Health-promoting fermented milks

by
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"Comprehensive Development of Conditions for Student Talent Management at the Univesity of West Hungary"
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Introduction

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A legend

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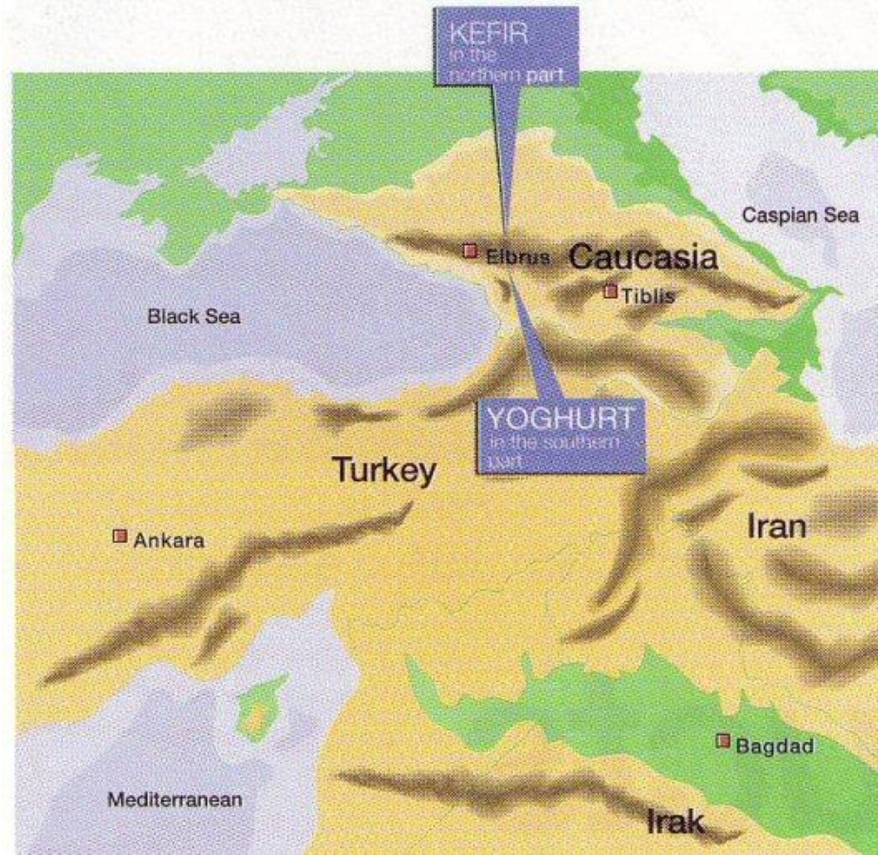
- Born on the slopes of Mount Elbrus in Caucasia.
  - Southern slopes: **yogurt** (name introduced in 8th century, changed to its present form in 11th century).
  - Northern slopes: **kefir** (in Turkish: kef = pleasurable), alcohol content up to 0.8%.



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The origin of fermented milks

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- Exact origin is difficult to establish.
- It could date from 10.000 to 15.000 yr ago.
- Way of life changed from food gathering to food producing.
- Today, fermented milks are manufactured worldwide.
- Approx. 400 generic names throughout the world for practically the same products.
- Primary **thermophilic** starters used for milk fermentations:
  - ***Streptococcus thermophilus***
  - ***Lactobacillus delbrueckii* subsp. *bulgaricus***
  - ***Lb. acidophilus***
  - ***Bifidobacterium* spp.**

