# Manufacturing technology for a *Spirulina*-enriched mesophilic fermented milk

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**Abstract** – The objectives of this research were: (1) to test the influence of a *Spirulina (Arthrospira) platensis* biomass on growth and acid production of various *Lactococcus* and *Leuconostoc* strains in milk, (2) to develop a *Spirulina*-containing commercial cultured milk fermented with the mesophilic lactic acid bacteria (LAB) strains selected, and (3) to run storage trials to determine the effect of the *Spirulina* biomass on viability of lactococci in the refrigerated product. Milk samples enriched with *S. platensis* at concentrations up to 0.8% were inoculated at the rate of 1% with the mesophilic LAB strains tested and then incubated at 30°C. The pH values and LAB counts of samples were measured at regular intervals. As part of the product development process, sensory tests were performed by untrained panelists to optimize the organoleptic properties of the final product, and then storage trials were carried out. Used at the rate of 0.3%, the *Spirulina* biomass significantly stimulated ($P < 0.05$) several of the mesophilic LAB strains screened. A technology for production of a *Spirulina*-enriched functional fermented milk has been developed. According to the results of ranking tests done by sensory panelists, optimum organoleptic properties were achieved in the product formulation prepared with the mixed culture of *Lactococcus lactis* subsp. *lactis* NCAIM B.2128 and *Lc. lactis* subsp. *cremoris* ATCC 19257, and supplemented with sucrose at 10%, *S. platensis* biomass at 0.3%, and strawberry-kiwifruit flavor at 1.5%. During the first 2 weeks of refrigerated storage at 4°C, the *S. platensis* biomass significantly increased ($P < 0.05$) the viability percentages of lactococci in the functional fermented milk developed.

**Keywords**: *Lactococcus* / *Leuconostoc* / *Spirulina* / cultured milk

## 1. INTRODUCTION

Because of the changing dietary habits of the general public, functional foods are products of interest to many people. Consumers would need to ingest considerably less medicine and artificially produced vitamin and mineral supplements if fermented milks were enriched with vitamins, proteins, essential fatty acids, and trace elements of natural origin. A simple way of attaining this goal is the use of cyanobacteria in the manufacture of cultured dairy foods (VARGA 1999, VARGA ET AL. 2002).

*Spirulina* (*Arthrospira*) *platensis* is a planktonic cyanobacterium species that forms massive populations in tropical and subtropical water bodies characterized by high levels of carbonate and bicarbonate and pH up to 11 (TOMASELLI 1997). The green colored *Spirulina*, which tastes like grass, is a valuable food supplement that has a wide range of beneficial nutritional effects. It typically contains 3% to 7% moisture, 53% to 63% protein, 4% to 6% lipids, 17% to 25% carbohydrate, 8% to 13% ash, 8% to 10% fiber, 1% to 1.5% chlorophyll-

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*a*, and a wide range of vitamins (BELAY 1997, COHEN 1997, VONSHAK 1997). The objectives of this work included:

- Testing the influence of the *S. platensis* biomass on growth of and acid production by various strains of *Lactococcus* (*Lc.*) *lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, and *Leuconostoc* (*Ln.*) *mesenteroides* in milk.
- Developing a production technology for a functional fermented milk manufactured with the LAB strains selected.
- Running storage trials to determine the effect of the *Spirulina* biomass on the survival of lactococci in the refrigerated product.

## 2. MATERIALS AND METHODS

The experiments were carried out in the accredited Microbiological Laboratory of the Institute of Food Science at the University of West Hungary (Mosonmagyaróvár, Hungary).

### 2.1. Monitoring the changes in acid production and viable cell counts of mesophilic lactic acid bacteria grown in milk

Milk samples enriched with *S. platensis* at different concentrations (*i.e.*, 0%, 0,3%, 0,5% or 0,8%) were inoculated at the rate of 1% with the mesophilic LAB strains to be tested. Incubations were performed in a water bath set at 30°C. The pH value of three replicate samples from all treatments was measured at regular intervals (*i.e.*, every 2 h) with an HI 8521 pH-meter and combined glass electrode standardized with pH 4.01 and 7.01 standard buffer solutions. The experiments were repeated twice.

