

INVESTIGATING ISOTHERMAL DSC METHOD TO DISTINGUISH BETWEEN COCOA BUTTER AND COCOA BUTTER ALTERNATIVES

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Abstract

In the article thermal behaviours of cocoa butter and representatives of the 3 classes of cocoa butter alternative fats were investigated using isothermal DSC-method. Besides traditional parameters, Avrami transformation and polar qualification system (adapted from NIR-technique) were used for data evaluations.

Using a new parameter, t_{\max}^* , the influence of the temperature change could be avoided. This parameter gave 100% success in classification of the investigated confectionery fats ($p < 0.05$).

In comparison with traditional Avrami parameters such as n and lgk measured at different temperatures, a new parameter (t^*) gave the best result in distinguishing confectionery fats (approx. 71% correctly classified). The classification improved using lgk and n together (79%).

Better classification could be achieved using polar qualification system. The percentage of correctly classified cases was 87.5% using either the point or the surface method ($p < 0.05$). In every case there was a clear borderline between cocoa butter-CBE fat and CBR-CBS fats.

Comparing Avrami method and PQS, it can be concluded that the latter is a more successful method in classification of unknown fat samples (pure cocoa butter alternative fats only). However PQS does not give any information about thermal behaviour of the sample.

Keywords: Avrami, cocoa butter, cocoa butter alternatives, DSC, isothermal, PQS

Introduction

Confectionery products consist of proteins, sugar and fats. The nature of the fat phase of confectionery products determines the properties of the final product. For example in chocolate the physical structure of the fat phase is largely responsible for 'snap' heat stability, mouth feel, flavour release and general consumer satisfaction.

One third of the chocolate is fat. The fat phase mainly consists of cocoa butter, which is one of the most expensive ingredients. Cocoa butter is needed to increase fat level in chocolate. Because of the high and unpredictable price of cocoa butter, there

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is a desire to replace cocoa butter with other fat. Today there are a number of fats suitable for total or partial replacement of the cocoa butter component in confectionery product.

The group of cocoa butter alternatives can be divided into 3 main classes: cocoa butter equivalents (CBE), cocoa butter replacers (CBR) and cocoa butter substitutes (CBS). These fats are compatible with cocoa butter in various ratios. The essential difference is between CBR and CBS fats, that contrary to cocoa butter and CBR fats, CBS fats contain lauric acid. Therefore they are less compatible with cocoa butter, then CBR or CBE fats.

The aim was to find a useful tool for the quality assurance to distinguish between cocoa butter and cocoa butter alternatives, using the differences in their thermal behaviour. The secondary aim was to find a good method for evaluating DSC (differential scanning calorimetry) data.

A representative from each class of the cocoa butter alternative fats was analysed using an isothermal differential scanning method. Thermal behaviours of the fats were compared in order to classify them.

Materials and methods

Materials: cocoa butter (CB) from unknown origin, Akomax (CBE fat), Akopol (CBR fat) and Nobletan (CBS fat).

SETARAM microDSC III was used for the isothermal investigations.

Isothermal measurement method: All measurements were started from 25°C. 30 mg±1 mg sample was heated up from 25 to 60°C, where 30 min holding time was applied. During this time all crystals were melted. After melting all fat samples were cooled down to the measuring temperatures (17–22°C, at each integer value). The choice of the correct cooling rate is very important. At higher rates (fast cooling) the lower melting forms of cocoa butter (form I and II) are produced, but using moderate cooling rates the formation of the more stable form IV is forced [5, 6]. The cooling rate used in this case was 1.2°C min⁻¹.

Methods of data evaluation

Traditional

In the traditional way of evaluation of differential scanning calorimetry (DSC) data various parameters are used according to Fig. 1.

Avrami transformation

The general approach for description of isothermal phase transformation kinetics is the Avrami equation originally developed for polymers. In the 1940s, various authors independently developed this kinetic formulation, which is sometimes called the Johnson–Mehl–Avrami–Komogorov or Avrami kinetics equation [1, 4].