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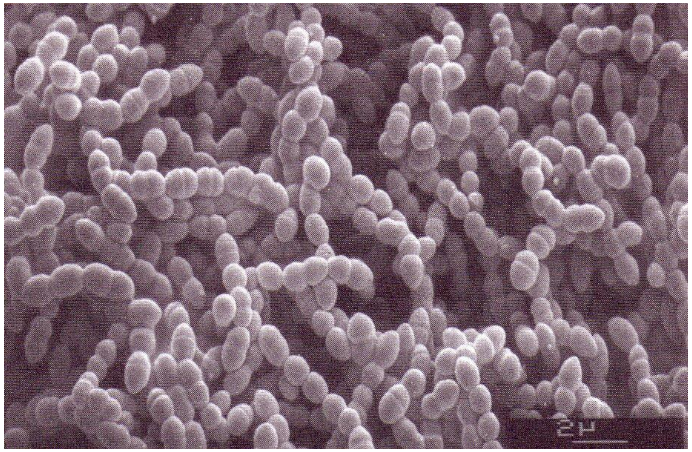
Streptococcus thermophilus

- Occurs in milk in long chains of 10-20 spherical/ovoid cells.
- Optimum growth temperature: **37°C**.
- Ferments lactose homofermentatively: **L(+)** lactic acid.
- Growth and metabolism are inhibited above **1% lactic acid**.
- **Glucose**, fructose and mannose are metabolized, but the fermentation of **galactose**, maltose and sucrose is strain specific.
- Displays **limited proteolytic ability**. Its source of nitrogen is, at least initially, free amino acids occurring naturally in milk.



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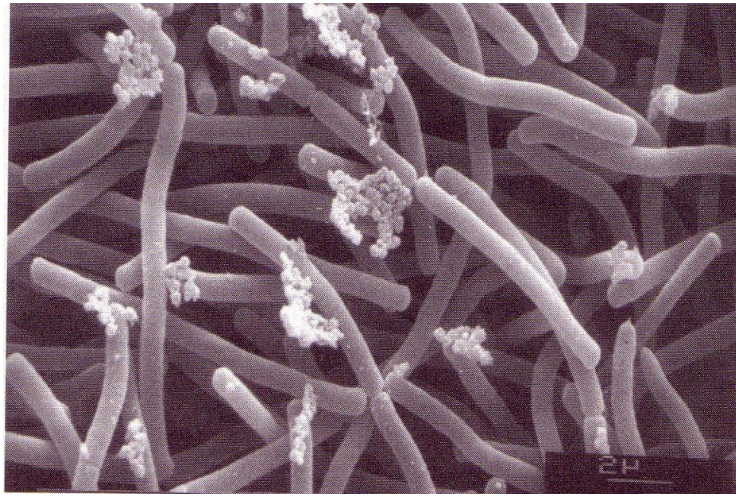
Lactobacillus delbrueckii subsp. *bulgaricus*

- Occurs in milk as chains of 3-4 short rods with rounded ends.
- Optimum growth temperature: **45°C**.
- Ferments lactose homofermentatively: **D(-)** lactic acid.
- **Glucose**, fructose, **lactose** and, in some strains, **galactose** are all utilized by this species.
- **Hydrolyzes casein**, by means of a wall-bound proteinase, to release peptides. However, its peptidase activity is limited.



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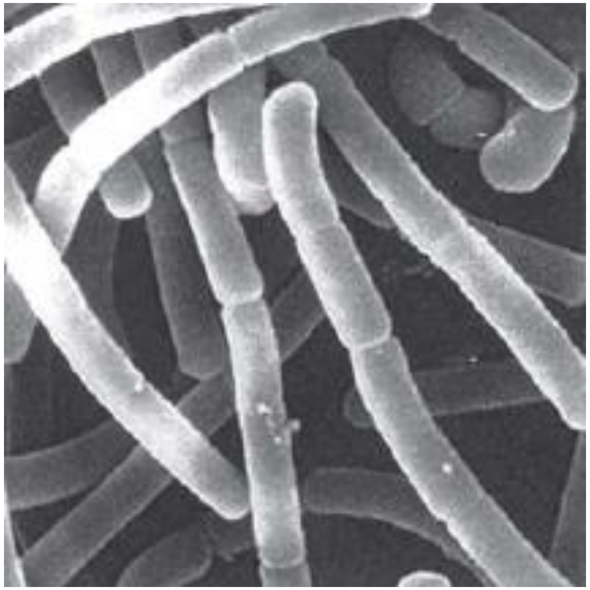
Lactobacillus acidophilus

- Occurs singly, in pairs, or in short chains.
- Obligately homofermentative species.
- Optimum growth temperature: **45°C**.
- Found in the distal end of the small intestine**small intestine**.
- Many strains are extensively used as **probiotics**. The **health benefits** associated with probiotic strains include:
  - reduction of gastrointestinal symptoms in lactose-intolerant individuals;
  - relief from symptoms of constipation;
  - treatment of infantile diarrhea;
  - activity against *Helicobacter pylori*;
  - immune-enhancing effects;
  - stimulation of phagocytosis in humans.



