*Journal of Thermal Analysis and Calorimetry, Vol. 82 (2005) 249–252*

# DETECTION OF PROBIOTIC MICROBES BY DSC (ISOTHERMAL) AND THAT OF THE STRUCTURE BY ELECTRON MICROSCOPE

*B. Schäffer*[*]*, B. Szily and S. Szakály*

Hungarian Dairy Research Institute, H-7623 Pécs, Tüzér u. 15, Hungary

Spreads with a fat content of 30-50% (these are called 'butter creams' in Hungarian and currently outsell butter) are generally made by post-heat treatment, and consequently contain no probiotic lactic acid bacteria. The Hungarian Dairy Research Institute (HDRI) has developed a spread that contains live lactic acid bacteria on the order of $10^7$/g, some part of that are probiotic. The major advantage of the probiotic spread compared to the post-heat treated one is that it spreads easily and without sticking both at cold (0–5°C) and room temperature (20-24°C). This good spread ability arises from its structure, while its probiotic quality derives from the proliferation of probiotic microbes. During recent experiments the extent of probiotic microbe proliferation was examined by isotherm calorimetry, the microstructure was studied by electron microscope, and nutritional biological characteristics were measured (according to the ethical codex) by human clinical studies.

By deconvolution of the heat flow curves it was determined that the probiotic cell count is in the order of $10^7$/g, far in excess of the internationally accepted cutoff, $10^6$/g. Structural research revealed that the fat globules in probiotic spread are distributed homogeneously, with an average diameter of less than 0.5 μm. The findings from the human clinical examinations showed that the reduction in the blood cholesterol level occurring after consumption did not differ in the total cholesterol. The probiotic spread decreased significantly greater reduction in the supposedly harmful LDL cholesterol in the third hour following consumption, with less of a reduction in the HDL cholesterol level.

***Keywords:*** *cholesterol profile of the blood, electron microscope, isotherm calorimetry, probiotic spread*

## Introduction

Consumption of high (at least 80%) fat content fats (butter, margarine) used for spreading on bread has been mostly replaced by the so-called spreads in last decades. Their common characteristics are the good cold spread ability (at refrigerator temperature) and most of them are made from different vegetable or animal fats or mixture of them [1–8]. In Hungary spreads of 36–40% fat content made exclusively from milk fat and are called butter creams. Butter creams distributed almost 20 years are produced by post-heat treatment technology (therefore they do not contain live microbes) and colloid chemically mainly they have an oil-in-water emulsion structure. The latter guarantees their nutritional benefit: the absorption of fat from them is mostly corpuscular, therefore they can be also consumed by patients suffering from chronic pancreatitis [9].

As the reception of probiotic products is very positive both from nutritional and marketing points of view the Hungarian Dairy Research Institute (HDRI) has developed the probiotic butter creams and such a production technology for their producing in which post-heat treatment is not applied and the products are fermented in the final packaging [10]. The probiotic property of the product is provided by Prebiolact-2 culture used besides the butter culture for the purpose of fermentation [10, 11].

Our experiments aimed at detection of microbes of probiotic butter creams originating from the mixture of butter culture and probiotic culture and estimation of their ratio by an isotherm calorimetric method. We also wanted to examine the emulsion structure of the product and to determine their several human physiological effects by clinical examinations.

## Materials and methods

### *Dairy materials*

Probiotic butter creams were made by the procedure developed by HDRI [5, 6]. Fermentation was carried out by adding 1% butter culture and 1% Prebiolact-2 culture. Butter creams were fermented until pH=4.7 was reached, and then they were cooled down to 4°C and cold ripened at this temperature for 24 h.