To monitor the changes in viable cell numbers of the lactococci strains selected, microbial counts of the samples were enumerated at h 0, h 6, and h 12 of the fermentation process in M17 agar using the pour-plate technique. The LAB strains used in the trials were obtained from the following culture collections: Belgian Co-ordinated Collection of Microorganisms (BCCM), National Collection of Agricultural and Industrial Microorganisms (NCAIM), Hungarian Dairy Research Institute Inc. / Chr. Hansen (HDRI/CH), American Type Culture Collection (ATCC).

### 2.2. Developing a functional fermented milk manufactured with mesophilic lactic acid bacteria and *Spirulina* biomass

As part of the product development process, three ranking tests were performed by 5, 11, and 12 sensory panelists, respectively, in an attempt to optimize the organoleptic properties of the final product. The samples were ranked according to the intensity of their sensory properties, with overall taste being the main ranking parameter.

### 2.3. Influence of the *Spirulina* biomass on mesophilic lactic acid bacteria during refrigerated storage of the model product

The *Spirulina*-enriched and control fermented milks used in the storage trials were manufactured at the pilot plant of the Hungarian Dairy Research Institute (Mosonmagyaróvár, Hungary). Antibiotic-free raw milk containing (per $dm^3$) 36.5 g of fat, 31.5 g of protein, 47 g of lactose, and 7 g of ash served as raw material. It was heated to 90°C and held for 10 min. In the case of the cyanobacterial product, *S. platensis* was added to the heat-treated milk cooled to 70°C. Plain milk and *Spirulina*-fortified milk were homogenized at a pressure of 18 MPa (180 bar) in a high pressure homogenizer. They were cooled to 30°C, and were inoculated with the starter culture selected by the sensory panelists. Incubation took approximately 10 h at 30°C. Sucrose at 10% and flavoring substances at 1.5% were then added during stirring at

pH 4.7. Thereafter, both *Spirulina*-enriched and control fermented milks were filled into 21 sterile, tightly capped centrifuge tubes (30 $cm^3$) each. The products were stored in a refrigerator at 4±1°C. Three cyanobacterial and three control samples were taken at weekly intervals, and their *Lactococcus* spp. counts were enumerated. The experimental program was repeated twice.

## 3. RESULTS AND DISCUSSION

### 3.1. Changes in acid production and viable cell counts of mesophilic lactic acid bacteria grown in milk

#### *3.1.1. Optimum concentration of the* Spirulina *biomass*

*Spirulina* levels capable of effectively stimulating acid production of lactococci were determined using *Lc. lactis* subsp. *lactis* Ha-2 and *Lc. lactis* subsp. *cremoris* W-24. The cyanobacterial biomass, used at 0.1% to 0.8%, was found to significantly increase ($P < 0.05$) the rate of acid development by lactococci between h 6 and h 12 of the fermentation process (data not shown). For this reason and because of organoleptic and economic considerations, further trials were run with *Spirulina* use at 0.3% (w/v).

#### *3.1.2. Effect of the* Spirulina *biomass on single strains of mesophilic lactic acid bacteria*

The effects of 0.3% (w/v) *Spirulina* on acid development by two strains of *Lc. lactis* subsp. *lactis* (NCAIM B.2125 and NCAIM B.2128), two strains of *Lc. lactis* subsp. *cremoris* (NCAIM B.2124 and ATCC 19257), four strains of *Lc. lactis* subsp. *lactis* var. *diacetylactis* (NCAIM B.2122, NCAIM B.2123, NCAIM B.2126, and NCIM B.2127), one strain of *Ln. mesenteroides* subsp. *cremoris* (NCAIM B.2120), and one strain of *Ln. mesenteroides* subsp. *dextranicum* (NCAIM B.1658) were monitored during fermentation. The results are shown in *Table 1*. Negative numbers mean that *Spirulina*-enriched samples had higher pH than controls, whereas positive numbers indicate that the pH of cyanobacterial samples was lower than that of controls.