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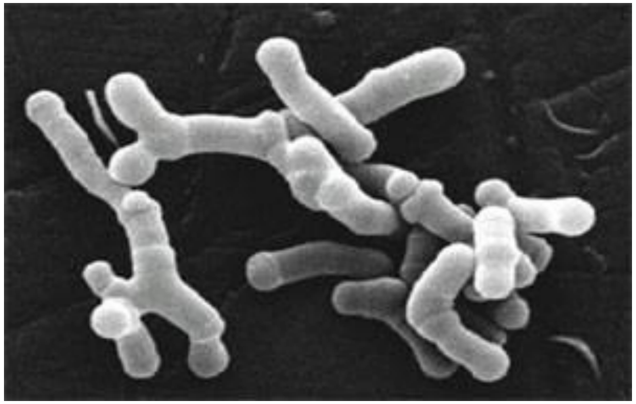
Bifidobacterium spp.

- Show bacillar form, with some strains developing **V, Y, X**, or other shapes.
- 29 described species with 14 occurring in humans.
- Predominant species in the **large intestine**, especially in the proximal colon.
- Produce **acetic and lactic acids** in the ratio of 3:2.
- Optimum temperature: **37°C** for strains of human origin.
- Optimum **pH: 6.5-7.0** (no growth below pH 5.0).
- Grow better in **human** than in bovine **milk**.
- Human species utilize glucose, galactose and lactose.
- **Lack of proteolytic activity**: poor growth in milk.



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Influence of various substances on thermophilic dairy starter bacteria during manufacture & storage of cultured milks

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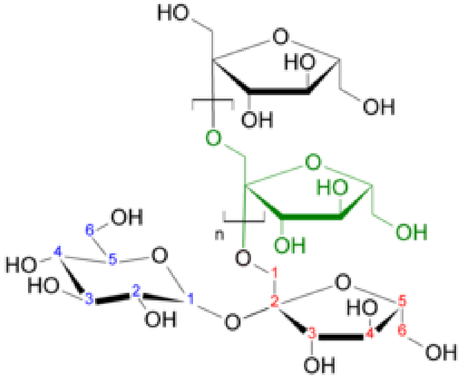
Classic prebiotics

- Prebiotic oligosaccharides are increasingly added to foods for their health benefits. Some oligosaccharides that are used in this manner are **fructooligosaccharides** (FOS), xylooligosaccharides (XOS), and galactooligosaccharides (GOS).
- **Fructooligosaccharides** (FOS), which are found in many vegetables, consist of linear chains of **2-7 fructose** molecules.
- **Inulin** has a much higher degree of polymerization (**up to 60 fructose** molecules) than FOS. Almost all chains terminate in one **glucose** molecule. These compounds can be only partially digested by humans.
- When oligosaccharides are consumed, the undigested portion serves as **food for the intestinal microbiota**. Depending on the type of oligosaccharide, different bacterial groups are stimulated or suppressed.
- Studies have demonstrated positive effects on calcium and other **mineral absorption**, immune system effectiveness, bowel pH, and intestinal regularity. Correlations have also been made with other positive health factors, but more research is required.



Inulin

Trials with oligofructose and inulin

- The **objective** of these studies was to investigate the influence of **oligofructose** and **inulin** on the microbial flora of probiotic fermented milks during refrigerated storage.