### *Isothermal calorimetric measurements*

The thermal experiments were performed in the Biophysical Department that has a rich experience in

* Author for correspondence: mtkipecs@axelero.hu

*1388–6150/$20.00*

*Akadémiai Kiadó, Budapest, Hungary*
*Springer, Dordrecht, The Netherlands*

wide field of application of this method [12–21]. To carry out calorimetric examinations so called butter cream ferment was prepared in the following way: 100 g fat-free sterile milk was measured into the measuring sacket of Stomacher device to which 2 g probiotic butter cream was added and the mixture was made homogeneous in Stomacher device (2 min). The homogeneous mixture was fermented at the production temperature of probiotic butter creams (30°C) until pH=4.7 was reached (approx. 8 h), cooled down to 4°C, and cold ripened for 24 h. To perform the direct isotherm measurements approx. 450 mg fat-free sterile milk and 50 mg butter cream ferment were measured into a mixing batch vessel, and the same amount of distilled water into the reference one. This was left at 30°C until thermal equilibrium was reached, and then the butter cream ferment was injected into sterile milk together with reference water. The heat flow curve of microbe proliferation was recorded for 18 h on a SETARAM Micro DSC-II calorimeter at 30°C under isotherm conditions.

In order to analyse the isotherm heat flow curves the deconvolution program developed by us [22] was applied and heat flow values referring to one microbe (or Cfu) measured for butter culture and Prebiolact-2 culture respectively were calculated.

*Electron microscopic investigation*

In order to examine the emulsion structure approx. 1 $mm^3$ piece of the samples was fixed in glutaraldehyde, then in osmiumtetroxide, dehydrated in alcohol series and imbedded in epoxy resin. Electron microscopic photos were taken of thin cross-sections by the help of a JEOL 1200 EX type electron microscope in transmission mode.

*Clinical test*

In so-called 'empty stomach loading' examinations were carried out with probiotic butter cream and control product every second week where the same persons were involved. The process of the loading examination was the following. In separate cases the participants ate on an empty stomach 50–50 g fat-equivalent amount of the test-products with 2 pcs of rolls max. in 15 min and drank after a glass (2 dl) of water. Then during 360 min in every $30^{th}$, $60^{th}$ and $120^{th}$ min blood samples were taken and examined for blood lipid profile, namely the control of total cholesterol-, - HDL-, LDL-cholesterol-, and triglyceride-(fat-)level [9].

## Results

Figure 1 shows the isotherm heat flow curve of the butter cream ferment. It is obvious that the curve could be decomposed into three Gaussian ones, the maximum of the first one is after the $5^{th}$ h, the second appears in $6^{th}$ h and it is followed by the third in $8^{th}$ h. The second Gaussian curve is characteristic for the proliferation of Prebiolact-2 and the third one refers for butter culture.

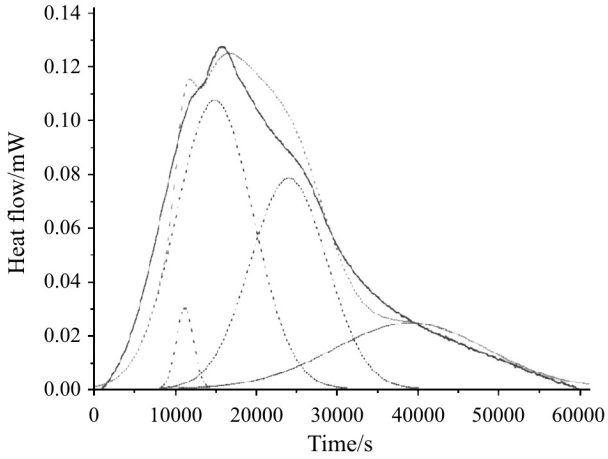


**Fig. 1** Decomposed heat flow curve of butter cream ferment

The values determined by the deconvolution program [22] are demonstrated in Table 1. Data characterizing Prebiolact-2 and butter culture are especially emphasized, because the ratio of two types of microbes in butter cream ferment can be calculated according to the proliferation heat produced by them.

Figure 2 shows the electron microscopic photo of the structure of probiotic butter cream.