*Table 1. Influence of 0.3% (w/v)* Spirulina *biomass on acid production of the mesophilic lactic acid bacteria strains tested*

| Strain | Average decrease in pH during fermentation compared to controls | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | h 0 | h 2 | h 4 | h 6 | h 8 | h 10 | h 12 | h 14 |
| NCAIM B.2125 | -0.07 | -0.07 | -0.17 | -0.16 | -0.10 | -0.02 | 0.00 | 0.00 |
| NCAIM B.2128 | -0.05 | -0.04 | +0.03 | **+0.25** | **+0.38** | **+0.22** | **+0.16** | **+0.14** |
| NCAIM B.2122 | -0.05 | -0.04 | +0.02 | +0.10 | +0.26 | +0.43 | +0.50 | +0.51 |
| NCAIM B.2123 | -0.06 | -0.06 | -0.06 | +0.01 | **+0.08** | +0.03 | +0.03 | +0.01 |
| NCAIM B.2126 | -0.06 | -0.07 | **-0.10** | -0.14 | +0.01 | +0.03 | +0.03 | **+0.03** |
| NCAIM B.2127 | **-0.04** | **-0.03** | **-0.02** | **+0.22** | **+0.54** | **+0.61** | **+0.51** | **+0.58** |
| NCAIM B.2124 | **-0.06** | -0.03 | **+0.04** | **+0.14** | **+0.30** | **+0.19** | **+0.18** | **+0.14** |
| ATCC 19257 | **-0.05** | **+0.03** | **+0.12** | **+0.56** | **+0.59** | **+0.14** | **+0.04** | **+0.06** |
| NCAIM B.2120 | -0.07 | -0.04 | 0.00 | **+0.12** | **+0.53** | **+0.86** | **+0.92** | **+0.90** |
| NCAIM B.1658 | **-0.05** | **-0.05** | **-0.07** | -0.03 | +0.09 | **+0.10** | **+0.09** | **+0.10** |

-: Retardation of acid production.
+: Stimulation of acid production.
**Bold number**: Significantly different at the $P = 0.05$ level (n = 6).

Means of the initial pH values (at h 0) of *Spirulina*-enriched samples were higher than those of controls because the cyanobacterial biomass is of alkaline character (an aqueous

solution containing 3 g/dm$^3$ *Spirulina* has a pH of 9.9) and it also possesses considerable buffering capacity.

Used at the rate of 0.3% (w/v), *Spirulina* significantly increased ($P < 0.05$) the acid production of *Lc. lactis* subsp. *lactis* NCAIM B.2128, *Lc. lactis* subsp. *lactis* var. *diacetylactis* NCAIM B.2127, *Lc. lactis* subsp. *cremoris* ATCC 19257, *Lc. lactis* subsp. *cremoris* NCAIM B.2124 and *Ln. mesenteroides* subsp. *cremoris* NCAIM B.2120 during the fermentation process (*Table 1*).

*Figure 1* illustrates the changes in viable numbers of *Lc. lactis* subsp. *lactis* NCAIM B.2128, *Lc. lactis* subsp. *lactis* var. *diacetylactis* NCAIM B.2127 and *Lc. lactis* subsp. *cremoris* ATCC 19257 in cyanobacterial and control samples.

