of ribosome melting [5, 9].

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