- Oligofructose- and inulin-supplemented and control fermented acidophilus-bifidus-thermophilus (**ABT**) **milks** were produced using a fast fermentation starter culture as the source of *Lactobacillus acidophilus* (A), bifidobacteria (B), and *Streptococcus thermophilus* (T).

- Products were stored at 4°C for 42 d. Microbiological analyses and acidity measurements were performed at weekly intervals.

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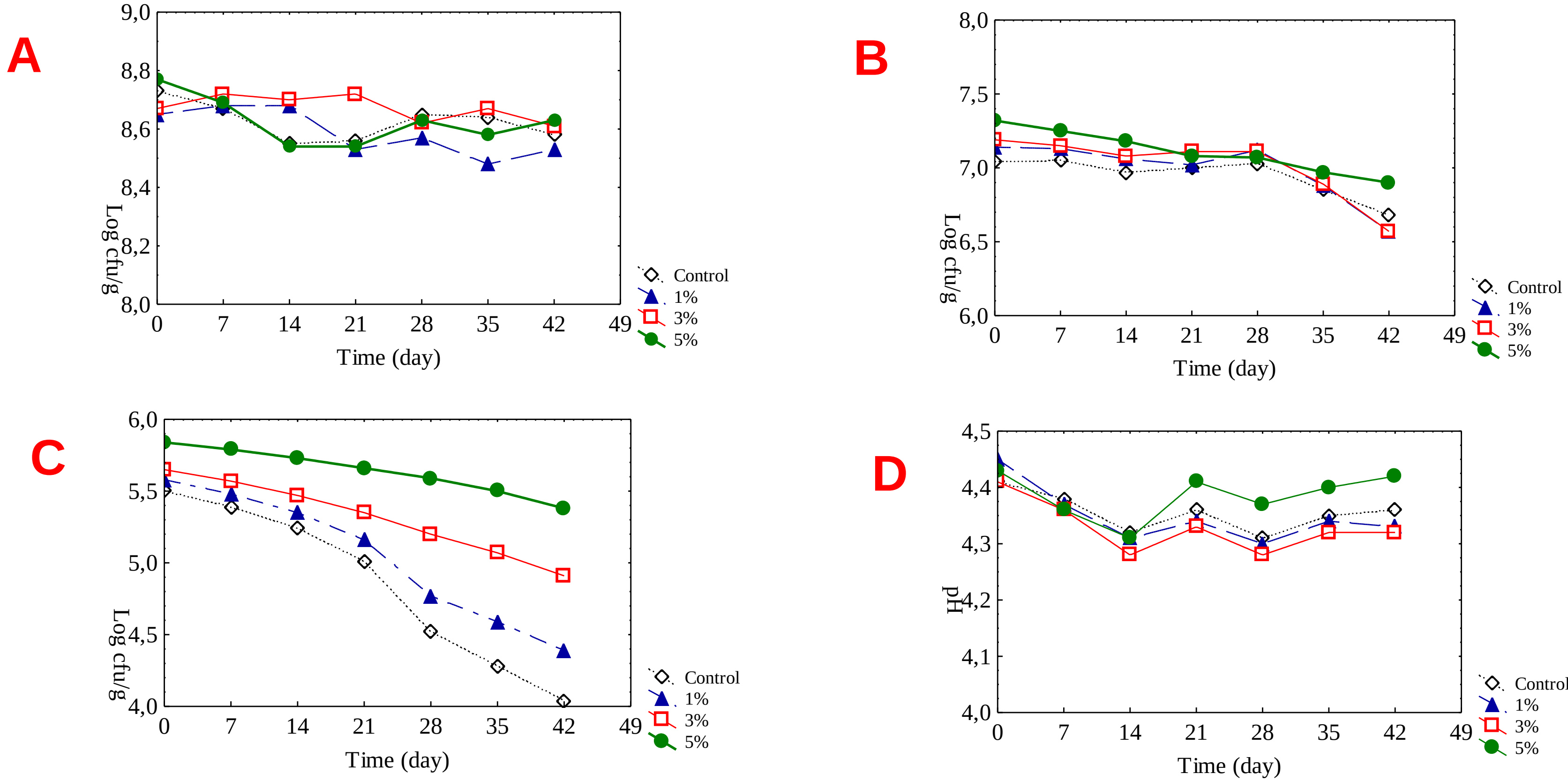

