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ISOPERIBOL CALORIMETRY AS A TOOL TO EVALUATE THE IMPACT OF THE RATIO OF EXOPOLYSACCHARIDE-PRODUCING MICROBES ON THE PROPERTIES OF SOUR CREAM

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The quality of sour cream production from homogenised cream in the 1970's was highly improved. The heat resistance of product remained badly, that is, it precipitated in hot food. The Hungarian Dairy Research Institute (HDRI) has elaborated a technology that eliminates this disadvantageous characteristic: it is the use of exopolysaccharide (EPS)-producing lactic acid bacteria. This bacterium produces no aroma, and the proliferation optima of EPS-producing and aroma-producing lactic acid bacteria cultures do not coincide. Detection of these two bacteria was done until now by gene technology, that is expensive and long lasting one. We have applied (at first as we know) isotherm calorimetric method to follow the simultaneous proliferation of these bacteria and it was determined that: both lactic acid bacteria cultures proliferate well at the non-optimal temperature of 30°C and the thermophilic EPS-producing culture was faster than that of the mesophilic aroma-producer. The two cultures do not inhibit each other in mixed culture, and the ratio in mixed culture was 79% EPS-producer and 21% aroma-producer.

Keywords: *electron microscopy, exopolysaccharides, isoperibol calorimetry, sour cream*

Introduction

Sour cream (together with the coarse grain quarg) is an ancient Hungarian dairy product originating from the Western part of Hungary that is approximately the same age as the appearance of the Hungarian people in the Carpathian basin. A large amount of sour cream is consumed even nowadays first of all in the Carpathian basin. Production of sour cream on an industrial scale began in the 1870's and until 1970 almost exclusively the so-called draining method of sour cream production was applied, then within a short time the industry turned to the technology of homogenisation [1, 2]. The technology by homogenisation is more economic compared to the draining technology, and in addition results in a product with high viscosity and consistency firmness without syneresis, having a long shelf life [6]. The only disadvantage of the homogenisation is that sour cream made by this technology precipitates in hot foods and this phenomenon only to a small extent can be decreased by changing the technological parameters of the process [3].

The Hungarian Dairy Research Institute (HDRI) has elaborated a technology that eliminates this disadvantageous characteristic [4]. One element of the technology is the use of exopolysaccharide (EPS)-producing lactic acid bacteria. The EPS-producing cultures provide the probiotic effect and their proliferation optimum is near 37°C. However, as this lactic acid bacterium produces no aroma, aroma-producing lactic acid bacteria must also be used during production in order to ensure the characteristic flavour of sour cream. This could be a butter culture (which produces first of all diacetylactis) with a proliferation optimum around 22–24°C.

Since the proliferation optima of EPS-producing and aroma-producing cultures do not coincide, there is a question of a shared temperature at which microbes of both cultures can fulfil their functions (EPS- and aroma-production). The technological experiments showed that both the heat-resistance and the original flavour of the product could be provided by fermentation at 30°C [4]. To the question of the ratio of proliferation of microbes of two different cultures at this temperature the answer was sought by isotherm calorimetric examinations because until now the gene technology was used for it, but this is expensive and time consuming. The other reason of our choosing is that calorimetry is widely used in food physics and it was shown when reactions and/or transitions take place within a food system, their kinetic parameterisation can be approached by careful analysis of isothermal heat flow curves [5]. It is a useful tool to monitor the shelf life of foods [6–9] too. In an early paper microcalorimetry was used to follow the growth of bacteria in milk [10], for the evaluation of bacteriological quality of seafood [11], to look for the thermal con-

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sequences of irradiation of bacteria [12] and the microbial degradation [13, 14]. Our calorimetric experiments were performed in the Biophysical Department exploiting their experience in the field of thermal analysis of biological macromolecules [15–17].

Experimental

Materials and methods

Chemicals

Glutaraldehyde, osmiumtetroxide and ethylalcohol were purchased from Reanal (Hungary).

Dairy materials

Among the butter cultures the mesophilic butter culture, marked CHN-22 of firm Hansen, was chosen organoleptically as a good aroma-producing one. Raw milk was supported by MIZO Pécs Dairy Factory, Hungary.

Viscosity measurements

EPS-production was measured by viscosity values, based on which Prebiolact-2 culture isolated by HDRI was selected. The measurements were performed at room temperature with 572 rpm and 9.5 Hz using a rotation type Bohlin Visco 88 BV (Sweden) viscometer.

Electron-microscopic measurements

To prove the EPS-production, raw milk fermented by Prebiolact-2 culture (1 mm^3) was fixed in glutaraldehyde then in osmiumtetroxide, dehydrated in alcohol series, imbedded in epoxy resin, then electron microscopic photos were taken by a JEOL 1200 EX type electron microscope in transmission mode at magnification of 40.000. For the sake of comparison we have investigated milk fermented with aroma producing butter culture.