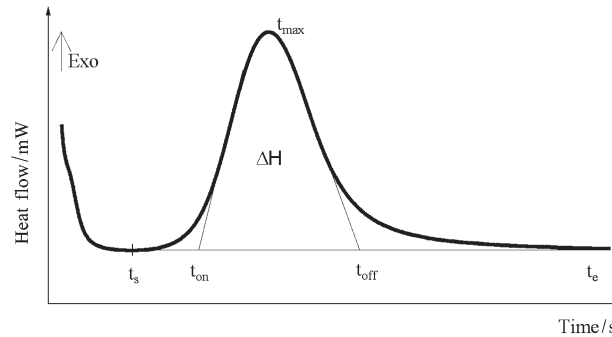


Fig. 1 Isothermal DSC curve. t_s =start of crystallisation, t_{on} =onset time, t_{max} =peak time, t_{off} =offset time, t_e =end of crystallisation, ΔH =enthalpy of the crystallisation

Isothermal Avrami kinetics is concerned with the overall crystallisation process including nucleation and growth. The Avrami equation is given as:

$$1-x=\exp(-kt^n)$$

where x is fraction of crystal transformed at time t during crystallisation, k is crystallisation rate constant which depends primarily on temperature, n is the Avrami exponent relating to the dimensionality of the transformation.

By transforming the Avrami equation an equation of a line is given:

$$\lg(-\ln(1-x))=\lg k+n\lg t$$

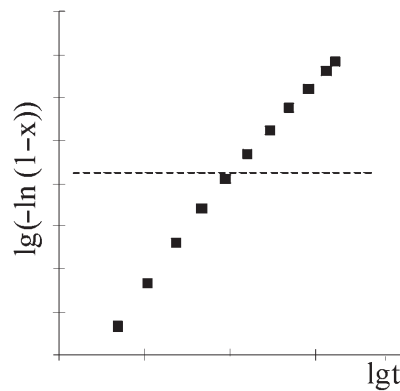


Fig. 2 Transformed isothermal crystallisation curve (Avrami line)

Plotting the left side of the equations vs. $\lg t$ a straight line is given. The slope is n , and from its intercept k can be calculated (Fig. 2).

Polar qualification system, PQS

The polar qualification system – a new data evaluation and qualification system used in NIR techniques – was introduced at the 3rd International Conference on Near Infra-red Spectroscopy [3].

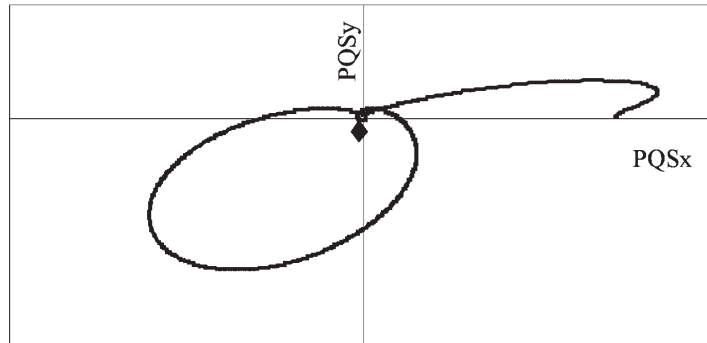


Fig. 3 Heat-flow curve and its gravity centre in the polar qualification system

The method is based on the evaluation of a ‘quality point’ which is given by the centre of its spectrum. In this case the heat-flow curve is represented in a polar co-ordinate system (Fig. 3).

The centre of the spectrum, the quality point, can be defined in 3 different ways.

According to the POINT method the centre is defined as the centre of gravity of unit masses placed in each spectral point. The formulas for calculating the x - y co-ordinates with point method are:

$$x = \frac{1}{k} \sum_{i=0}^{k-1} V_{\lambda_i} \cos i\alpha \qquad y = \frac{1}{k} \sum_{i=0}^{k-1} V_{\lambda_i} \sin i\alpha$$

where V_{λ_i} : spectral value (heat-flow) measured at λ_i wavelength (in this case time)

$$k = (\lambda_{\max} - \lambda_{\min})/s$$

k : number of spectral data

s : difference between 2 time points

$$\alpha = 360/k$$

According to the LINE method the centre is defined as centre of gravity of a wire shaped as the polar heat-flow spectrum. (L is the length of the spectral line.)

$$x = \frac{1}{2L} \sum_{i=0}^{k-1} \sqrt{(V_{\lambda_i} \cos i\alpha - V_{\lambda_{(i+1)}} \cos(i+1)\alpha)^2 + (V_{\lambda_i} \sin i\alpha - V_{\lambda_{(i+1)}} \sin(i+1)\alpha)^2} \cdot (V_{\lambda_i} \cos i\alpha + V_{\lambda_{(i+1)}} \cos(i+1)\alpha)$$

$$y = \frac{1}{2L} \sum_{i=0}^{k-1} \sqrt{(V_{\lambda_i} \cos i\alpha - V_{\lambda_{(i+1)}} \cos(i+1)\alpha)^2 + (V_{\lambda_i} \sin i\alpha - V_{\lambda_{(i+1)}} \sin(i+1)\alpha)^2} \cdot (V_{\lambda_i} \sin i\alpha + V_{\lambda_{(i+1)}} \sin(i+1)\alpha)$$

$$L = \sum_{i=0}^{k-1} \sqrt{(V_{\lambda_i} \cos i\alpha - V_{\lambda_{(i+1)}} \cos(i+1)\alpha)^2 + (V_{\lambda_i} \sin i\alpha - V_{\lambda_{(i+1)}} \sin(i+1)\alpha)^2}$$