Survival of *Streptococcus thermophilus* (**A**), *Lactobacillus acidophilus* (**B**), and *Bifidobacterium* spp. (**C**) in **oligofructose**-supplemented and control fermented ABT milks; and changes in pH (**D**) of these products during storage at 4°C

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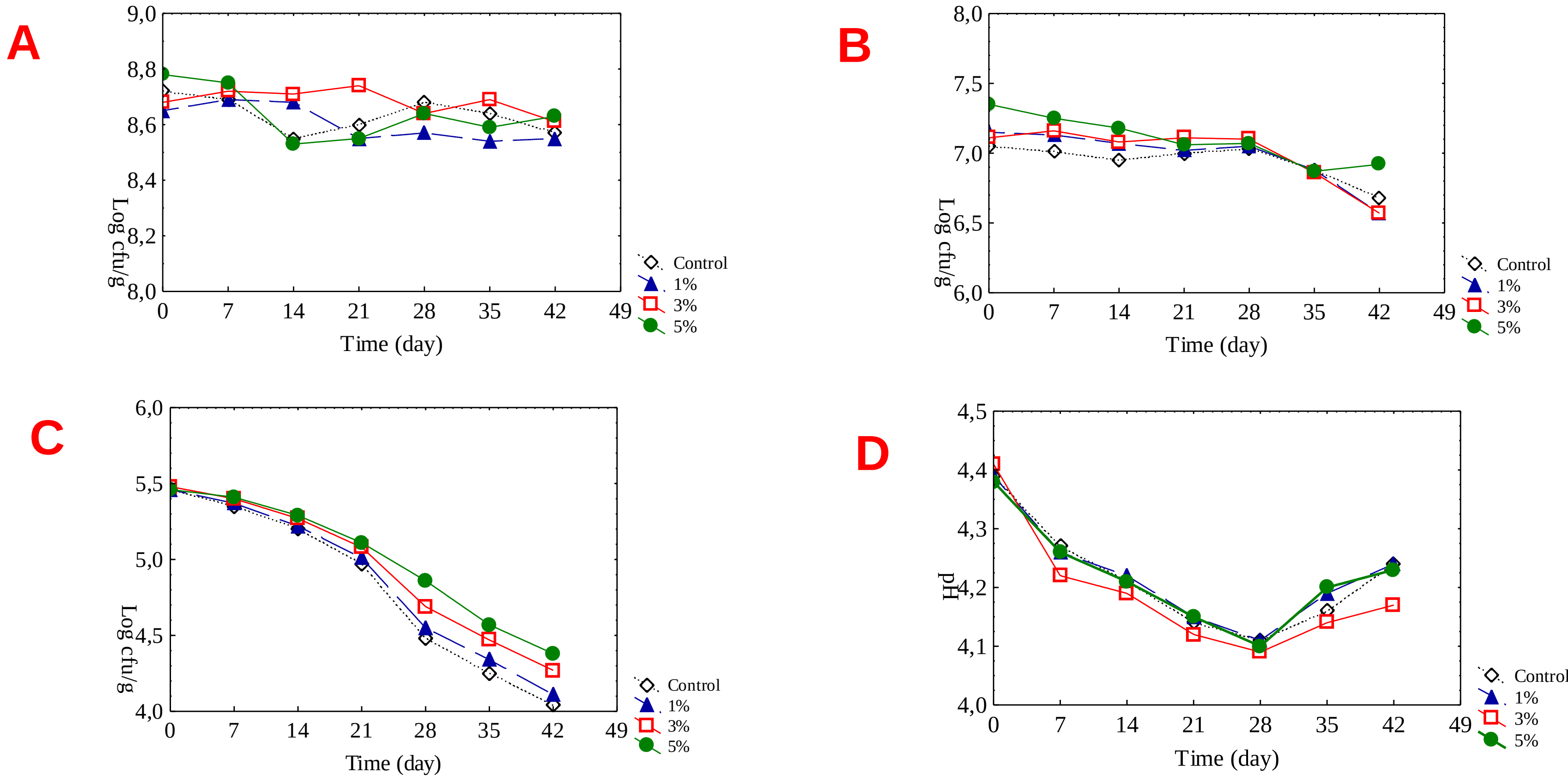