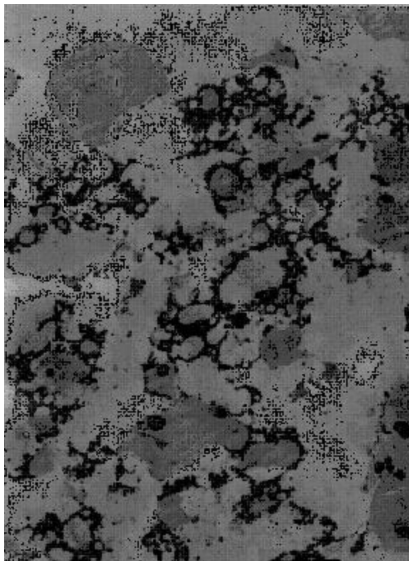

**Fig. 2** Electron microscopic structure of probiotic butter cream

**Table 1** Data characteristic for and calculated from the isotherm heat flow curve of butter cream ferment ($t$= time at the peak of heat flow, $H$=calorimetric enthalpy, $H°$=$H$ normalized for the mass ($m$=0.558 g), $C$=microbe count)

| Parameters of Gaussian curve characeristic in mixed culture | $t$/s | $H$/mJ | $H°$/mJ g$^{-1}$ | $H°$/C·10$^{-8}$ | C·10$^{8}$/Cfu | Percentile ratio of microbes of cultures |
|---|---|---|---|---|---|---|
| Prebiolact-2 culture | 24150 | 1357 | 2432 | 491 | 4.95 | 81 |
| Butter culture | 30013 | 766 | 1373 | 1212 | 1.13 | 19 |

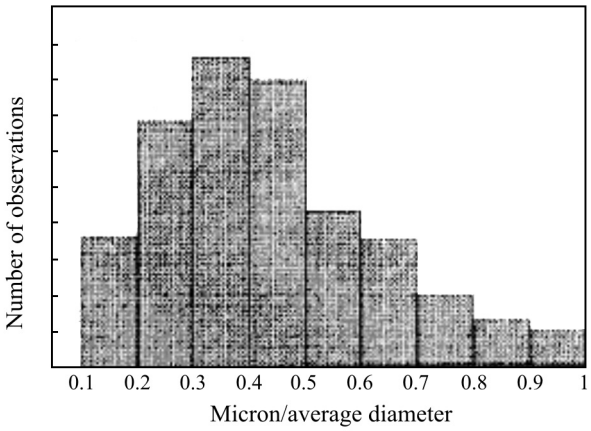


**Fig. 3** The size of fat globules of probiotic butter cream

It is undoubtedly shown that the colloid chemical structure is oil-in-water emulsion. The size of fat globules can be well measured; their distribution according to the size is demonstrated in Fig. 3.

In Fig. 4 the change of the cholesterol-profile of the blood is shown after consumption of 50–50 g fat of probiotic butter cream and margarine.

Figure 5 shows the relative digest ability of fat from probiotic butter cream and other six foods of different emulsion-structure in 3$^{rd}$ h after consumption.

## Discussion and conclusions

It is proved by our results that if the experiments are well prepared, then the existence of probiotic lactic acid bacteria culture (Prebiolact-2) besides the other lactic acid bacteria culture (butter culture) could be detected in probiotic butter cream by isotherm calorimetric method. The condition of demonstration is to make a so-called butter cream ferment as a first step, i.e. the live lactic acid bacteria micro flora being in the product should be proliferated in fat-free milk. This butter cream ferment contains the microbes of probiotic butter cream and due to its low viscosity it is suitable to be injected into the fat-free sterile milk of low viscosity in a mixing batch vessel as a lactic acid bacteria culture.

Taking into consideration that the proliferation of microbes from the product was studied during twice inoculation, the 35:65% ratio of butter culture and Prebiolact-2 culture can be only supposed, determination of exact ratios requires further research. However, it can be surely stated that the probiotic microbes are present in probiotic butter creams at least in the order of $10^6$, as their total plate count reaches the order of 4–9·$10^8$.