At the SURFACE method the centre is defined as the centre of gravity of the surface surrounded by the polar spectrum. (s is the surface surrounded by the spectral curve.)

$$x = \frac{1}{s} \sum_{i=0}^{k-1} \frac{V_{\lambda_i} \cos i\alpha + V_{\lambda_{(i+1)}} \cos(i+1)\alpha}{3} \frac{V_{\lambda_i} V_{\lambda_{(i+1)}} \sin\alpha}{2}$$

$$y = \frac{1}{s} \sum_{i=0}^{k-1} \frac{V_{\lambda_i} \sin i\alpha + V_{\lambda_{(i+1)}} \sin(i+1)\alpha}{3} \frac{V_{\lambda_i} V_{\lambda_{(i+1)}} \sin\alpha}{2}$$

$$s = \frac{1}{2} \sum_{i=0}^{k-1} V_{\lambda_i} V_{\lambda_{(i+1)}} \sin\alpha$$

Results and discussion

Traditional parameters

From the original heat-flow curves it can be seen that at higher temperatures the intensity of the crystallisation decreased. This can be observed from the decreased peak heat-flow values and the flattening of the curve. The onset time of the crystallisation (t_{on}) also increased.

Observations were made according to the class of the fats as follows:

- CBR and CBS fats started to crystallise at lower temperatures (in the range of 17–20°C), before reaching the isothermal condition. They finish crystallisation rapidly; their heat-flow maximums (peaks) were higher than cocoa butters or CBE's.
- CBE fat crystallised in the investigated temperature range only between 17–20°C in such a way, that it is of use for comparison. At higher temperatures the heat-flow change of the crystallisation is at the sensibility limit of the equipment, and the crystallisation is very slow, which can be seen from the almost flat heat-flow curves.
- Cocoa butter crystallises as well between 19–22°C. It gives 'valuable' responses. At lower temperatures crystallisation was observed during cooling, before the isothermal condition was reached.

From investigation of the fat thermograms, it can be concluded that the best temperature for comparison of pure confectionery fats is 20°C (Fig. 4). At lower temperatures CBR and CBS fats crystallised too fast. At higher temperatures than 20°C CBE fat crystallised too slow, and its released heat-flow was too small, close to the resolution limit of the equipment used. Figures 5–7 show the changes in the traditional parameters vs. temperature.

As seen from Fig. 5 at 17°C the peak of the CBS fat is the highest, but above 17°C the peak heat-flow of the CBR fat is significantly higher than for other fats.

In cases of CBR, CBS and CBE fats with increasing temperature the peak heat-flow decreases. Cocoa butter showed a slight increase in peak heat-flow values between 17–18°C, but above 19°C it decreases, too. An explanation for this could be, that at lower temperatures the cocoa butter starts to crystallise already in the cooling phase, that means the actual recorded heat-flow curve is a combination of the crystal-

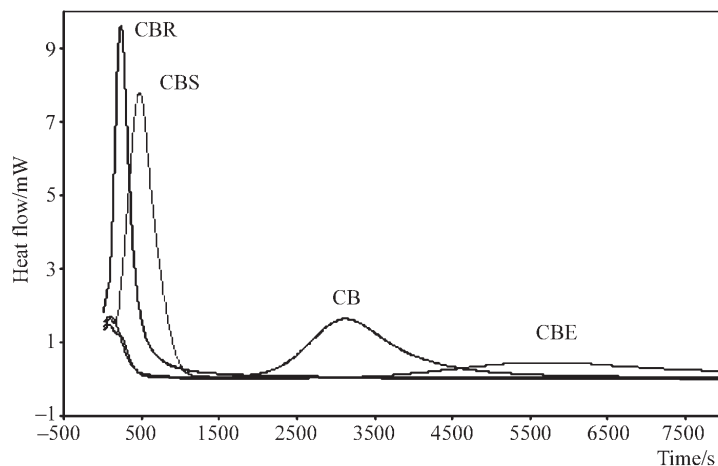


Fig. 4 Isothermal heat-flow curves of cocoa butter and cocoa butter alternatives at 20°C