Survival of *Streptococcus thermophilus* (**A**), *Lactobacillus acidophilus* (**B**), and *Bifidobacterium* spp. (**C**) in **inulin**-supplemented and control fermented ABT milks; and changes in pH (**D**) of these products during storage at 4°C

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In **summary**, the commercial oligofructose and inulin products tested had – although to varying degrees – beneficial effects on the viability of bifidobacteria in refrigerated fermented ABT milks.

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Honey

- In recent years, there has been increasing interest in the use of natural food additives and incorporation of health-promoting substances into the diet.
- Honey has been gaining interest as a substitute sweetener in various foods because of its healthy and natural image. Honey-sweetened products are viewed as value-added and consumers are willing to pay up to 13% more for them compared to products containing other sweeteners.
- Honey is a natural syrup containing primarily fructose (38.4%) and glucose (30.3%). Other sugars in honey include maltose (7.2%), sucrose (1.3%), and various **oligosaccharides** (**up to 10.9%**).
- Its low pH makes honey compatible with many food products in terms of acidity.
- Honey–yogurt combinations are relatively uncommon.

Trials with honey

- **Honey**-enriched and control **yogurts** and **fermented ABT milks** were produced.
- Products were stored at 4°C for 6 wk. Microbiological analyses and pH measurements were performed at weekly intervals.

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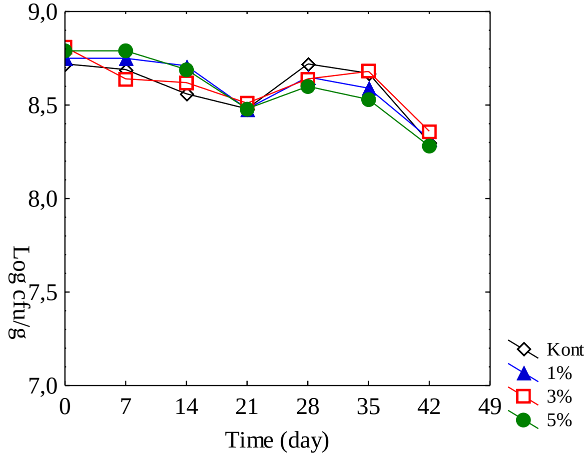

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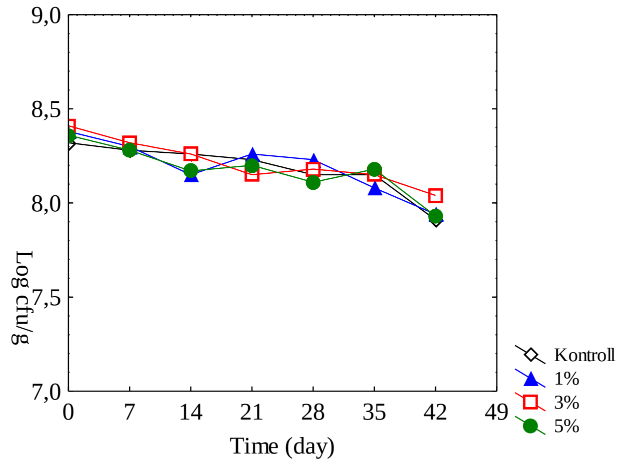

