

DECOMPOSITION OF DSC CURVES OF DAIRY PRODUCTS WITH GAUSSIAN FUNCTIONS

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DSC measurements have wide application in the field of biochemical/biophysical research to look into the melting behaviour of muscle proteins in different intermediate states of ATP hydrolysis cycle as well as in the field to develop dairy products. It was a demand to develop a deconvolution program to evaluate and interpret the background of DSC scans such a way that the decomposition into a proper function has to be simple and quick. There should be possible to choose the proper parameters as well as to calculate the data of decomposing curves with the simultaneous graphic representation of them including the resultant DSC scan as the sum of decomposing functions. In most cases Gaussian function is used for this purpose. Our main field of interest is the food physics where e.g. the proliferation of different probiotic bacteria follows a lognormal distribution, its first derivative is a Gaussian one, for this reason we have chosen it.

The main parts of this PC program are:

- the process of fitting and calculation of Gaussian functions,
- a multiple step correction of these function (optimal fitting),
- graphic representation of all functions.

An example is shown for its application how to analyse the melting of butter fat.

Keywords: dairy products, deconvolution, DSC scans, Gaussian function

Introduction

We have widely used the differential scanning calorimetric method in the field of muscle research and to develop new dairy products during the last decades [1–9]. The isotherm calorimetric method served to differentiate from each other the proliferated milk acid bacteria in the product being derived from probiotic or no probiotic cultures during the development of probiotic (sour cream, butter cream, coarse grain quarg, yaourt) milk products.

It was necessary both in the muscle research and in the dairy one to analyze the obtained DSC curves, to decompose them into more simple curve (believing that these curves could be attached to some structural units of the investigated samples). The selection of the proper function for a deconvolution process depends on the nature of investigated problem. In the spectroscopy it is widely accepted that each unknown spectrum can be considered as a linear combination of several reference spectra [10, 11]. Freire *et al.* [12] analyzing a lot of biological thermal denaturation have realized that Gaussian functions are the best fitting one. Refinement of this deconvolution resulted in a lot of new approaches [13–17]. Gaussian function

seemed to be the most convenient for our purpose too, because e.g. the proliferation of some microbe follows that kind of time function where the first derivative is a classical Gaussian function [18].

The main goal of this work was to develop a user friend deconvolution program that decomposes the DSC scans into Gaussian function such a way that

- the data processing is simple and quick,
- the more important parameters could be chosen by the user,
- the program evaluates and plots graphically the characteristic parameters of the curves.

Experimental

Materials and methods

Preparation of butter

Cream obtained from raw milk in winter period was used in the experiments. The fatty acid composition of its fat corresponded to that detailed in our previous work [7]. Cream was ripened physically and by the so-called winter ripening method, i.e. cold–warm–cold process was used.

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DSC experiments

A Setaram Micro DSC-II type scanning calorimeter was used in calorimetric measurements, exploiting the experience of the Biophysical Department in the field of thermal analysis of biological macromolecules [19, 20]. Mass of the sample changed between 700–900 mg and conventional Hastelloy batch vessels were used in the experiments. The reference material was ethyl alcohol. The measurements were carried out in the temperature range of 0–50°C with the heating and cooling rate of 0.3 K min⁻¹. In all cases the experiments were started after having thermal equilibrium when the fluctuation of heat flow was below 0.5 μW.

Deconvolution

We have started the development of our deconvolution program with the thermal denaturation curves of pure proteins of a muscle actomyosin system [21], where the DSC curves were the heat flow-temperature function. Our working hypothesis was an observation of Freire [12], that on the basis of the shape of heat flow-temperature curves they can be decomposed into the sum of Gaussian functions having the next general form:

$$f(t) = he^{-\frac{\lambda^2(t-s)^2}{2}} \quad (1)$$

where t is the temperature (or in isotherm case the time), s is the temperature (or the time in isotherm case) at the maximum of the function, h is the heat flow at the maximum of the function and λ is the reciprocal of the half value of the function.

Before starting the decomposition into Gaussian curves we have to correct the baseline shift of DSC curves other way the problem cannot be solved. The deconvolution can be performed according to the next steps taking into consideration all the circumstances:

- baseline correction of heat flow curves,
- to fit the individual Gaussian functions and
- correction of the parameters of separate Gaussian functions for best fitting.