Isotherm calorimetric experiments

To detect the microbe proliferation in mixed culture, cream of 20% fat content was used, which was heat-treated, homogenised by the method described in the HDRI-technology [8] and sour cream was made from it. Fermentation of sour cream production was separately performed with butter culture, Prebiolact-2 culture and with their 50–50% mixture. To perform isotherm calorimetric measurements sour cream ferment was produced previously in the following way: 100 g fat free sterile milk was put into the measuring bag of Stomacker equipment and after adding 2 g sour cream to it the mixture was homogenized (for ~2 min). This homogenized mixture was fermented on 30°C until reaching pH=4.7 (for ~8 h) and cooling to 4°C, and at this temperature it was cold ripened for 24 h. 450 mg fat free sterile milk and 50 mg sour cream ferment from the proper sort was measured into the separated chambers of a mixing batch vessel (Setaram) for calorimetric measurements. The same quantity of distilled water was used as a reference.

The sample holders were put into the Setaram Micro DSC-II calorimeter operating in isotherm mode. They were left there until thermal equilibrium was reached, then the sour cream ferment was inoculated into the sterile milk. The heat flow curve of microbe proliferation was recorded at 30°C for 16 h under isotherm conditions.

Data handling of heat flow curves

The separate proliferation of probiotic and aromatic bacteria was demonstrated with the aid of deconvolution of heat flow curves [18]. This program performed all the graphic presentation and thermal data calculation.

Microbiological test

Parallel to the isotherm calorimetric investigation sour cream ferment was produced by milk fermentation reaching pH=4.7 using 1000 fold quantities of calorimetric protocol. It was cooling to 4°C, stored for 24 h and the total plate count was determined by an international standard method.

Results and discussion

Electron microscopic picture of butter culture that gives the aromatic taste of sour cream can be seen in Fig. 1. It is quite well shown that the microbes have a shape of coccus. Figure 2 demonstrates the electron microscopic photo of a microbe of Prebiolact-2 culture providing the high viscosity of sour cream. An EPS cloud – which cannot be seen in Fig. 1 – might be observed in this picture surrounding the bacteria of coccus shape similar to the microbes of butter culture. The isotherm heat flow-time curve of a ferment made from sour cream containing 20% fat fermented with butter culture is presented in Fig. 3. The graph could be decomposed into two Gaussian functions with maxima at 2 and 6.5 h. Figure 4 shows the heat flow-time scan of a ferment produced from sour cream of 20% fat content fermented with Prebiolact-2 culture. We could decompose it into three Gaussian curves with maxima at 3, 4.5 and 8.5 h. Comparing Figs 3 and 4 we can conclude that Gaussian peak at 8.5 characterises the butter culture while the peak

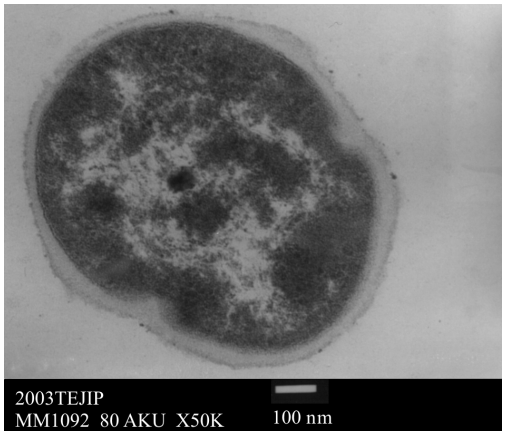

Fig. 1 Electron-microscopic picture of butter culture responsible for the aromatic taste of sour cream

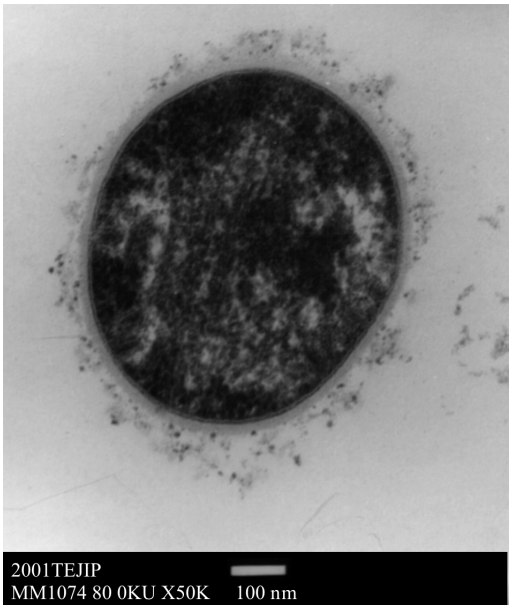

Fig. 2 Electron-microscopic photo of a microbe of Prebiolact culture providing the high viscosity of sour cream

