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# Examination of growth of probiotic microbes by an isoperibolic calorimetry

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**Abstract** Due to the increasing consumers' interest in up-to-date nutrition nowadays the production of main part of fermented dairy products (e.g. yogurt, kefir) is made by using probiotic microbes. The majority of this product group are the flavoured variations, the sweetener of which is, first of all, still refined sugar (e.g. saccharose). Honey of natural origin, consequently preferred from the nutritional physiological point of view, is suitable to replace this refined carbohydrate. In our experiments we have sweetened the most frequently used milk containing of 1.6 and 3.6% fat with generally used saccharose of 10%, and the difference in the dry material content was equilibrated by drink water of 3% (control product). The experimental product was sweetened with robinia honey of 13% (dry material content was 77%). The fermentation was performed with a probiotic culture of 5%, which was clinically tested to be probiotic. The fermentation process was conducted in isotherm regime at 36 °C during 18 h in batch wessels using SETARAM Micro DSC II calorimeter. The calorimetric enthalpy was proportional to the probiotic microbe counts generated during the fermentation. Due to our experiments, we have come to the conclusion that honey instead of hindering much rather stimulates the growth of probiotic microbes. At sample pairs sweetened by saccharose and acacia honey, respectively, the higher enthalpy was measured at samples containing honey in all cases.

**Keywords** Probiotic microbes · Sweetening by saccharose and honey · Isotherm fermentation

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## Introduction

Due to the increasing interest of consumers in up-to-date nutrition, the so-called functional foods were born, which besides their nutrients contain one or more definitely health protective (bioavailable) components [1]. In the field of dairy products, the bioavailability of different souring microbes has already been hinted at in 1907, when the long age of Balkan mountain shepherds was explained by their consumption of a huge amount of yoghurt [2]. In the latest decades, the probiotic and prebiotic fermented dairy products have significantly spread among functional dairy products. The beneficial effects of these products have also been summarized in a special book in Hungary [3], and their therapic effects were also published in case of patients suffering from diarrhoea [4].

The main part of probiotic dairy products consumed all over the world consists of flavoured variations, the sweetener of which is still, first of all, refined sugar (e.g. saccharose). Consumers can rightly wish to replace this component not preferred from the nutritional physiological point of view by a natural sweetener, favourably by honey.

In our work, in the framework of the project OMFB-00938/2009 supported by the Hungarian State, we wanted to find the answer whether replacement of sweetening

saccharose by honey could influence the growth of probiotic microbes. To begin with, we wanted to examine whether the amount of microbes being formed during fermentation will be changed. Considering dependence of measurable colony-forming microbe number in the product after fermentation on different factors (e.g. storing temperature/time), it was practical to use a method which provided information directly on growth of microbes. During our previous studies, we came to the conclusion that the isoperibolic calorimetry method was suitable to follow the growth of microbes [5], and the ratio of probiotic microbes [6] could be determined by deconvolution of the curve [7].

## Materials and methods

In our experiments, the growth of microbes of the Hungarian culture of trade name Prebiolact-2 was examined. The probiotic properties of the culture are proved by clinical tests [8].

At the determination of recipes of control and experimental samples, we took into consideration that the milk composition and carbohydrate content of samples should have been the same, and they could differ only in carbohydrate-free component content of honey. The recipes in accordance with the above conditions are shown in Table 1. The carbohydrate content of acacia honey was 77.0%.

During processing the samples, the minimum heat treatment was applied in order to protect the biologically active components of honey to the greatest degree. That is why the available homogenized UHT-milk was used as raw material, in which granulated sugar and acacia-honey were solved, respectively, at 60 °C.

900–900 mg of milk samples were measured into the batch wessels of 1000 mg capacity in the way that the filled in amounts should have been the same by pairs in the range of ±2 mg. A pair of one c-milk and m-milk samples were made from the same raw material and were inoculated by the same culture. The inoculation was made by 50–50 μL Prebiolact-2 culture injected in the batch cells, then the closed cells were agitated intensively for 10 s. Distilled water was used as reference, the amount of which was the same as that of the sample in range of ±0.1 mg.

The inoculated sample together with the reference sample were put into a SETARAM Micro DSC-II (France, Lyon) ultra-sensitive scanning calorimeter, and after setting in the heat balance at 36 °C, the proliferation curves were plotted under isotherm condition with data collection of 1100 min. Within a 10–15 min after inoculation by culture, the measurements were always started, so the microbes began to grow only afterwards (it was checked and proved at the very early stage of the isotherm proliferation).

900 g from c-milk of the first sample pair was inoculated by 50 mL Prebiolact-2 culture (a thousand time higher amount than used in the isotherm proliferation examinations), then it was fermented at 36 °C for 1100 min, cooled down to 5 °C and the probiotic total plate count was measured within 24 h in an accredited microbiological laboratory using the MSZ EN ISO 4833:2003 method.

**Table 1** Composition of the examined samples

| Component's | | |
|---|---|---|
| Denomination | Amount/g/100 g | |
| | Control (c-milk) | Experimental (m-milk) |
| Whole milk, 3.6% fat content | 87.0 | 87.0 |
| Granulated sugar | 10.0 | – |
| Drinking water | 3.0 | – |
| Acacia-honey | – | 13.0 |
| Altogether | 100.0 | 100.0 |

## Results and discussion

An isotherm curve of c-milk is shown in Fig. 1, the probiotic microbe number of which was also measured after fermentation with a thousandfold amounts.