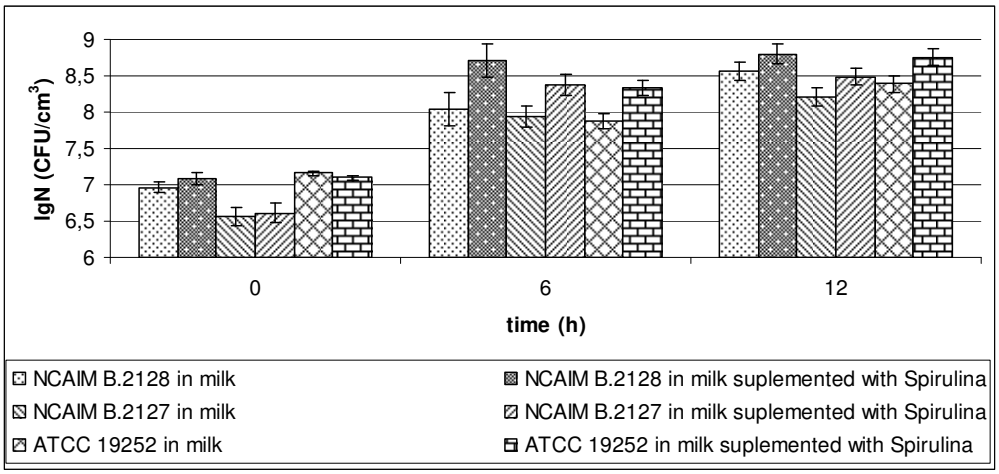


*Figure 1. Changes in viable cell counts of* Lactococcus lactis *subsp.* lactis *NCAIM B.2128,* Lc. lactis *subsp.* lactis *var.* diacetylactis *NCAIM B.2127, and* Lc. lactis *subsp.* cremoris *ATCC 19257 during fermentation in milk and in* Spirulina*-enriched milk (whiskers indicate 95% confidence intervals of means; n = 6)*

By h 6 of the fermentation process, *Spirulina* enrichment resulted in a significant increase ($P < 0.05$) in the growth rates of all three strains tested. In the case of *Lc. lactis* subsp. *lactis* var. *diacetylactis* NCAIM B.2127 and *Lc. lactis* subsp. *cremoris* ATCC 19257, significant differences ($P < 0.05$) were observed in viable counts at h 12 as well (*Figure 1*).

### 3.2. Development of a functional fermented milk manufactured with mesophilic lactic acid bacteria and *Spirulina* biomass

According to the results of ranking tests done by sensory panelists, optimum organoleptic properties were achieved in the product formulation prepared with the mixed culture of *Lc. lactis* subsp. *lactis* NCAIM B.2128 and *Lc. lactis* subsp. *cremoris* ATCC 19257, and supplemented with sucrose at 10%, *Spirulina* biomass at 0.3%, and strawberry-kiwifruit flavor at 1.5%. *Figure 2* illustrates the manufacturing technology of the novel functional fermented milk developed.