Characteristics of the computer program

Hardware: The requirements to perform a successful run of our program are:

- Intel processor (or other compatible with it, at least 486),
- Microsoft Windows 95 or NT 3.5 (or the newer versions),
- 8 Mb memory,
- at least 1 Mb place on hard disc.

File menu: Menu points that could be chosen are as follows: Load – Save – Save as – Save graph – Printer settings – Graph printing – Exit.

Data manipulation menu: User can choose from the next menu: Data – Graphic representation (plot) – exchange X and Y values – To reverse the positive or negative heat flow – Normalisation to zero – Baseline correction.

Variable menu: One can choose among the possibilities: Calculation of maxima – Save of maxima – Load the maxima – Calculation of Gaussian functions – Save of Gaussian functions – Load of Gaussian functions.

Windows menu: The next steps can be chosen: Close – Fit on each other – Fit beside each other – Fit below each other.

Results and discussion

Baseline correction of heat flow curves

The user can choose the starting and final points of the new baseline. Generally two points are wanted in a case of a conventional heat flow curve but in case of well separated curves it is possible to choose more (arbitrary number) starting and end points too. In latter case the baseline correction can be performed for each baseline determined by each starting – end point set. The slope in front of the chosen starting point is identical in each case with the slope of straight line determined by the starting and end points. The procedure of a baseline correction is demonstrated on a DSC scan of thermal denaturation of an actomyosin system (Figs 1a–d). It can be seen quite well on the Fig. 1 that in case of raw DSC scan (a) as the first step we fit a coordinate system (b), after it a new baseline with its own slope is risen between the two chosen points (c) and finally the data will be converted to the new baseline, therefore a new DSC curve of corrected baseline is created (d).

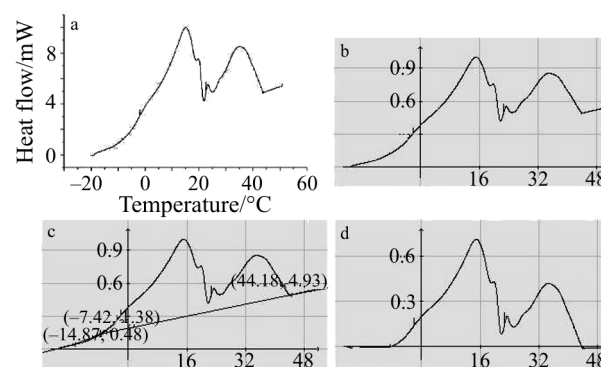


Fig. 1 Baseline correction of DSC scan of thermal denaturation of muscle fibre: a – raw curve, b – normalised for zero, c – fitting a new baseline and d – the conversion of the DSC curve for the new baseline (the vertical axis is the heat flow and the horizontal one is the temperature)

Fitting the individual Gaussian curves

As a first step to perform the fitting, we have to determine the local maxima, that will be the maxima of the individual Gaussian curves. Those t values will be chosen by the program to be local maxima where the first derivative of the heat flow curve is zero and the second one is negative. This means that at this place the magnitude of heat flow at $t-1$ and $t+1$ is smaller than that of t . The user can delete those false maxima that are the consequences of the fluctuations in the measurement. After choosing the proper maxima we can start to fit the Gaussian curves described by Eq. (1). Supposing that the value of heat flow function at t equal to $g(t)$ then according to the least square method we have to calculate the minimum of the next function:

$$\sum_{t=t_1}^{t_2} [f(t) - g(t)]^2 \quad (2)$$

h, λ and s are the unknown variables of functions thus we can find the minima where the first derivatives with respect to this variables are zero, that is

$$\begin{aligned} \frac{d}{dh} \left\{ \sum_{t=t_1}^{t_2} \left[\ln \frac{h}{g(t)} - \lambda^2 \frac{(t-s)^2}{2} \right]^2 \right\} &= 0 \\ \frac{d}{d\lambda} \left\{ \sum_{t=t_1}^{t_2} \left[\ln \frac{h}{g(t)} - \lambda^2 \frac{(t-s)^2}{2} \right]^2 \right\} &= 0 \quad (3) \\ \frac{d}{ds} \left\{ \sum_{t=t_1}^{t_2} \left[\ln \frac{h}{g(t)} - \lambda^2 \frac{(t-s)^2}{2} \right]^2 \right\} &= 0 \end{aligned}$$