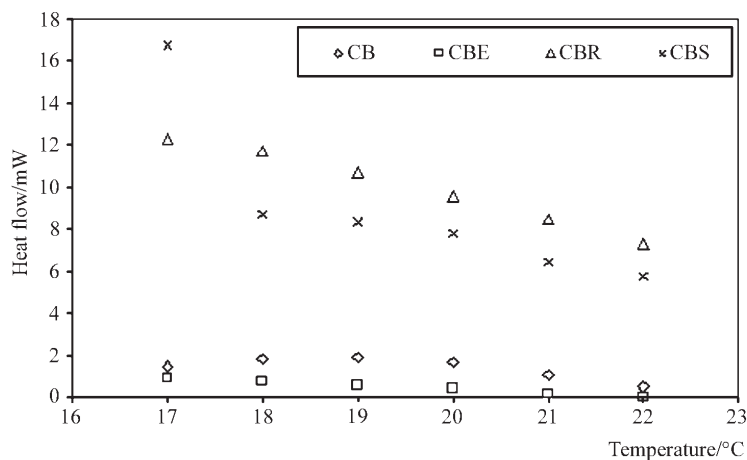


Fig. 5 Heat-flow maximums vs. temperature

lisation during cooling and the isotherm crystallisation process. It is a lower value, because a part of the crystals were crystallised before measurement started.

As seen on Fig. 5 a possible distinction parameter could be the measured heat-flow of the crystallisation under isothermal circumstances between 17–22°C. If it is higher than 4 mW, the unknown fat sample is not pure cocoa butter or CBE fat.

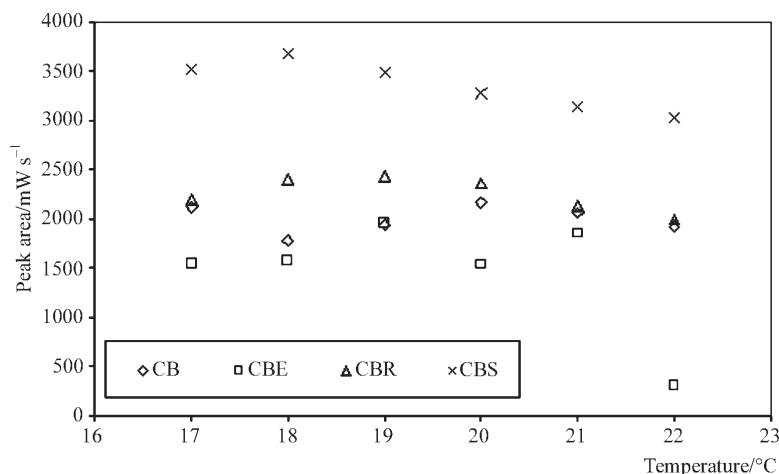


Fig. 6 Peak area vs. temperature

The effect of temperature increase on the area under the peak is not significant (Fig. 6). This parameter alone is therefore not suitable for distinguishing confectionery fats. CBE at 22°C showed a very low value for peak area. The reason for this is, that the crystallisation hasn't finished within 3 days, therefore the calculated area is an estimated value, and it doesn't represent the fully crystallised state.

It is also remarkable, that the peak area is a parameter, which is calculated using the inflection points of the heat-flow curve and the baseline. There are different ways to draw a baseline, and it can influence the calculated value. If we suppose, that all the substance crystallises (the heat-flow curve returns to baseline), the dH value should be more or less the same, but only if we have the same crystal forms. As mentioned, cocoa butter has 6 different crystal forms. Only, if the temperature is above the melting point of the α form (approx. 18°C), can we be sure, that β' form is produced (using the same cooling rate).

For CBR and CBS fats it is a different story. They can crystallise directly in β' form. But, they start to crystallise during cooling, so the recorded heat-flow doesn't represent the total crystallisation.

CBE tends to crystallise at higher temperatures (>20°C) very slow, so the calculated heat flow is an estimation, which may cause problems by comparison.

Figure 7 shows the changes of peak time (t_{max}) vs. temperature. As it can be seen, the peak time increases with the increase in temperature. This is not so remarkable in case of CBR and CBS fats, because they crystallise in the investigated temperature range very fast.

CBE fat at 22°C showed a smaller peak time than expected. The reason for this can be found in the uncertainty of the recognition of the maximum, because the heat-flow curve is flat. Therefore there is not one maximum point, but a time range of maximum points ('plateau').

