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Influence of chicory inulin on the survival of microbiota of a probiotic fermented milk during refrigerated storage

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Abstract - Inulin-supplemented and control probiotic fermented milks were produced using a dairy starter culture containing *Streptococcus thermophilus* CHCC 742/2130, *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12, and were then stored at 4 °C for 6 weeks. Microbiological analyses were performed at weekly intervals. The results showed that the presence of inulin at 1 to 5% (wt/v) did not influence significantly ($P > 0.05$) the survival rates of either *S. thermophilus* or *L. acidophilus*. However, the addition of inulin at 5% (wt/v) had a significant beneficial effect ($P < 0.05$) on the viability of bifidobacteria after 28 days of refrigerated storage.

Key words: bifidobacteria, inulin, fermented milk, storage, survival.

In recent years, several *Lactobacillus* and *Bifidobacterium* species have received attention as probiotic organisms, and have been incorporated into a wide range of dairy products (Klein *et al.*, 1998; Biavati *et al.*, 2000). Regulatory authorities around the world are looking for assurance that a probiotic product can deliver viable starter organisms at sufficient numbers to the large intestine in order to provide a benefit to the consumer. Levels of at least 10^6 to 10^7 CFU/ml should be present at the time of consumption if a health claim is to be made. The selective stimulation of the growth of probiotic bacteria is called prebiosis. Inulin is a soluble dietary fibre that has been studied extensively, and may provide the best evidence of prebiotic effects in humans (Van de Wiele *et al.*, 2004). Although the effects of prebiotics on colonic bifidobacteria have been investigated to some extent, there are limited reports on the influence of prebiotics on probiotic organisms stored in dairy foods. Therefore, the main objective of this study was to monitor the effect of commercial inulin on viability of the characteristic microbiota of a fermented acidophilus-bifidus-thermophilus (ABT) milk.

A 12% (wt/v) non-fat dry milk solution (4 l) was prepared, divided into four equal portions and supplemented with commercial chicory inulin (Raftiline GR; Orafti, Tienen, Belgium) at levels of 1, 3, or 5% (wt/v), whereas the fourth batch was devoid of inulin and served as a control. The process milks thus prepared were then pasteurised at 90 °C for 10 min. The ABT-5 probiotic starter culture, which consisted of *Lactobacillus acidophilus* La-5 (A), *Bifidobacterium lactis* Bb-12 (B) and *Streptococcus thermophilus* CHCC 742/2130 (T), was kindly supplied in freeze-dried direct vat set form by Chr. Hansen (Hørsholm, Denmark). It was added to the heat-treated process milks cooled to 40 °C at the rate of 0.2 U/l corresponding to 2% (v/v) conventional bulk starter. Incubation took 6 h at 37 °C to reach a pH value of 4.5 to 4.6. Thereafter, the fermented ABT milks were cooled to 15 °C in ice water, and each of the four batches was separated into 21 fractions that were transferred in sterile tightly capped centrifuge tubes (30 ml; Sarstedt, Nümbrecht, Germany). After 24 h of cooling at 8 °C (d 0), the samples were stored at refrigeration temperature (4 °C). The entire experimental program was repeated three times.

Three tubes of all four products were taken at each sampling time, i.e., after 0, 7, 14, 21, 28, 35 and 42 days of storage. Samples were removed aseptically from centrifuge tubes and diluted by mixing 10 ml with 90 ml of 0.1% peptone water. Further dilutions were made as required.

Streptococcus thermophilus CHCC 742/2130 was enumerated by the standard pour-plate method using M17 agar (Oxoid, Basingstoke, United Kingdom). The pH of the medium was 6.9 ± 0.1. The inoculated plates were incubated at 37 °C for 48 h under aerobic conditions. *Streptococcus thermophilus* CHCC 742/2130 formed lenticular colonies with a diameter of 1 to 2 mm. Colony-forming units (CFU), expressed as log per millilitre, were used to report survival of streptococci.

MRS-maltose agar (Anonymous, 1995) with pH 6.2 ± 0.1 was used for enumeration of *L. acidophilus* La-5. The plates were incubated at 37 °C for 72 h. Anaerobic culture jars (2.5 l) were employed to generate anaerobic conditions, atmospheric oxygen being absorbed by means of AnaeroGen AN 25 sachets (Oxoid). The counts were expressed as log CFU/ml. The lactobacilli identified on the basis of colonial type were confirmed by microscopic examination. *Lactobacillus acidophilus* La-5 was Gram-positive rods with rounded ends.

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TABLE 1 – Survival of *Bifidobacterium lactis* Bb-12 in inulin-supplemented and control fermented acidophilus-bifidus-thermophilus (ABT) milks during refrigerated storage at 4 °C

Storage time (days)	Control		Inulin 1.0% (wt/v)		Inulin 3.0% (wt/v)		Inulin 5.0% (wt/v)	
	Log CFU/ml*	%	Log CFU/ml*	%	Log CFU/ml*	%	Log CFU/ml*	%
0	5.46 ± 0.22 ^{A}	100.0	5.46 ± 0.24 ^{A}	100.0	5.48 ± 0.18 ^{A}	100.0	5.46 ± 0.17 ^{A}	100.0
7	5.35 ± 0.10 ^{A}	77.3	5.37 ± 0.30 ^{A}	80.4	5.40 ± 0.09 ^{A}	83.1	5.41 ± 0.24 ^{A}	88.2
14	5.20 ± 0.26 ^{A}	54.8	5.22 ± 0.19 ^{A}	57.8	5.27 ± 0.22 ^{A}	62.3	5.29 ± 0.24 ^{A}	67.5
21	4.97 ± 0.20 ^{A}	32.5	5.01 ± 0.09 ^{A}	35.3	5.08 ± 0.19 ^{A}	39.6	5.11 ± 0.21 ^{A}	44.8
28	4.48 ± 0.27 ^{A}	10.5	4.55 ± 0.16 ^{A}	12.3	4.69 ± 0.22 ^{AB}	16.3	4.86 ± 0.25 ^{B}	25.2
35	4.25 ± 0.18 ^{A}	6.2	4.34 ± 0.12 ^{A}	7.7	4.47 ± 0.30 ^{AB}	9.7	4.57 ± 0.21 ^{B}	12.8
42	4.04 ± 0.25 ^{A}	3.8	4.11 ± 0.28 ^{AB}	4.5	4.27 ± 0.24 ^{AB}	6.2	4.38 ± 0.19 ^{B}	8.3

