THE INFLUENCE OF INULIN ADDITION ON THE DEVELOPMENT AND THE ACTIVITY OF BIFIDOBACTERIUM IN MILK

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Abstract: *One of the prebiotic products used nowadays for the stimulation of bifidobacteria is inulin, a soluble fiber belonging to the fructans group-chain of fructose units ending or not in one glucose unit. This study aims to analyze the way in which the addition of these oligosaccharides in different percentages of 2%, 4%, 6% on a powder milk medium that has been reconstituted with 12% s.u. improves the growing speed and the fermentative activity of these probiotic bacteria. The data obtained have revealed that the doses of 4% added inulin in the culture medium is enough for an optimum improvement of the bifidobacteria viability.*

Keywords: *inulin, bifidobacteria, milk*

Introduction

The first applications of the bifidobacteria have aimed at the milk industry because milky products are the best holders for introducing these bacteria in the human body. Bifidobacteria grow slowly and acidify the milk slowly, which correlates with a large amount of fermentation time, they need a low redox pottential especially at the beginning of the growing phase and the supplementation with growing factors is often indispensable [Gomes and Malacta, 1999]. Due to the fact that bifidobacteria have a slow development in milk, which is considered an "artificial" medium for these bacteria because it contains amino acids and peptides with a small molecular mass in insufficient quantities (0.1 g/l), their association with other substances that stimulate their growth occurs. Among these, a growing interest has been shown for a specific group of oligosaccharides, fructooligosaccharides, which can be synthesized from natural sources (inulin) or synthesized from sucrose. These carbohydrates are fermented only by some specific bifidobacteria or lactobacilli stems, which can influence the selection of probiotic bacteria selection. The studies carried out in vitro have shown that fructooligosaccharides are fermented by bifidobacteria, which has also been confirmed in vivo through tests with healthy volunteers [Suskovic J. and his co-workers, 2001]. The tests in vivo have shown that a use of 15 g/day of oligofructose and inulin is accompanied by a remarkable growth of the number of bifidobacteria in the colon. Therefore, the use of oligofructose led to obtaining a population of predominant bifidobacteria in the intestinal microbiote. Inulin is a soluble fiber belonging to the fructans group-chain of fructose units ending or not in one glucose unit. Dietary fibers are generally vegetable substances-parts of plant cells-that are not or very little digested, reabsorbed or metabolized by the digestive enzymes of the upper part of the digestion system and are subsequently more or less fermented or metabolized by the microbial flora of the large intestine (colon) [Costin, 1999]. Therefore, native inulin may be added to milk in order to increase their fibre content and to develop new functional claims or health claims like bifidogenic or probiotic effect.

Materials and methods

The inulin produced by Consucra (Belgium) was used in percentages of 2% (P2 sample), 4% (P3 sample), 6% (P4 sample) to enrich the based medium of skimmed milk reconstituted with 12% s.u. degreased. The physical-chemical characteristics of the base medium have been determined using the Lactostar device.

The samples supplemented with inulin and the blank sample (P1) have been inoculated with a probiotic culture of bifidobacteria *Bifidobacterium lactis* (figure 1), delivered by Enzymes&Derivates under the commercial name DI-PROX BF 801 in the same percentage in all the samples (3%). After inoculating it with lactic bacteria, the thermostating of the samples was performed at a temperature of 37˚C for 24h. Right after the inoculating it, at intervals of 0h, 3h, 6h, 12h, 24h after sowing these samples, including the blank sample P1 (base medium without inulin), the comparison within cells through the direct Breed counting method has been determined in order to establish the evolution of the lactic bacteria population. At the same time, for the same intervals of time, the fermentative activity has been monitored by determining the titrable acidity, which was then expressed in Thorner degrees.

Results and Discussion

The base medium of powder milk reconstituted with 12% skimmed s.u. showed the following characteristics: 3.1% fat, 4.16% protein, 4.11% lactose, 10.39% SNF, freezing point -0,540˚C, 0.83% mineral salts.

The results obtained have shown that inulin, when added to the base medium with a concentration of 2, 4 and 6%, has a pozitive role in the process of multiplying probiotic lactic bacteria, the number of cells obtained being larger in these samples than in the blank sample.