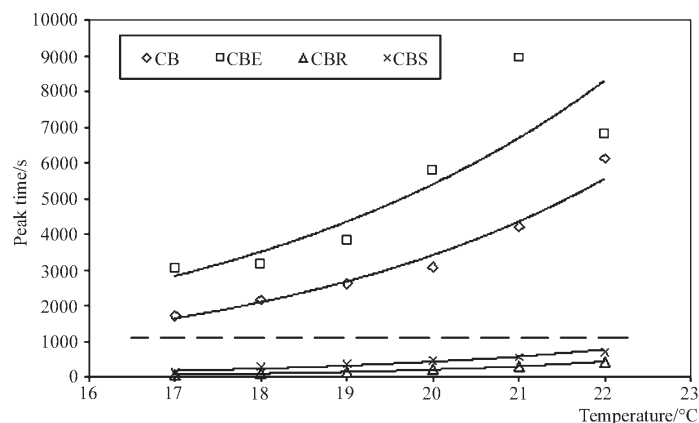


Fig. 7 Peak time (t_{\max}) vs. temperature

In the investigated temperature range it can be concluded, that CBR and CBS fats have their maximum heat-flow (peak) within 20 minutes, cocoa butter and CBE fat reach their maximum after that time.

The relation between peak time (t_{\max}) and temperature can be described by an exponential function. Table 1 shows the parameters of the fitted function. The values of the correlation coefficients are higher than the critical values at 99% significance level ($p < 0.01$) so the model is acceptable [2].

Table 1 Parameters of the exponential curves describing the relationship between temperature and t_{\max}

$y = Ae^{Bx}$	Cocoa butter	CBE	CBR	CBS
A constant	26.723	73.637	0.1874	1.4333
B constant	0.2427	0.2148	0.3531	0.28863
R^2	0.9764	0.8335	0.9804	0.9245
r	0.9881	0.9130	0.9902	0.9615

Using $\ln t_{\max}$ the exponential relationship between t_{\max} and temperature can be transformed into a linear function (Fig. 8, Table 2, $p < 0.05$). This transformation is shown from 17–22°C in Fig. 8. At higher temperatures it is difficult to locate the maximum points by CBE fat.

Table 2 Parameters of the line describing the relationship between temperature and $\ln t_{\max}$

$y = Ax + B$	Cocoa butter	CBE	CBR	CBS
A constant	0.1929	0.2099	0.416	0.3754
B constant	4.1912	4.3683	-2.8136	-1.2533
R^2	0.9936	0.8607	0.982	0.9198
r	0.9968	0.9277	0.9899	0.9591

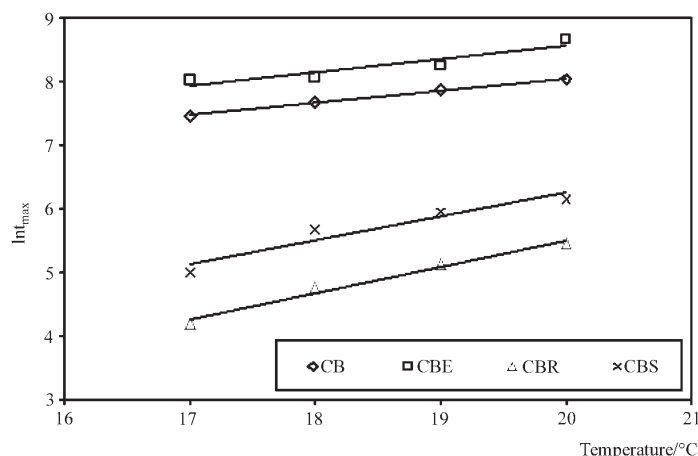


Fig. 8 Changes of the $\ln t_{\max}$ parameter vs. temperature

Using the fact that the correlation between t_{\max} value and temperature can be described by a straight line, every t_{\max} value in the investigated temperature range can be transformed to a value 'measured' at 20°C (t_{\max}^*), which can be further used for statistical evaluations. The algorithm is shown in Fig. 9.

Variance analysis shows that the t_{\max}^* values of the investigated fat samples were significantly different at 95% significance level (Fig. 10, Table 3).

