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DETECTION OF PROBIOTIC MICROBES IN PROBIOTIC CHEESE SPREADS BY ISOTHERMIC CALORIMETRY

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The probiotic cheese spreads developed by the Hungarian Dairy Research Institute (HDRI) have a better Ca:P ratio than traditional brands and spread easily and without sticking even cold, further, their live lactic acid bacteria content gives them additional advantages as well. These advantages derive from the fact that a probiotic culture may also be employed during fermentation, through which the cheese spread may become probiotic if the culture proliferates. The international specification is a probiotic cell count of at least 10^6/g. However, during fermentation the probiotic cheese spread must also use other non-probiotic cultures to produce the right taste. Given that the microorganisms in the cultures are all cocci, their indication in a mixed environment is difficult and time-consuming. It appears possible, therefore we have used the isothermic DSC method due to their differences in heat production.

In order to analyze the calorimetric curves a deconvolutional program was devised which decomposed them into Gaussian curves. It was confirmed that probiotic bacteria proliferated in the probiotic cheese spreads, and their ratio was greater than 40% with a total plate count of $2–7 \cdot 10^8$/g. Accordingly, the probiotic cheese spread developed by HDRI contains an order of magnitude more probiotic bacteria than the internationally accepted cell count of 10^6/g.

Keywords: *cheese ferment, DSC, probiotic cheese spread*

Introduction

The live flora spreads and their production procedure [1] developed by the Hungarian Dairy Research Institute (HDRI) make it possible to produce probiotic cheese spreads as well. These probiotic cheese spreads have the following advantages compared to the traditional processed ones [2–9]. The original Ca:P ratio (1.5:1) of raw materials which is very favourable from the nutritional physiological aspect is maintained. They are well 'fat-like' spreadable even at refrigerator (5°C) temperature.

Among the live microorganisms they also contain probiotic microbes at least on order of internationally accepted 10^6.

The probiotic cheese spreads are made by the same technology as live flora cheese spreads, with the difference that to the fermentation on the top of butter culture providing the requested flavour, probiotic culture (Prebiolact-2) is also used [10]. Both of the cultures consist of lactic acid bacteria strains, every member of which is of coccus shape [10]. Identification of them in a mixed environment could be solved only by gene examination, and would be both time-consuming and expensive.

Our experiments aimed at identification of microorganisms originating from probiotic cheese spreads, butter culture and Prebiolact-2 culture in a mixed environment by isothermic DSC method. To the experiments the heat amount of different cultures determined by the deconvoluted curves recorded by isothermic DSC method, which is developed at formation of a single microbe (or Cfu colony) was used [2].

Materials and methods

Dairy materials

For experimental purposes probiotic cheese spreads were made of Pannónia cheese (Hungarian one, Emmenthal type cheese) based on the patent of HDRI titled 'Live flora spreads and process for their production' [1] in such a way that the fermentation was carried out by adding 1% butter culture and 1% Prebiolact-2 culture. The cheese spreads were fermented until pH = 4.9 was reached, were cooled down to 4°C, then were cold ripened at this temperature for 24 h.

To carry out calorimetric examinations so called cheese ferment was prepared according to the following method. 100 g fat-free sterile milk was measured in measuring sack of Stomacher device, then 2 g probiotic cheese spread was added and the blend was homogenized in the Stomacher device (2 min). The homogeneous blend was fermented at production temperature of probiotic cheese spreads (30°C) until

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pH = 4.7 was reached (approx. 8 h), cooled down to 4°C, and then cold ripened for 24 h.

Isothermic calorimetry

The calorimetric curve of microbe proliferation was recorded for 18 h in a SETARAM Micro DSC-II calorimeter at 30°C under isothermic conditions in the Biophysical Department of University Pécs. Their thermoanalytical group has an excellent skill in the investigation of thermodynamic properties of different biological and food macromolecules [11–26]. To the direct calorimetric measurements approx. 450 mg fat-free sterile milk and approx. 50 mg cheese ferment were measured into a mixing batch vessel and the same amount of distilled water into the reference cell. After thermal equilibrium has been reached at 30°C the cheese ferment was injected into sterile milk and at the same time the aliquote quantity of reference water was mixed in the reference cell.

Data evaluation

In order to analyse the isotherm calorimetric curves a deconvolutional program developed by us [2] was applied using the heat amounts falling to 1 microbe (or Cfu) measured for butter culture and Prebiolact-2 culture respectively.

Results

Figure 1 shows the isotherm calorimetric curve of cheese ferment. It is obvious that the curve can be broken into three Gaussian curves: the maximum of the first is after 5th h, that of the second appears in about 6th h and the third's is after the 8th h. Based on the preliminary experiments the second Gaussian curve is characteristic for the proliferation of Prebiolact-2 culture, and the third curve for that of butter culture.

The values obtained by the deconvolutional program are given in Table 1. We especially pointed out the values characteristic for Prebiolact-2 and butter cultures based on which the ratio of the two-type microbes being in cheese ferment can be calculated according to the proliferation heat produced by them.

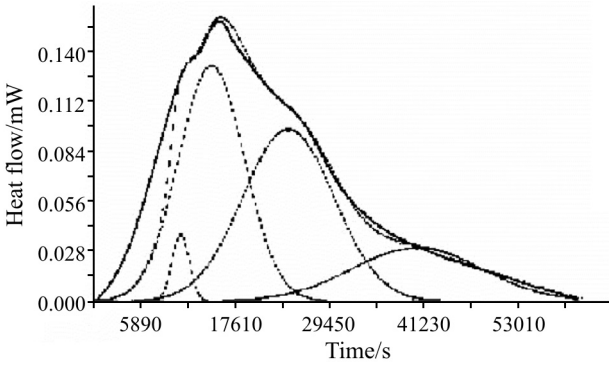

Fig. 1 Deconvoluted calorimetric curve of cheese ferment

Discussion and conclusion

It is proved by the results of experiments that, if appropriately prepared, the presence of probiotic lactic acid bacteria culture (Prebiolact-2) can be detected besides the other lactic acid bacteria culture (butter culture) in probiotic cheese spread by isotherm calorimetric method. The condition of detection is to make so-called cheese ferment as a first step, i.e. the live lactic acid bacteria microflora being in the product should be cultivated in fat-free milk. This cheese ferment contains the microbes of probiotic cheese spread and due to its low viscosity it can be injected as lactic acid bacteria culture into the mixing batch vessel to the fat-free sterile milk which has also a low viscosity.

Considering that the proliferation of microbes of the product has been examined during twice inoculation, the ratio of butter culture and Prebiolact-2 culture 55:45% can only be supposed, to determine the exact ratio further experiments are needed. However, it can be surely stated that probiotic microbes are present at least in the order of 10^6 in probiotic cheese spreads, as the total plate count of them reaches the order of $2–7 \cdot 10^8$ [11].

Table 1 Data characteristic for the calorimetric curve of cheese ferment and values calculated from them

Glaussian curve characteristic for mixed culture	*S*/s	*H*/mJ	*M*/g	*H*°	*H*°/C·10^{-8}	C·10^8/Cfu	Percentile ratio of cultures
	6975	174					
Prebiolact-2 culture	13880	467	0.556	2612	1212	2.16	55
Butter culture	21600	1452		856	491	1.71	45
	36750	435					

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