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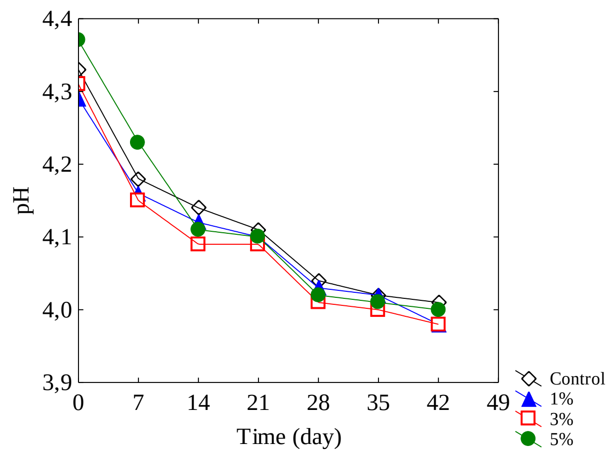

Survival of *Streptococcus thermophilus* (**A**) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (**B**)
in control and **honey**-enriched yogurts;
and changes in pH (**C**) of these products during storage at 4°C

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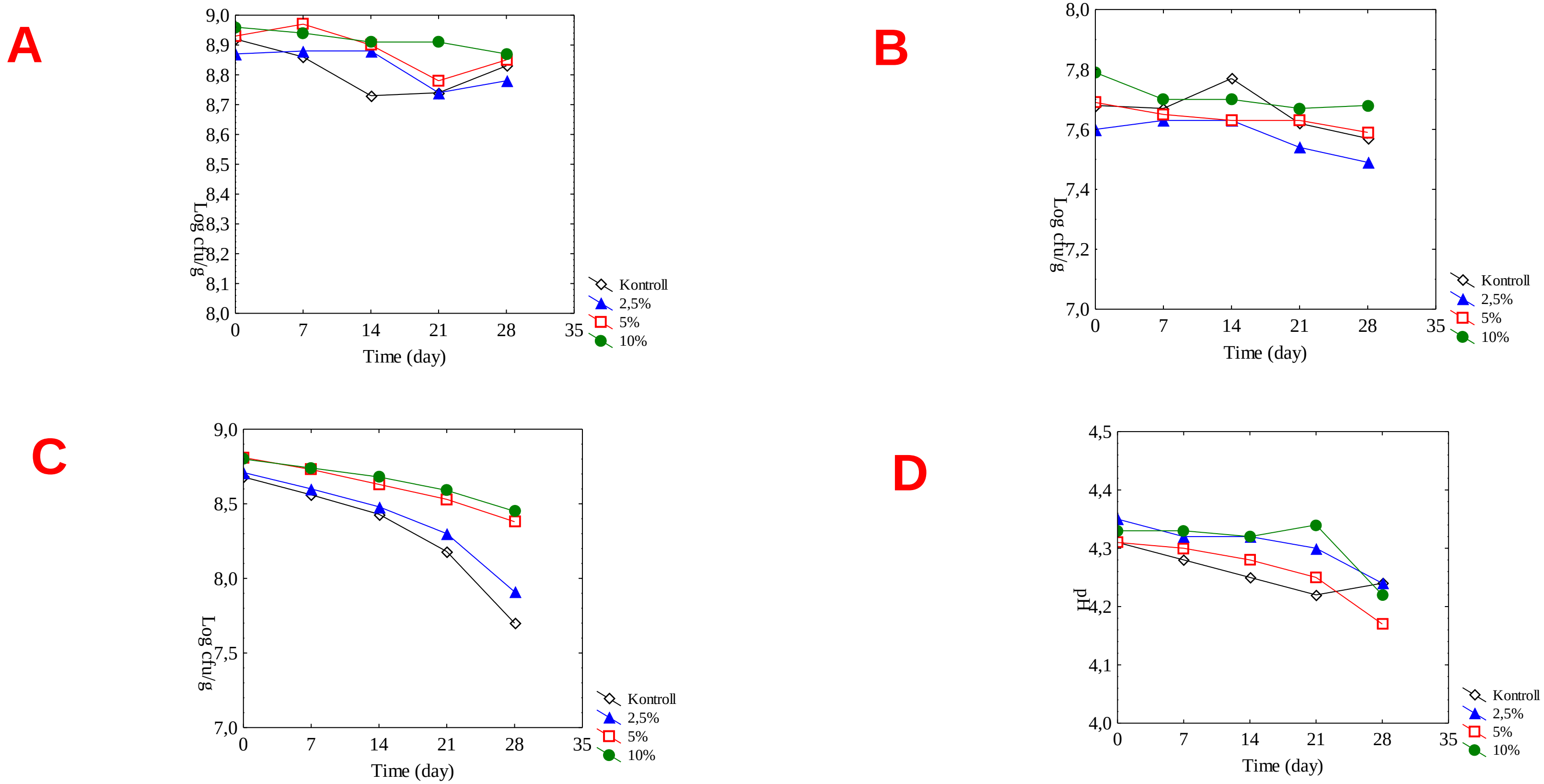