* Values are means ± SD based on nine observations (three samples, three replicates). Log CFU/ml means with different letters in the same row are significantly different ($P < 0.05$).

Nalidixic acid (0.030 g), neomycin sulphate (0.200 g), lithium chloride (0.600 g) and paromomycin sulphate (0.250 g), all obtained from Sigma (St. Louis, Mo., USA), were suspended in distilled water (100 ml), and then sterilised by filtering through Millipore filters (Millipore, Bedford, Mass., USA) with pore diameter of 0.22 µm. The pH of the solution was adjusted to 7.3 ± 0.1 with 0.1 *M* sodium hydroxide (NaOH) before sterilisation. A 5-ml aliquot of this antibiotic solution was added to 100 ml of MRS agar (pH 6.2 ± 0.1; Oxoid) immediately before use. The culture medium thus prepared was used for the enumeration of *B. lactis* Bb-12. The plates were incubated at 37 °C for 5 days. Anaerobic conditions were generated using anaerobic culture jars (2.5 l) and AnaeroGen AN 25 sachets (Oxoid). The counts were expressed as log CFU/ml. The bifidobacteria colonies identified were irregularly shaped or lenticular, and were corroborated by microscopic observation.

The data obtained were subjected to analysis of variance using the general linear model procedure of STATISTICA data analysis software system, version 6.1 (StatSoft Inc., Tulsa, Okla., USA). Significant differences among the log CFU/ml means were determined by using Duncan's multiple comparison test at $P < 0.05$ (StatSoft).

The addition of inulin did not influence significantly ($P > 0.05$) the growth and survival of coccus-shaped starter bacteria during fermentation and subsequent refrigerated storage of the fermented ABT milks (data not shown). *Streptococcus thermophilus* was the most numerous starter culture component both at the beginning and at the end of the 42-day storage period. Food regulations in Hungary require fermented dairy products to contain lactic acid bacteria of starter culture origin at concentrations of at least 10^7 CFU/ml at the time of consumption (Anonymous, 2004). Even the counts of streptococci exceeded largely this value throughout the entire storage period.

Ranging from 7.05 to 7.35 log CFU/ml, the initial counts of *L. acidophilus* La-5 were found to be approximately 1.5 log cycles lower than those of *S. thermophilus* CHCC 742/2130 (data not shown). After a period of stagnation, a gradual decrease in viable numbers of lactobacilli was observed. The addition of inulin had no significant effect ($P > 0.05$) on the survival of *L. acidophilus* La-5 during storage. Our results are consistent with previous reports by Bozanic *et al.* (2001) on the inability of inulin to stimulate the growth and survival of *L. acidophilus* La-5 in both fermented bovine and caprine milks during 28 days of storage at 5 °C.

As shown in Table 1, the initial levels of *B. lactis* Bb-12 were somewhat lower than the suggested minimum level of 10^6 CFU/ml (Anonymous, 2004). The low pH of fermented milks may affect adversely the survival of bifidobacteria because their growth is retarded considerably below pH 5.0 (Scardovi, 1986; Biavati *et al.*, 2000). This would appear to be the reason that the loss of viability of *B. lactis* Bb-12 during storage was more pronounced than was that of the other lactic acid bacteria. However, the addition of inulin at 5% had a significant beneficial effect ($P < 0.05$) on their survival rates after 28 days of refrigerated storage. In a study by Shin *et al.* (2000), inulin was found to be the least effective in retaining the viability of *Bifidobacterium* spp. Bf-1 and Bf-6 among the carbohydrate sources tested. In accordance with our findings, a 5% concentration of inulin was needed to observe significant differences ($P < 0.05$) compared to controls (Shin *et al.*, 2000). Carbohydrates with a degree of polymerisation exceeding 10, as is the case for inulin, do not seem to be ideal substrates for bifidobacteria. Very little is known about the mechanism of carbohydrate uptake by bifidobacteria. However, it appears likely that the substrate transport system may be more efficient for dimeric and oligomeric carbohydrate sources (Varga *et al.*, 2003).

In conclusion, the results of this research demonstrated that the presence of inulin at 1 to 5% (wt/v) did not influence significantly ($P > 0.05$) the survival of either *S. thermophilus* CHCC 742/2130 or *L. acidophilus* La-5 in fermented ABT milks during storage at 4 °C. As one would expect, *B. lactis* Bb-12 were highly susceptible to acid injury. Their counts fell more sharply than those of lactobacilli and streptococci; however, the addition of inulin at 5% (wt/v) improved significantly ($P < 0.05$) their viability after 28 days of refrigerated storage. In this study, the commercial inulin tested was found to have stimulatory properties for *B. lactis* Bb-12 and, thus, it might be suitable for improving the viability of bifidobacteria to some extent in refrigerated fermented milks.

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