at 4.5 h is a fingerprint for the Prebiolact-2 culture. Table 1 contains data of total plate counts and Gaussian curves of Figs 3 and 4 for sour creams produced with butter and Prebiolact-2 cultures. It was calculated the liberated heat during creation of a single microbe from the measured data which can be seen also in Table 1. Figure 5 shows the isotherm heat flow-time curve of a ferment made from sour cream of 20 % fat content fermented with a 50–50% mixture of butter and Prebiolact-2 cultures. It can be seen quite well that during decomposition four Gaussian curves can be obtained with a maxima at 3.5, 4.4, 7.2 and 11.3 h. The second peak is related to the proliferation of Prebiolact-2 while the third one to the proliferation of butter culture.

The values obtained by the deconvolution are given in Table 2. We especially pointed out the values

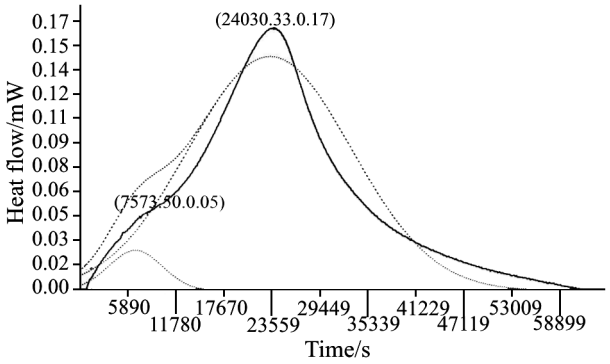

Fig. 3 Isotherm heat flow–time curve of a ferment prepared from sour cream of 20% fat content fermented with butter culture

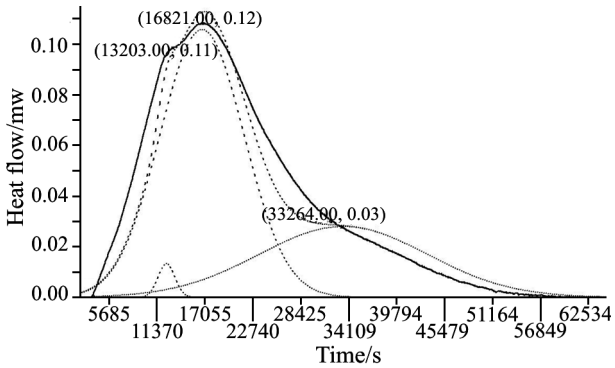

Fig. 4 Isotherm heat flow–time curve of a ferment prepared from sour cream of 20% fat content fermented with Prebiolact-2 culture

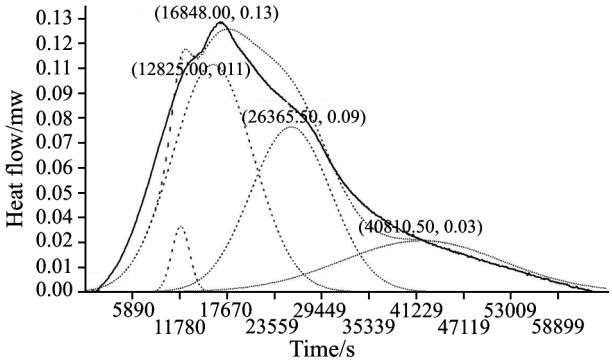

Fig. 5 Isotherm heat flow–time curve of a ferment prepared from sour cream of 20% fat content fermented with 50–50% mixture of butter and Prebiolact-2 cultures

characteristic for Prebiolact-2 and butter cultures based on which the ratio of the two-type microbes being in sour cream ferment can be calculated according to the proliferation heat produced by them.

It is proved by the experiments that during the production of so-called heat-resistant sour cream (precipitation in hot food is less than in case of traditional sour cream) both the Prebiolact-2 culture and butter culture

Table 1 Measured and calculated data of heat flow curves and microbe counts

Sample		t/s	H/		H_0^1/mJ g^{-1}	$C\cdot10^8$/cfu g^{-1}	$H_0^1/C\cdot10^{-8}$/mJ cfu^{-1}
name	M/g		mJ	%			
Butter culture	0.556	6912	228	7.2	5282	4.4	1200
		23476	2937	92.8			
Prebiolact-2 culture	0.568	12447	36	1.6	2879	5.8	496
		16659	1635	71.4			
		33385	619	27.0			

M – the total mass of sample, t – the times belonging to the matched Gaussian curves, H – the area under Gaussian curves that is the heat production, H_0^1 – the heat production normalised to unit sample mass, C – the total plate count measured after fermentation till pH=4.7, H_0^1/C – the heat production corresponding to a single microbe creation (or microbe colony)