Table 3 Means of t_{\max}^* with 95% confidence intervals 1=CB; 2=CBE; 3=CBR; 4=CBS

Fat	Count	Mean	Stdn. Error (individual)	Lower limit	Upper limit
1	4	3130.38	31.3712	3030.55	3230.22
2	4	5271.1	286.123	4360.52	6181.67
3	4	246.7	9.01498	218.011	275.39
4	4	524.186	37.8461	403.743	644.629
Total	16	2293.09			

From the statistical evaluations can be concluded that t_{\max}^* parameter is suitable for distinguishing the different classes of confectionery fats.

Although t_{\max} parameter depends on the temperature, using the described transformation into t_{\max}^* above, the values can be compared. Table 3 shows the limits of t_{\max}^* values for classification of an unknown fat samples ($p < 0.05$).

For t_{\max} values measured at less than 20°C this transformation can be used without any restrictions. At temperatures over 20°C the correlation between t_{\max} and temperature has to be investigated further.

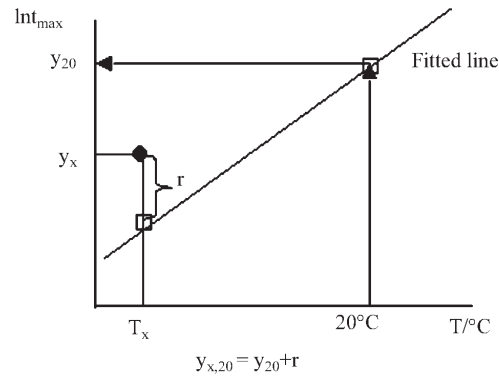


Fig. 9 Algorithm for transformation of t_{\max} measured at any temperature

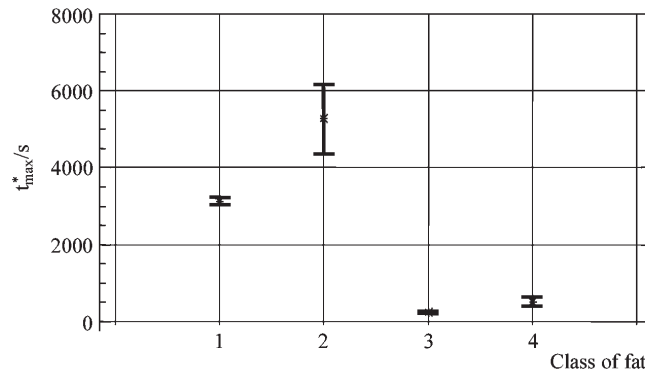


Fig. 10 Means and 95% confidence interval for t_{\max}^* 1=CB; 2=CBE; 3=CBR; 4=CBS

Avrami parameters

For evaluations of isothermal differential scanning calorimetry measurements the Avrami transformation is commonly used. As examples heat-flow curves measured at 20°C transformed into Avrami lines can be seen at Fig. 11. Table 4 shows the parameters of the equations.

Table 4 Avrami lines of pure confectionery fats by 20°C

$y=Ax+B$	CB	CBE	CBR	CBS
n	6.4193667	4.572	2.2823	2.9792
$\lg k$	-22.72493	-17.478	-5.5201	-8.1271
$t^* (y=0)$	3.5401176	3.822835	2.418657	2.727947
R^2	0.9812083	0.9908	0.9941	0.9995
r	0.9905551	0.995389	0.997046	0.99975

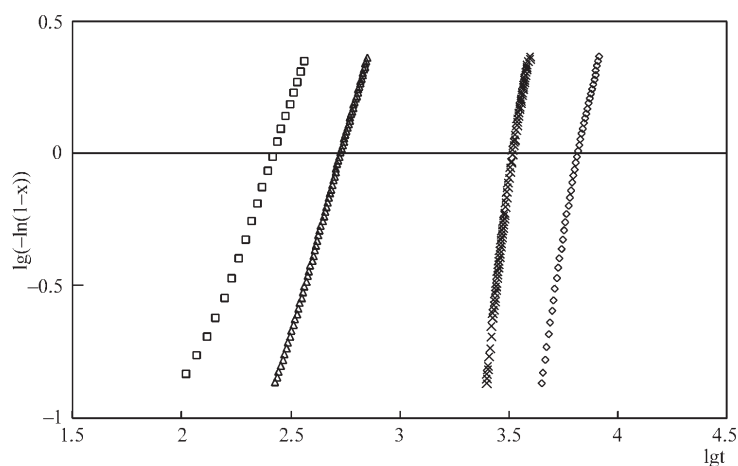


Fig. 11 Avrami lines of cocoa butter and cocoa butter alternatives (20°C)

To describe the crystallisation behaviour another parameter (t^*) was introduced. This parameter is related to the speed of the crystallisation and the crossing point of the Avrami straight line and $\lg t$ abscissa gives it. t^* is evaluated from the time taken to crystallise a proportion of the sample given by $(e-1)/e$ (approx. 63%).