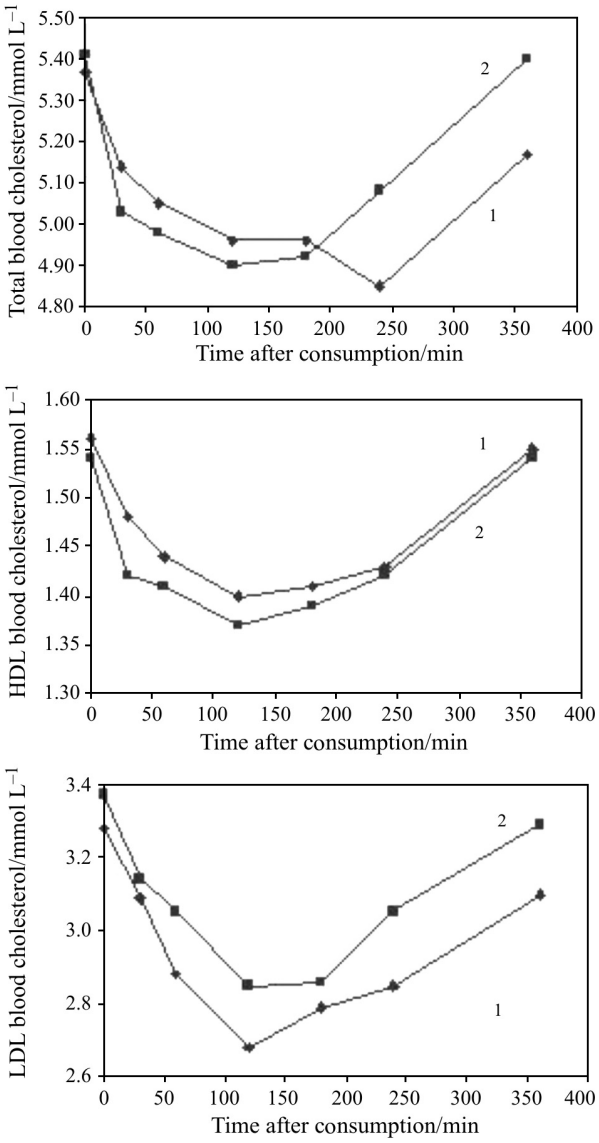


**Fig. 4** Change of blood cholesterol profile in man after consumption of 50 g fat of probiotic 1 – butter cream and 2 – margarine each

It is proved by electron microscopic examinations that the colloid chemical structure of butter creams is oil-in-water emulsion, in which the average

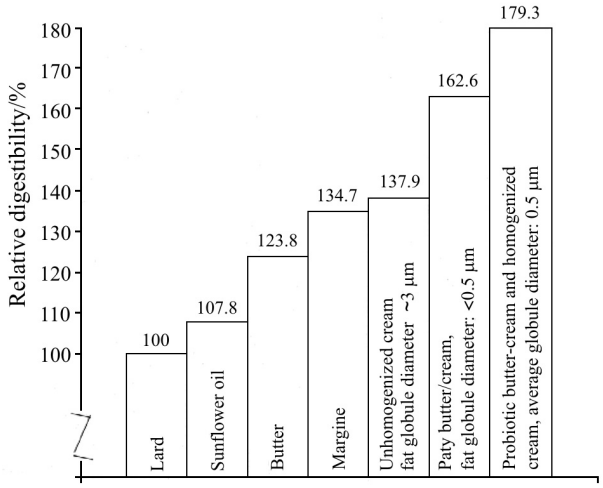


**Fig. 5** Relative digestibility (absorption rate) of fat of probiotic butter cream and other six foods in 3rd h after consumption in man (lard=100%)

diameter of fat globules is about 0.5 μm and most of them are 0.3–0.4 μm in size.