It could be well seen in Fig. 1 that the Prebiolact-2 culture contains two different microbe groups which can

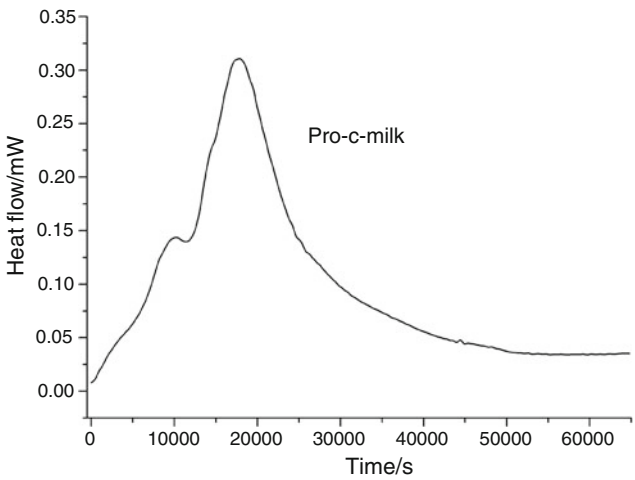


**Fig. 1** The isotherm fermentation curve of the c-milk sample (exotherm deflection is upward)

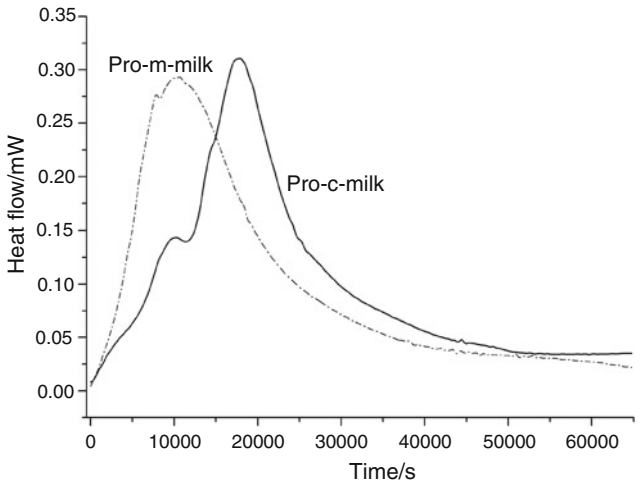


**Fig. 2** The isotherm curves of fermentation of the c- and m-milk samples

well be separated from each other. Microbes of one group grow rapidly, the heat flow shows the maximum value at 172 min. The growth of the other microbe group is slower, the maximum of heat flow is at 300 min, but the amount of this group is significantly higher.

The enthalpy value calculated from the integral of the total isotherm curve is −4,55 J/g. After fermentation made by using 1000-fold amount of culture $5.4 \times 10^8$ CFU/g alive microbe number was measured in the sample, hence the heat amount liberating at the formation of a colony-forming microbe is $0.84 \times 10^{-8}$ J.

In Fig. 2, the isotherm proliferation curves of fermentation of milk sweetened by sugar (pro-c-milk) and milk sweetened by honey (pro-m-milk) made from the same raw material are demonstrated.

**Table 2** Enthalpy values (*H*) of isotherm curves of c- and m-milks divided in three fermentation series and the relevant colony-forming microbe numbers (*M*)

| Serial number | Sign of the sample | *H* /J/g | $M \times 10^8$/CFU/g |
|---|---|---|---|
| 1 | c-Milk | 4.55 | 3.82 |
| | m-Milk | 4.97 | 4.17 |
| | Δm–c | 0.42 | 0.35 |
| | Δ% | 9.20 | 9.20 |
| 2 | c-Milk | 3.87 | 3.25 |
| | m-Milk | 3.98 | 3.31 |
| | Δm–c | 0.11 | 0.09 |
| | Δ% | 2.80 | 2.80 |
| 3 | c-Milk | 5.05 | 4.24 |
| | m-Milk | 5.36 | 4.50 |
| | Δm–c | 0.31 | 0.26 |
| | Δ% | 6.10 | 6.10 |
| Average Δm–c | | 0.28 | 0.24 |
| Average Δ% | | 6.00 | 6.00 |

It is obvious from Fig. 2. that the descending phase of the isotherm curves of fermentation of both samples is similar. The heat flows of quicker (maximum at 132 min for m-milk, and 172 min for c-milk) and slower growing (at 10190 min for m-milk and 18000 min for c-milk) microbe groups can be well distinguished from each other. The heat flow maximum of the quicker growing part is higher at the isotherm curve of fermentation of m-milk.

In Table 2, the enthalpy values, the colony-forming microbe numbers belonging to them, the differences and average values, measured in three series, are summarized.

## Conclusions

Results of our experiments have proved that microbes of probiotic Prebiolact-2 culture grow well in both milks sweetened by either saccharose or honey. The descending phase of the isotherm curves following the fermentation process according to the heat production of microbes is similar, in both situations basically two microbe groups are indicated, the growth of one is quicker and that of the other is slower. The enthalpy values obtained by integration of the curves in all the three series were higher at fermentation of milks sweetened by honey, indicating production of more probiotic microbes. The increase was 0.28 J/g, i.e. 6% on average. This corresponds to increase of $24 \times 10^6$ CFU/g, which is 24 times higher than the minimum $10^6$ CFU/g determined for probiotic dairy products by international standards.

It can be conluded that the replacement of saccharose by honey does not decrease but stimulates the growth of probiotic microbes. It could be proposed by our investigation to substitute the sugar by honey in some probiotic products because it has a double stimulating effects: at one side it evokes quicker microbe proliferation and at the other side this way the total probiotic microbe count is greater in the final product. We could say that the isoperibolic technique can be applied in a wide range of the RD field in case of different food products too [9].

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