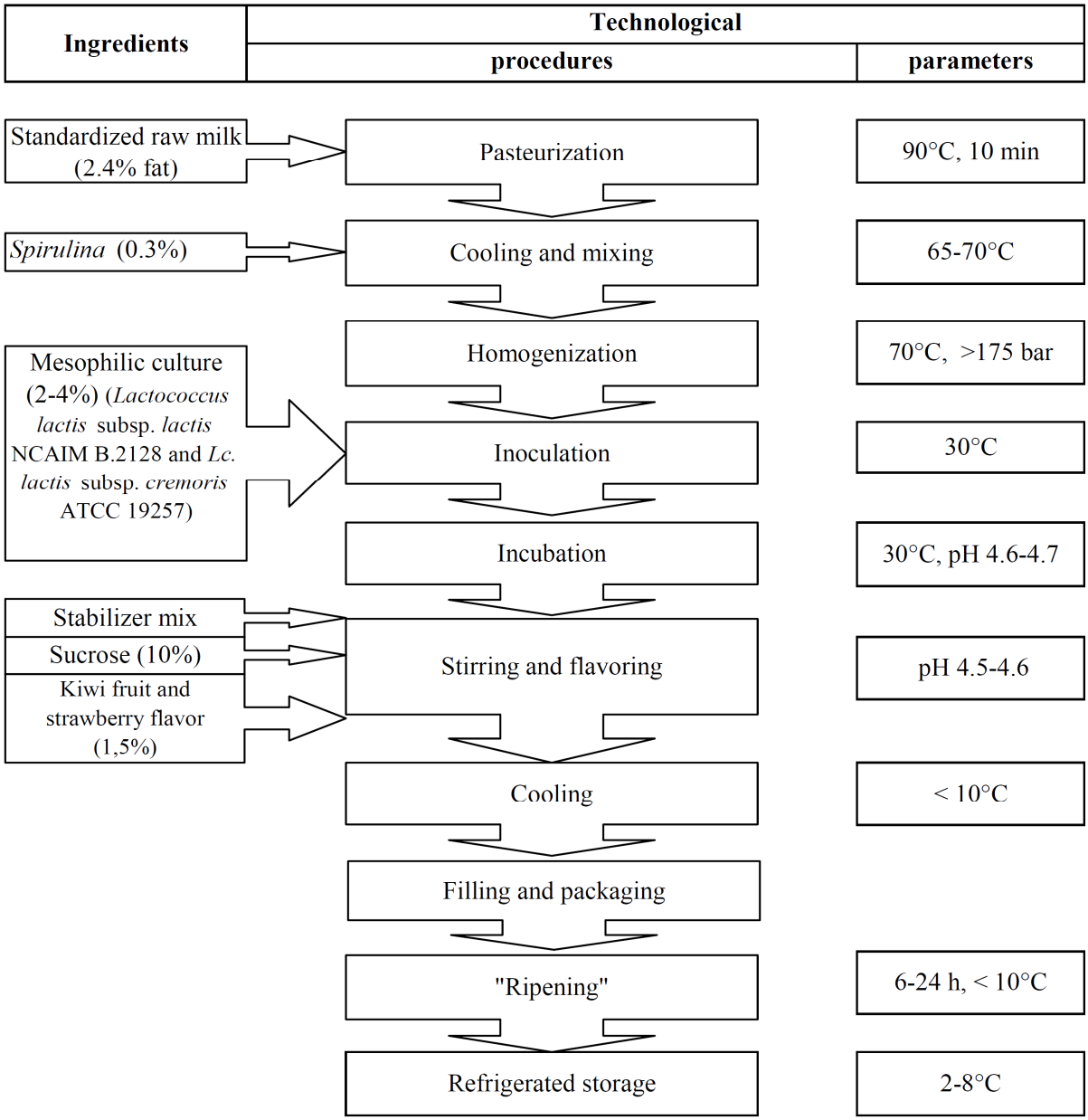


*Figure 2. Technology of manufacture for the novel* Spirulina*-enriched functional fermented milk*

### 3.3. Influence of the *Spirulina* biomass on shelf life of the newly developed product

During the first 2 wk of refrigerated storage at 4±1°C, the *Lactococcus* counts were significantly higher ($P < 0.05$) in the cyanobacterial fermented milk than in the control product (*Table 2*), confirming earlier reports on the stimulatory effects of *Spirulina* on coccus-shaped starter LAB (VARGA 1999). It is worth mentioning that an increase was observed in viable cell counts of both products during the first week of storage, however, viability percentages declined slowly thereafter. The lactococci counts of the two products did not differ significantly ($P > 0.05$) at the end of the 6-wk storage period (*Table 2*).

*Table 2. Viability of lactococci in* Spirulina*-enriched and control fermented milks during storage at 4°C*

| Storage time (day) | *Lactococcus* count ($Log_{10}$ CFU/$cm^3$)* | | *Lactococcus* survival (%)** | |
|---|---|---|---|---|
| | Control | *Spirulina*-enriched | Control | *Spirulina*-enriched |
| 0 | 8.53 ± 0.05 | **8.65 ± 0.07** | 100.00 | 100.00 |
| 7 | 8.66 ± 0.17 | **8.92 ± 0.18** | 133.78 | 186.00 |
| 14 | 8.49 ± 0.17 | **8.79 ± 0.23** | 91.21 | 137.03 |
| 21 | 8.47 ± 0.05 | 8.65 ± 0.16 | 86.78 | 100.92 |
| 28 | 8.39 ± 0.10 | 8.26 ± 0.17 | 71.83 | 40.72 |
| 35 | 7.57 ± 0.11 | 7.57 ± 0.12 | 11.05 | 8.34 |
| 42 | 7.44 ± 0.07 | 7.43 ± 0.05 | 8.06 | 6.09 |

* Values are means ± SD, based on 6 observations (3 samples × 2 replicates).
** Values are means calculated from *Lactococcus* count ($Log_{10}$ CFU/$cm^3$) means.
**Bold number**: Significantly different from the respective control ($P < 0.05$).

Food regulations in Hungary require fermented milks to contain LAB of starter culture origin at concentrations of at least $10^7$ CFU/g over the shelf life of the product (CODEX ALIMENTARIUS HUNGARICUS COMMISSION 2004). The lactococci counts in both products largely exceeded this value throughout the entire storage period (*Table 2*).

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