This parameter is more objective than the parameters traditionally used for Avrami transformations, such as t_s or t_c which are determined in a more subjective way, since they are the points where the baseline and the heat-flow curve differ.

Using SPSS for Windows 6.0 statistical software, it was investigated which parameter is the most useful to distinguish between cocoa butter and cocoa butter alternatives.

The results of the statistical investigations can be summarised as follows: t^* allows 70.83% of fats to be correctly classified in respect to all measuring temperatures (Fig. 12, $p < 0.05$). Using $\lg k$ the classification was 58.33% correct, n gave the worst result with 50% correctly classified fat samples.

There was a clear difference between the groups of CB/CBE fat and CBR/CBS fats. This difference was not recognisable using n as classification factor.

Therefore it was statistically proven, that t^* is more useful to classify cocoa butter alternative fats than the traditional Avrami parameters.

Using multifactorial discriminant analysis the effectiveness of distinguishing between confectionery fats can be improved. Using t^* parameter together with any other parameter gave the same result for classification (70.83%), but using t^* , $\lg k$ and n together the classification was 75% successful ($p < 0.05$). The best classification was achieved using n and $\lg k$ (Table 5).

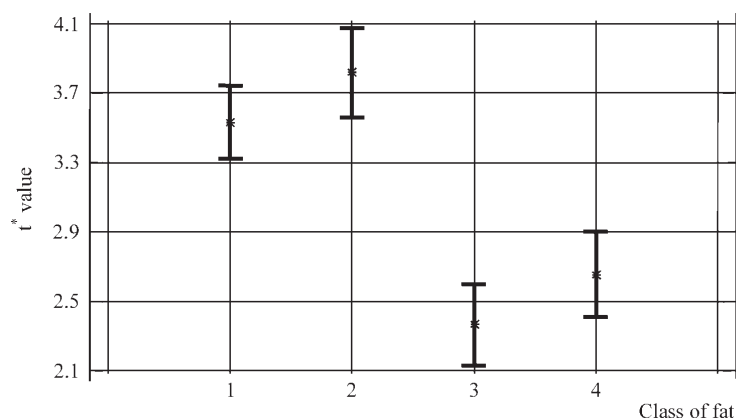


Fig. 12 Means and 95% confidence intervals of t^* parameter. 1=cocoa butter; 2=CBE fat; 3=CBR fat; 4=CBS fat

Table 5 Results of the discriminant analysis for n and $\lg k$

Group 1=cocoa butter; Group 2=CBE fat; Group 3=CBR fat; Group 4=CBS fat

Actual Group	Cases	1	2	3	4
Group 1	6	4	1	0	1
		66.70%	16.70%	0.00%	16.70%
Group 2	6	1	5	0	0
		16.70%	83.30%	0.00%	0.00%
Group 3	6	0	0	5	1
		0.00%	0.00%	83.30%	16.70%
Group 4	6	0	0	1	5
		0.00%	0.00%	16.70%	83.30%

Percent of 'grouped' cases correctly classified: 79.17%

Table 6 Constants of the discriminant function

	Function 1	Function 2
n	4.870077	2.49824
$\lg k$	1.552555	0.505899
(Constant)	0.675118	-3.06042

Using the discriminant function it is possible to classify an unknown fat sample (pure fat only) according to its calculated Avrami parameters.

Quality points in the polar qualification system

The advantage of the polar qualification system is that it marks so-called 'quality points', which represent the individual fat samples on a quality plane. Figure 13

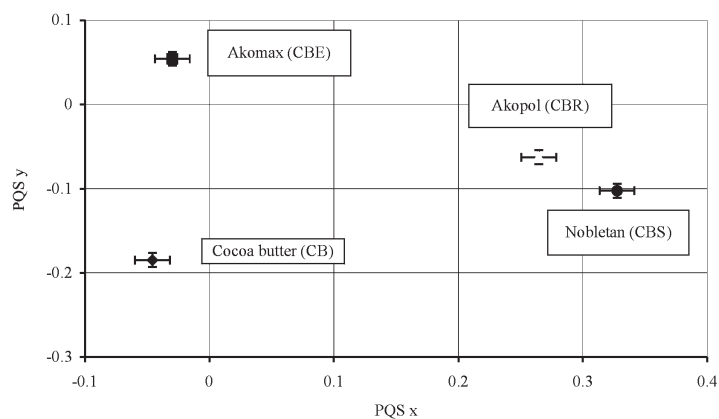


Fig. 13 Quality points of confectionery fats (point method, 20°C)

shows that the quality points of the investigated fat samples can be clearly distinguished.