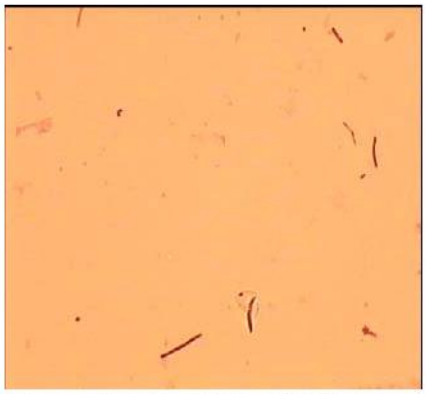

Figure 1. Picture showing the bifidobacteria Bifidobacterium lactis in milk reconstituted P1 (with 12% s.u.) after 3 hours of thermostating at 37˚C

From the analysis obtained, it has been noticed that after 3 h of thermostating, the increase of the cells number is proportional with the increase of the inulin dose, the number of lactic bacteria being ten times bigger then the initial one. The most numerous population of lactic bacteria was obtained in the medium with 6% inulin (P4) but the growing speed of the bacteria from this medium is similar to that of the bacteria from the medium with 4% inulin (P3) during the whole process of thermostating of the analyzed samples.

After 24 h of thermostating at a temperature of 37˚C, the lactic bacteria have reached a maximum number of $13.18 \cdot 10^{12}$ cells/cm^3 in a medium with 6% inulin (P4). The increase of the lactic bacteria population in 24 hours of thermostating was ~100 bigger than the blank sample, pointing at the stimulating effect that inulin has on the bifidobacteria activity.

In case of using inulin for stimulating the growth of probiotic bacteria, we recommend their use with a 4% concentration in order to obtain satisfying results.

At the end of the thermostating period, it has been noticed that the acidity obtained in inulin medium (figure 2) has registered a considerable growth in the first 24 hours of thermostating.

Table 1: The Evolution of the Probiotic bacteria population during 24h of termostating

Medium variants analyzed	The Evolution of the Probiotic Bacteria Population (ufc/cm^3)				
	0h	3h	6h	12h	24h
P1 (Blank sample)	$7.021 \cdot 10^{10}$	$7.28 \cdot 10^{10}$	$7.67 \cdot 10^{10}$	$12.2 \cdot 10^{10}$	$15.8 \cdot 10^{10}$
P2	$7.3 \cdot 10^{10}$	$7.6 \cdot 10^{10}$	$12.1 \cdot 10^{10}$	$15.6 \cdot 10^{10}$	$17.4 \cdot 10^{10}$
P3	$7.21 \cdot 10^{10}$	$4.80 \cdot 10^{11}$	$7.83 \cdot 10^{11}$	$1.41 \cdot 10^{12}$	$2.76 \cdot 10^{12}$
P4	$7.31 \cdot 10^{10}$	$7.53 \cdot 10^{11}$	$10.26 \cdot 10^{11}$	$7.11 \cdot 10^{12}$	$13.18 \cdot 10^{12}$

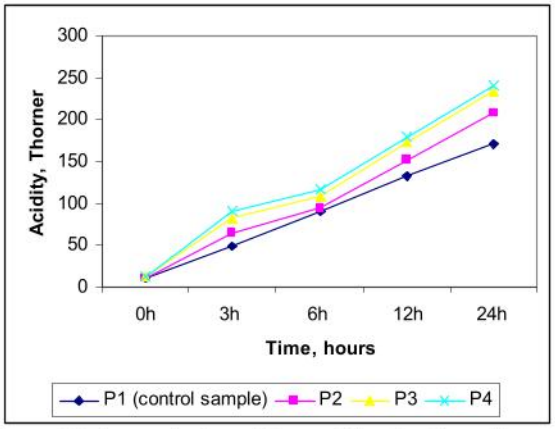

Figure 2. The evolution of the acidity for the milk variants supplemented with different doses of inulin

Generally, acidity growth has been proportional with inulin addition in the medium, thus being a strong relation between the evolution of the acidity and the number of cells in the medium. The highest value of the acidity was obtained in the P4 medium and was, after 24 hours of thermostating, 27% bigger than the blank sample.

Conclusions

The use of inulin for stimulating bifid bacteria in milk ensures a growing speed of the probiotic bacteria 100 times bigger than that in the milk with no ingredient added. We recommend the medium variant P3 for planting bifidobacteria as this medium leads to obtain numerous populations of probiotic bacteria.

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