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Examination of the growth of probiotic culture combinations by the isoperibolic batch calorimetry

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Abstract

Probiotic ("intestinal friend") foods, and probiotic dairy products in particular, have substantially increased in number recently. In the production of some of these products a problem is caused by the fact that the thermotolerant microbes that produce the probiotic effect do not generate an aroma; the taste of the product is given by aroma-producing mesophilic microbes. The growth optima of the two microbe groups do not coincide. Given that heat is released in the course of microbe reproduction, the isoperibolic calorimetry method appears the best and fastest for monitoring the process. During the experiments the thermotolerant Prebiolact, owned by the Hungarian Dairy Research Institute (HDRI) and clinically verified to be probiotic, and Hansen Company's CHN-22 mesophilic butter culture were examined bred separately and together. The cultures were incubated at 30 °C, at which temperature prior studies had shown both lactic acid bacteria cultures were capable of growing. The cultures were injected into milk, then the microbe growth heat flow-time curve over 18 h was taken on the Setaram Micro DSC-II calorimeter at 30 °C under isothermic conditions. Analysis of the heat flow curves led to the following conclusions. Both lactic acid bacteria cultures grow well at the non-optimal temperature of 30 °C, thermotolerant Prebiolact somewhat faster and mesophilic CHN-22 slower. The two cultures do not impede each other in mixed cultures; the growth peaks of the two cultures were easily isolated on the power–time curve by a deconvolution program. In sum it can be stated that mixed cultures of the two cultures examined can be used to produce dairy products which are probiotic, but their taste character (e.g. aroma) is determined by the butter culture. To date we have elaborated production procedures for probiotic butter cream and heat-resistant sour cream using the two cultures in a mixed one.

Keywords: Isoperibolic batch calorimetry; Ratio of probiotic and aromatic microbes; Microbial growth

1. Introduction

In the area of sour dairy products the consumption of probiotic products is steadily increasing throughout the world. By now the Japanese YACULT counts as a classic, and has spread to the entire world. The Hungarian Dairy Research Institute (HDRI) also began developing probiotic dairy products 4 years ago. The basis for this were the microbe strains isolated in an international project in the 1980s, the culture used in this work was produced from them [1]. In wide-ranging in vitro and human clinical examinations it was determined that the Prebiolact culture was expressly probiotic. The consumption of kefir containing the Prebiolact culture had the effect of increasing the percentage of probiotic bacteria in the feces of the consumer from an original 12.7 to 72%, within which the increase of Bifidobacteria was 60 fold. The reason for this is that Prebiolact produces exopolysaccharide [2], which is the nutrient for Bifidobacteria. This effect does not occur in the consumption of regular kefir.

In the production of dairy products aroma-producing bacteria must be used along with probiotic bacteria for the taste effect. The growth optimum, however, is different for the bacteria in the two cultures, 37 °C for Prebiolact, and 24 °C for the aroma-producing butter culture. In their combined use fermentation must be conducted at a temperature where both are growing well. However, as both consist of lactic acid bacteria which are coccus shaped, their identification in combined environments (by genetic examination, perhaps) is rather difficult. Although the employment of isoperibolic calorimetry primarily examines microbe inactivation

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[3] and cell denaturation of bacteria [4,5], it also describes the exothermic process which is undergone under isothermic conditions on the impact of microbe reproduction, at the time of its peak matches the time of the inflexion point on the growth curve [6].

In light of the above, an isotherm method was elaborated by which the rate of growth of the two cultures in a combined environment could be measured.

2. Materials and methods

During the experiments the thermotolerant Prebiolact and Hansen's CHN-22 mesophilic butter culture were examined bred separately and together. The cultures were incubated at 30 °C, at which temperature prior studies had shown both lactic acid bacteria cultures were capable of growing. For the isotherm examinations approximately 450 mg sterile non-fat milk (pH 6.65) and 25 mg culture (pH 4.5) were poured separately into a mixing batch vessel (lower volume maximum: 500 μl and the upper one is 200 μl) and left at 30 °C until heat equilibrium was obtained. Then the culture was injected into milk, then the microbe growth heat flow-time curve over 18 h was taken on the SETARAM Micro DSC-II calorimeter at 30 °C under isotherm conditions.