The co-ordinates of the quality points are easy to calculate (Table 7).

Table 7 x - y co-ordinates of pure fats by 20°C, calculated using point method ($p=0.05$; $\sigma_x=0.014$; $\sigma_y=0.0083$)

Type of fat	x co-ordinate	y co-ordinate
Cocoa butter	$-0.0459 \pm \sigma_x$	$-0.18491 \pm \sigma_y$
CBE fat	$-0.02989 \pm \sigma_x$	$0.054214 \pm \sigma_y$
CBR fat	$0.264604 \pm \sigma_x$	$-0.06261 \pm \sigma_y$
CBS fat	$0.32751 \pm \sigma_x$	$-0.10254 \pm \sigma_y$

Table 8 Discriminant analysis on quality points of pure fats calculated by point method
1=cocoa butter (CB), 2=CBE, 3=CBR, 4=CBS

Actual Group	Cases	1	2	3	4
Group 1	6	5	1	0	0
		83.30%	16.70%	0.00%	0.00%
Group 2	6	2	4	0	0
		33.30%	66.70%	0.00%	0.00%
Group 3	6	0	0	6	0
		0.00%	0.00%	100.00%	0.00%
Group 4	6	0	0	0	6
		0.00%	0.00%	0.00%	100.00%

Percent of 'grouped' cases correctly classified: 87.50%

Discriminant analysis on x - y co-ordinates was used to check the effectiveness of the classification (Table 8).

Comparing the 3 methods of the PQS data evaluation for the investigated temperature range, it was found that CBS fat is always distinguishable from other fats. Choosing surface method CBE fat could be separated from other fats. With point method only CBR fat could be distinguished from the other fats.

The percentage of grouped cases correctly classified was the same using either the point or the surface method (87.5%), using the line method it was only 83.33% ($p < 0.05$).

Conclusions

In the article thermal behaviours of cocoa butter and representatives of the 3 classes of cocoa butter alternative fats were investigated using isothermal DSC-method. Besides traditional parameters, Avrami transformation and polar qualification system (adapted from NIR-technique) were used for data evaluations.

At the evaluation of heat-flow curves, it must be noted that the parameters are dependent on the measuring temperature. The correlation between temperature and a parameter can not be easily described in every case (see: peak area). Using a new parameter, t_{\max}^* , the influence of the temperature change could be avoided. This parameter gave 100% success in classification of the investigated confectionery fats ($p < 0.05$).

Using Avrami transformations the influence of the temperature is still disturbing. In comparison with traditional Avrami parameters such as n and $\lg k$ measured at different temperatures, a new parameter (t^*) gave the best result in distinguishing confectionery fats (approx. 71% correctly classified). t^* is the crossing point of the Avrami straight line and the $\lg t$ abscissa. It is evaluated from the time taken to crystallise a proportion of the sample given by $(e-1)/e$ (approx. 63%). The classification improved using $\lg k$ and n together (79%).

Better classification could be achieved using polar qualification system. The percentage of correctly classified cases was 87.5% using either the point or the surface method ($p < 0.05$). In every case there was a clear borderline between cocoa butter-CBE fat and CBR-CBS fats.

Comparing Avrami method and PQS, it can be concluded that the latter is a more successful method in classification of unknown fat samples (pure cocoa butter alternative fats only). However PQS does not give any information about thermal behaviour of the sample.

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References

- 1 M. Avrami, *J. Chem. Phys.*, 8 (1940) 212.
- 2 Bányainé S. J., Perczelné Z. M., *Tartósított termékek statisztikai minőségellenőrzése*, Mezőgazdasági Kiadó, Budapest 1983.
- 3 K. Kaffka and L. S. Gyarmati, Qualitative (Comparative) analysis by near infrared spectroscopy. In: *Proceedings of the Third International Conference on Near Infrared Spectroscopy*, Eds R. Biston and N. Bartiaux-Thill, Agricultural Research Centre Publishing, Gembloux (Belgium) 1991, p. 135.
- 4 E. Woldt, *J. Phys. Chem. Solids*, 53 (1992) 521.
- 5 G. Ziegleder, *Fat Sci. Technology*, 92 (1990) 481.
- 6 M. Kellens and H. Raynaers, *Fat Sci. Technology*, 94 (1992) 94.