and

$$\ln h = \frac{\sum_{t=t_1}^{t_2} \{\ln[g(t)](t-s)\} \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} \{\ln[g(t)](t-s)^2\} \sum_{t=t_1}^{t_2} (t-s)^3}{\sum_{t=t_1}^{t_2} (t-s) \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} (t-s)^2 \sum_{t=t_1}^{t_2} (t-s)^3} \quad (8)$$

$$\begin{aligned} &\left\{ \sum_{t=t_1}^{t_2} [\ln(g(t))] \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} [\ln(g(t))(t-s)^2] \sum_{t=t_1}^{t_2} (t-s)^2 \right\} \left[\sum_{t=t_1}^{t_2} (t-s) \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} (t-s)^2 \sum_{t=t_1}^{t_2} (t-s)^3 \right] = \\ &= \left\{ \sum_{t=t_1}^{t_2} [\ln(g(t))(t-s)] \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} [\ln(g(t))(t-s)^2] \sum_{t=t_1}^{t_2} (t-s)^3 \right\} \left\{ \sum_{t=t_1}^{t_2} (t-s)^4 \sum_{t=t_1}^{t_2} 1 - \left[\sum_{t=t_1}^{t_2} (t-s)^2 \right]^2 \right\} \quad (9) \end{aligned}$$

The separate solution of these equations resulted in:

- the first derivative with respect to parameter h will be

$$\ln h \sum_{t=t_1}^{t_2} 1 = \lambda^2 \sum_{t=t_1}^{t_2} \frac{(t-s)^2}{2} + \sum_{t=t_1}^{t_2} \{\ln[g(t)]\} \quad (4)$$

- the first derivative with respect to parameter λ will be

$$\lambda^2 = 2 \frac{\sum_{t=t_1}^{t_2} \left\{ \ln \left[\frac{h}{g(t)} \right] (t-s)^2 \right\}}{\sum_{t=t_1}^{t_2} (t-s)^4} \quad (5)$$

- and the first derivative with respect to parameter s will be

$$\sum_{t=t_1}^{t_2} \left\{ \ln \left[\frac{h}{g(t)} \right] (t-s) \right\} = \lambda^2 \sum_{t=t_1}^{t_2} \frac{(t-s)^3}{2} \quad (6)$$

When we put together Eqs (4)–(6) then we get Eqs (7) and (8).

The next equality (Eq. (9)) comes from Eqs (7) and (8).

The only unknown is s in Eq. (9), which can be obtained in the following way: we calculate the absolute value of the difference between the two sides of the equation for all value being between t_1 and t_2 . The wanted parameter s of Gaussian function will be the minimum of them.

Substituting this s parameter into Eq. (7) parameter h remains the only unknown which can be achieved by Eq. (10).

Substituting the s and h parameters obtained this way into formula (5) – parameter λ can be calculated as

$$\ln h = \frac{\sum_{t=t_1}^{t_2} \{\ln[g(t)]\} \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} \{\ln[g(t)](t-s)^2\} \sum_{t=t_1}^{t_2} (t-s)^2}{\sum_{t=t_1}^{t_2} (t-s)^4 \sum_{t=t_1}^{t_2} 1 - \left[\sum_{t=t_1}^{t_2} (t-s)^2 \right]^2} \quad (7)$$

$$h = \exp \frac{\sum_{t=t_1}^{t_2} \{\ln[g(t)]\} \sum_{t=t_1}^{t_2} (t-s)^4 - \sum_{t=t_1}^{t_2} \{\ln[g(t)](t-s)^2\} \sum_{t=t_1}^{t_2} (t-s)^2}{\sum_{t=t_1}^{t_2} (t-s)^4 \sum_{t=t_1}^{t_2} 1 - \left[\sum_{t=t_1}^{t_2} (t-s)^2 \right]^2} \quad (10)$$

$$\lambda = \sqrt{\frac{\sum_{t=t_1}^{t_2} \left\{ \ln \left[\frac{h}{g(t)} \right] (t-s)^2 \right\}}{\sum_{t=t_1}^{t_2} (t-s)^4}} \quad (11)$$

Parameter correction of single Gaussian function

A simple estimation of Gaussian functions performed in arbitrary sequence during the deconvolution program would fit to the reality only in that case when they have had no any influence on each other. With respect to the fact that this requirement generally cannot be fulfilled there is need to determine the sequence and to correct the parameters of the estimated Gaussian functions.