For the microbe titer studies fermentation was conducted with a thousand times greater quantities at 30 °C until a pH of 4.5 was reached, then the microbe titer was determined.

3. Results and discussion

Fig. 1 gives the growth heat flow-time curve of the butter culture, and Fig. 2 that of the Prebiolact culture. The figures show that both cultures grow well at 30 °C. The butter culture reaches its growth maximum in 6.5 h, and the Prebiolact culture in 4.5 h. Fig. 3 shows the combined growth power–time curve for the two cultures. It can be clearly seen that the curve is a combination of curves equivalent to growth maximums of 4.5 and 6.5 h.

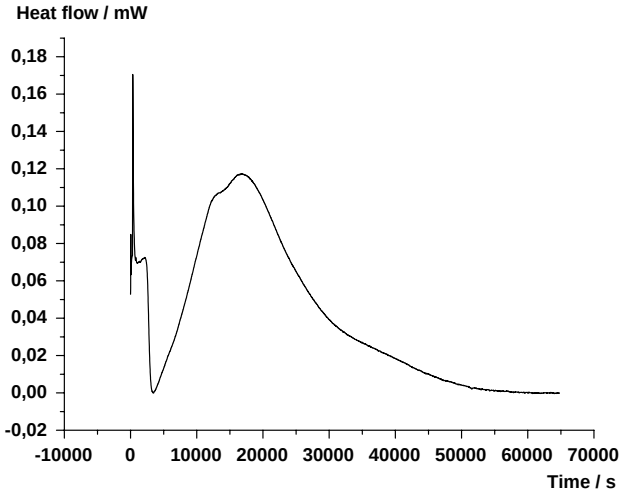

Fig. 2. Heat flow-time curve of a Prebiolact culture.

In order to show that the two cultures be separable even in combined environment, it seemed most practical to use a deconvolution through which the heat flow curve could be approximated by Gaussian curves [7,8]. A computer program was made for deconvolution. Fig. 4 shows the decomposition of the heat flow curve taken by the butter culture, and Fig. 5 the Prebiolact culture. It can be seen that the curves can be decomposed into three Gaussian curves, respectively, according to the number of different microbes being in the mixture.

Fig. 6 is the decomposition of the heat flow curve for the combined growth of the two cultures. Approximation in this case can be done by four Gaussian curves. It can be seen that the Gaussian curves for the growth maximums of 4.5 and 6.5 h can be easily separated, and on the basis of their areas the microbe titer ratios can be deduced against a known microbe titer.

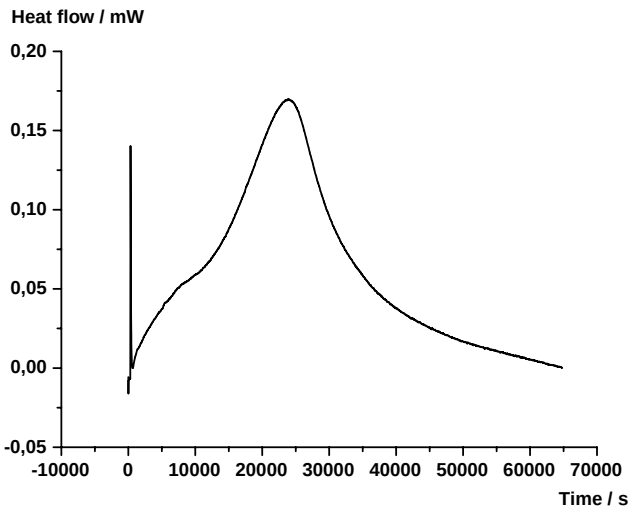

Fig. 1. Heat flow-time curve of a butter culture.