It was also determined that there is no significant difference between the effect of fats of probiotic butter cream and margarine exerted on the cholesterol profile of the blood, however, the probiotic butter cream is a little bit better from this point of view. This statement seems to be an important scientific finding because in the last 4–5 decades just the milk fat was blamed for the increase of cholesterol level of blood [9]. It can be also concluded that 'oil-in-water' emulsion type foods, at the top with the probiotic butter cream followed by cream homogenized in the same way, then with Party butter cream and non-homogenized cream are among the excellently digestable foods, compared to the 'water-in-oil' emulsion type products (margarine, butter, vegetable oil, lard) which can be worse digested. Adding to these facts that 100% of fat from probiotic butter creams and 70% of fat from Party butter cream are absorbed together with fat-soluble vitamins directly without enzymatic decomposition, taking care of liver, pancreas and the total digestive system, these products, from the point of view of absorption as well, can be justifiably called functional foods [1–3].

## Acknowledgements

This work was supported by FVM 19-d/2002 (Ministry of Agriculture and County Development) grant. The SETARAM Micro DSC-II was purchased from OTKA CO-272 grant.

## References

1 Z. Szakály and Gy. Széles, Proceedings of XXII CIGR World Congress and AgEng'94 Conference on Agricultural Engineering, Milano, 1994, pp. 1595–1600.
2 Z. Szakály, Gy. Széles, B. Szily and S. Szakály, Proceedings of XXVI International Congress in Work Science, Lillehammer, 1995, pp. 29–31.
3 B. Schäffer, S. Szakály, J. Belágyi and D. Lőrinczy, Hungarian Dairy J., 55 (1995) 22.
4 Z. Szakály, Gy. Széles, B. Keller and S. Szakály, Proceedings of International Conference on Agricultural Engineering, Madrid, 1996, pp. 1091–92.
5 B. Schäffer, D. Lőrinczy and S. Szakály, J. Thermal Anal., 47 (1996) 515.
6 B. Schäffer, S. Szakály, D. Lőrinczy and J. Belágyi, Milchwissenschaft, 54 (1999) 82.
7 B. Schäffer, S. Szakály, D. Lőrinczy and J. Belágyi, Milchwissenschaft, 55 (2000) 132.
8 Béla Schäffer, S. Szakály, D. Lőrinczy and Balázs Schäffer, J. Therm. Anal. Cal., 64 (2001) 659.
9 Gy. Mózsik, IDF Bulletin-230, Brussels, 1988, pp. 11–16.
10 B. Schäffer, S. Szakály, B. Szily and M. Zsinkó, European Patent Office, EP01940862.4 (2000).
11 S. Szakály, IDF Bulletin-230, Brussels, 1988, pp. 5–10.
12 D. Lőrinczy and J. Belágyi, Biochem. Biophys. Res. Com., 217 (1995) 592.
13 D. Lőrinczy and J. Belágyi, Thermochim. Acta, 259 (1995) 153.
14 D. Lőrinczy and J. Belágyi, Thermochim. Acta, 296 (1997) 161.
15 D. Lőrinczy, F. Könczöl, B. Gaszner and J. Belágyi, Thermochim. Acta, 322 (1998) 95.
16 D. Lőrinczy, J. Belágyi, Eur. J. Biochem., 268 (2001) 5970.
17 I. Domán, Gy. Tóth, T. Illés and D. Lőrinczy, Thermochim. Acta, 376 (2001) 117.
18 D. Lőrinczy, N. Hartvig and J. Belágyi, J. Biochem. Biophys.Method, 53 (2002) 75.
19 I. Gazsó, J. Kránicz, Á. Bellyei and D. Lőrinczy, Thermochim. Acta, 402 (2003) 117.
20 I. Domán, T. Illés and D. Lőrinczy, Thermochim. Acta, 405 (2003) 293.
21 Z. Szántó, L. Benkő, B. Gasz, G. Jancsó, E. Rőth and D. Lőrinczy, Thermochim. Acta, 417 (2004) 171.
22 Balázs Schäffer, Béla Schäffer and D. Lőrinczy, J. Therm. Anal. Cal., 2005 (accepted for publ.).

DOI: 10.1007/s10973-005-6898-y