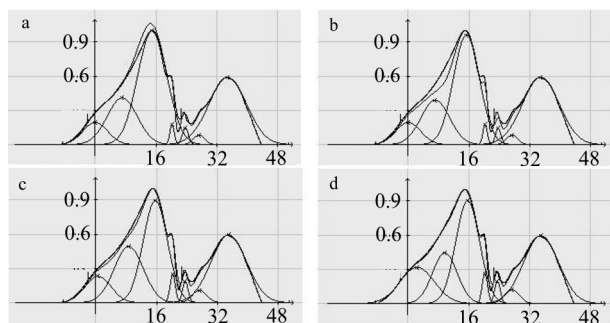


Fig. 2 Generated curves of the deconvoluted a – DSC scan, b – after the first, c – the second and d – the third parameter correction (the axes are the same as in Fig. 1)

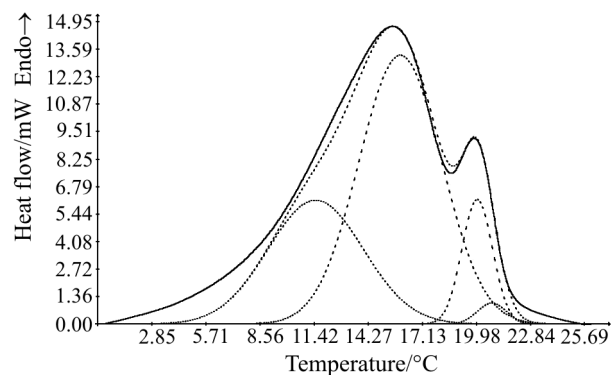


Fig. 3 Deconvolution of butter fat melting in that temperature range which is important from the point of view of spreadability

Table 1 The characteristic data of Gaussian function of Fig. 3 calculated by the program

$T_{\max}/^{\circ}\text{C}$	dH/dt /mW	$T_{1/2}/^{\circ}\text{C}$	Area ratio/%
11.48	6.12	0.4	30.71
15.95	13.29	0.46	57.76
20.01	6.16	1.245	9.93
20.79	1.047	1.3	1.6

T_{\max} and dH/dt are the temperature and heat flow at the maximum of fitting Gaussian function, while $T_{1/2}$ stands for the half width at T_{\max}

We have chosen the sequence of parameter estimation on the basis of h term. Parameters of that Gaussian function were estimated at first where the highest h could be waited, the others were done in descending sequence. The correction of parameters were calculated similarly, that is the rest of Gaussian functions were subtracted from Gaussian function being under correction and it was followed by the repeated calculation of parameters as described in ‘Fitting the individual Gaussian curves’.

The result of parameter correction is demonstrated in Figs 2a–d. The improvement of fitting of Gaussian function can be seen quite on Fig. 2 after one (b), twice (c) and triple (d) parameter correction compared to the deconvolution without parameter correction (a). The application of this deconvolution procedure can be seen in Fig. 3 where it was applied for decomposing of butter melting. In Table 1 are listed the most important thermal parameters of this procedure calculated by the program itself.

Conclusions

This program was tested in the field of muscle research to follow the effect of different nucleotides on the thermal stability of individual protein domains in different intermediate states of ATP hydrolysis cycle.

The heat flow curves were evaluated during the development of the following dairy products by this program:

- melting and crystallisation of milk fat during development of technology of cold spread able butter,
- thermal denaturation of proteins during development of technology of processed cheeses without peptization,

- examination of the growth of probiotic culture combinations during development of technology of probiotic dairy products and to control the isotherm adjacent proliferation of microbes.

This program provides quick and simple deconvolution and graphic representation of heat flow curves.

Acknowledgements

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