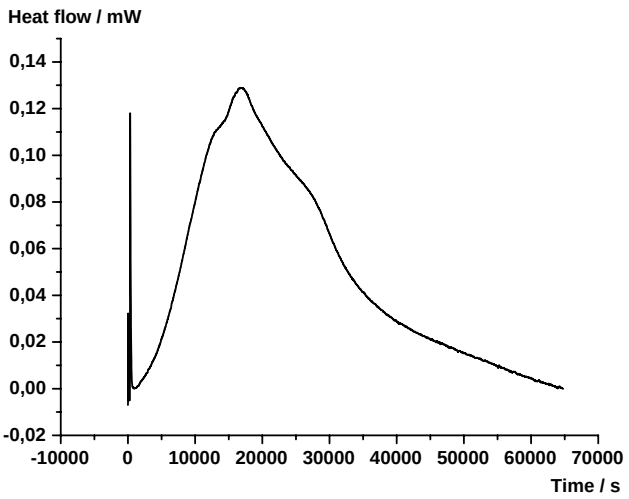

Fig. 3. Heat flow-time curve of a mixed culture (Prebiolact + butter).

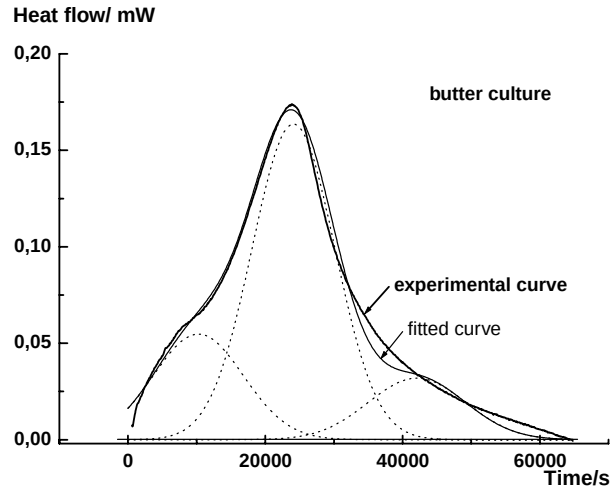

Fig. 4. Deconvoluted heat flow curve of a butter culture.

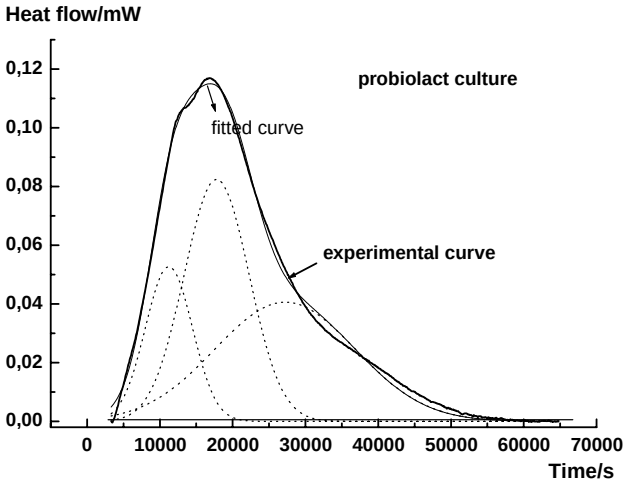

Fig. 5. Deconvoluted heat flow curve of a Prebiolact culture.