Table 2 Measured and calculated data of heat flow curves

Mixed culture		t/s	H/		H_0^2/mJ g^{-1}	$H_0^1\cdot10^{-8}/C$/ mJ cfu^{-1}	$C_c\cdot10^8$/cfu	R/%
Gaussian curve of	M/g		mJ	%				
Prebiolact-2 culture and butter culture	0.582	11853	8	3.2				
		15863	113	39.8	1950	496	3.9	71
		25583	112	39.5	1936	1200	1.6	29
		41850	50	17.5				

H_0^2 – heat production referred to unit sample mass, H_0^1/C – the heat production of a single microbe (from Table 1), C_c – calculated total plate count ($=H_0^2/H_0^1/C$), R – calculated percentage ratio of microbes of cultures.
The other symbols are the same that in Table 1.

proliferate. The EPS-production of Prebiolact-2 culture could be directly observed on the electron microscopic photos. This EPS-production ensures the appropriate viscosity of heat-resistant sour cream, its resistance to syneresis, well stirrability and, together with the technology, the heat tolerance [8]. The aroma-producing microbes of butter culture are responsible for the organoleptically characteristic properties of sour cream [1, 9].

Conclusions

Based on the measurements carried out by the isotherm calorimetric method and by the help of deconvolution program, the proliferation of both cultures could be directly detected at the fermentation temperature (30°C) of the technology. According to our knowledge this is the first application of isotherm calorimetry for detection of proliferation of probiotic bacteria in mixture with other one and their separation from each other. On the basis of heat production (Table 1) corresponding to creation of a single microbe, which had been calculated earlier, in fermented sour cream the ratio of microbes originating from Prebiolact-2 and butter culture was 71:29%, when the cream had been inoculated by the mixture of two cultures in 50:50% (Table 2). The procession of dairy products has initiated this study. The main point was to have a method that is able to identify the presence of different bacteria, to determine their participation in the final product, this way to control the quality and technological process. It is cheaper and quicker than the method using the gene technology, and with the aid of determination of the ratio of participating bacteria the producer of the product can be identified. Such question as the quantitative correlation between the properties of sour cream and the growth of the microbes lies in the field of basic research and it was out of our interest.

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References

1 B. Schäffer, J. Schrem and S. Szakály, Tejipar, 3 (1977) 65 (in Hungarian).
2 A. A. El-Nimr, S. Szakály and B. Schäffer, Eur. J. Dairy Sci., 3 (1975) 104.
3 B. Schäffer, G. Tomka and S. Szakály, Brief communications of XIX. Int. Dairy Congr. Delhi, 1E (1974) 659.
4 G. Óbert, B. Schäffer, S. Szakály and B. Szily, Heat resistant sour cream, their preparation and sour creams containing foods, Hungarian Patent Office, HP 0100317 (2001).
5 M. Riva and A. Schiraldi, Thermochim. Acta, 220 (1993) 117.
6 L. Franzetti, A. Galli, A. Perazzoli and M. Riva, Ann. Microbiol. Enzimol., 45 (1995) 291.

7 M. Riva, L. Franzetti, A. Galli and A. Schiraldi, Ann. Microbiol. Enzimol., 47 (1997) 199.
8 M. Riva, D. Fessas, L. Franzetti and A. Schiraldi, J. Thermal Anal., 52 (1998) 753.
9 M. Riva, D. Fessas and A. Schiraldi, Thermochim. Acta, 370 (2001) 73.
10 N. J. Berridge, C. M. Cousins and A. J. Cliffe, J. Dairy Res., 41 (1974) 203.
11 L. Gram, Int. J. Food Microbiol., 16 (1992) 25.
12 Cs. Mohácsi-Farkas, J. Farkas and A. Simon, Acta Alimentaria, 23 (1994) 157.
13 H. Teeling and H. Cypionka, Appl. Microbiol. Biotechnol., 48 (1997) 275.
14 T. Andlid, L. Blomberg, L. Gustafsson and A. Blomberg, Syst. Appl. Microbiol., 90 (1999) 145.
15 M. Kiss, J. Belágyi and D. Lőrinczy, J. Therm. Anal. Cal., 72 (2003) 565.
16 D. Lőrinczy, M. Kiss and J. Belágyi, J. Therm. Anal. Cal., 72 (2003) 573.
17 T. Dergez, F. Könczöl, N. Farkas, J. Belágyi and D. Lőrinczy, J. Therm. Anal. Cal., 80 (2005) 445.
18 B. Schäffer, B. Schäffer and D. Lőrinczy, J. Therm. Anal. Cal., 82 (2005) 531.

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