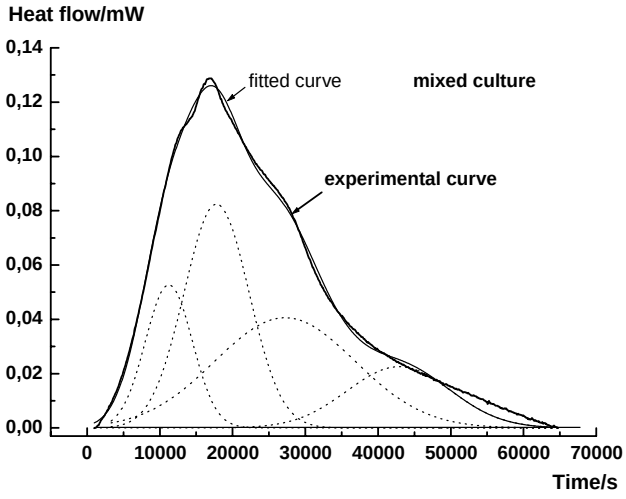

Fig. 6. Deconvoluted heat flow curve of a mixed culture.

4. Conclusions

The heat flow-time and microbe titer figures necessary for drawing conclusions are contained in Table 1. The table gives:

- the times (t) in second for the maxima of the matched Gauss curves,
- the area of the Gauss curves (ΔH) in milliJoule,
- the percent proportions of the areas (ΔH%),
- the amount (M) of the sample poured into the calorimetry vessel in gram and,
- the total microbe count (C) in colony forming unit (CFU)/g measured after fermentation to pH 4.7.

Taking the Gauss curves for the 4.5 and 6.5-h maxima indicated in the diagrams, for the Prebiolact and butter cultures we calculated:

- heat quantities applying to 1 g of sample ($\Delta H/M$) and,
- heat quantity equivalent to one microbe ($\Delta H/MC$).

Table 1
Data of heat flow-time curves and microbial titers (see explanation of symbols in the text)

Sample	t (s)	ΔH (mJ)	ΔH (%)	M (g)	$C \times 10^8$ (CFU)	$\Delta H/M$ (mJ/g)	$(\Delta H/MC) \times 10^{-8}$	Percent of culture
Prebiolact culture	12096	63	2.8	0.564	5.4	2651	491	100
	16686[a]	1495	66.8					
	30767	681	30.4					
Butter culture	7344	344	10.7	0.559			1212	100
	23423[a]	2561	80.3					
	36844	285	9.0					
Mixed culture	12690	33	1.2	0.576	5.9			
	15876[a]	1246	44.4			2163	Prebiolact: 75%; butter culture: 25%	
	25974[a]	1046	37.3			1816		
	40622	480	17.1					

[a] The data represent those Gaussian curves which were used for calculation.

The areas of the Gauss curves characteristic of the two individual cultures were divided by this heat quantity, yielding the microbe count figures for the two individual cultures. From this came the final result, that milk inoculated with Prebiolact and butter culture in 1:1 proportions will contain 75% probiotic bacteria and 25% butter culture bacteria at the end of fermentation. Symmetrical Gaussian curves were used during the decomposition of growth curves because the increase of number of individual bacterium stocks follows a lognormal distribution and its first derivative is a symmetrical Gaussian curve.

5. Application of the results

These results were utilized in the development of production procedures for two products. One was a version of traditional Hungarian sour cream which could be stirred well, had a high viscosity, and did not precipitate in the food during cooking [9]. The second was Vajkrém (butter cream), which has a 20-year-history in Hungary (and is called Spread in most countries of Europe), for which we developed a probiotic variant [10]. In both products the appropriate rate of growth for the two cultures was confirmed. The exopolysaccharide production of the Prebiolact culture increases viscosity and texture in proportion to its quantities [11], and aids stirrability and heat resistance in sour cream and ensures excellent cold spreadability (at 0–5 °C) in probiotic butter cream. In both products the butter culture ensures a pleasant taste and aroma.

Finally, it should be mentioned regarding the practical introduction of the products that the heat-resistant sour cream has been produced and distributed in large quantities in Hungary since March 2002, while the practical introduction of the probiotic butter cream is still in progress, and distribution is expected to begin in 2003.

Acknowledgements

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