Survival of *Streptococcus thermophilus* (**A**), *Lactobacillus acidophilus* (**B**), and *Bifidobacterium* spp. (**C**) in control and **honey**-enriched fermented ABT milks;
and changes in pH (**D**) of these products during storage at 4°C

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Enrichment of fermented milks with honey is recommended because honey:

- is a natural sweetener possessing a variety of beneficial nutritional properties,
- improves the sensory quality of the finished product,
- does not have an inhibitory effect on characteristic LAB in yogurt,
- improves viability of bifidobacteria in probiotic fermented milks.

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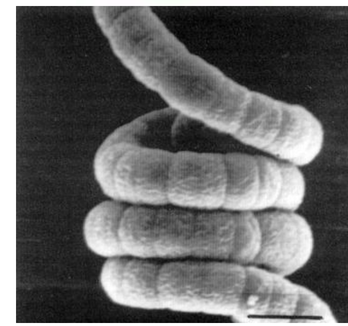
Microalgae

- **Microalgae** are photosynthetic microorganisms that can be used to produce high value compounds.
- Spray-dried microalgal biomasses typically contain
  - 3-7% moisture,
  - **45-70% protein**,
  - 15-25% carbohydrates,
  - 4-22% lipids,
  - 2-4% nucleic acid,
  - 7-13% ash,
  - 5-10% fiber, and
  - a wide range of vitamins and other biologically active substances.
- Microalgae have been commercially produced for approx. 40 years now with the main species grown being ***Chlorella*** and ***Spirulina*** for health food.
- ***Spirulina platensis*** is a filamentous cyanobacterium species, whereas ***Chlorella vulgaris*** belongs to single-celled green algae.



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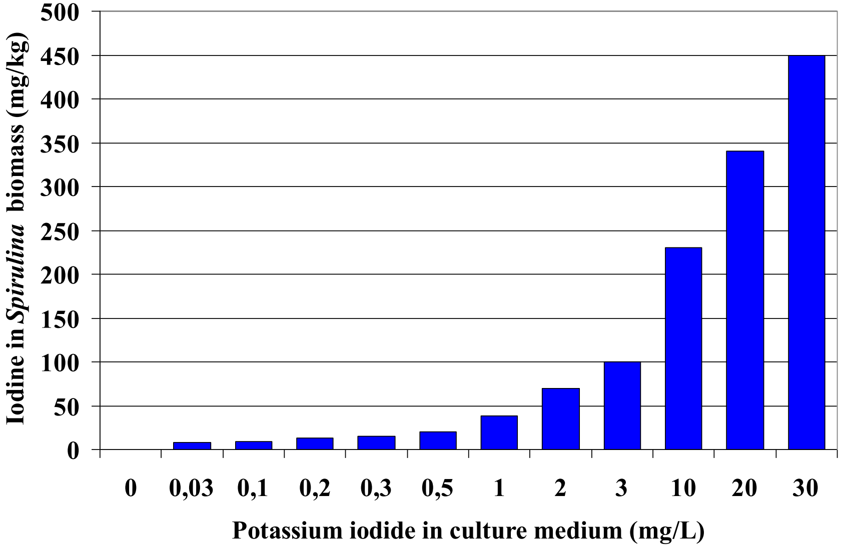
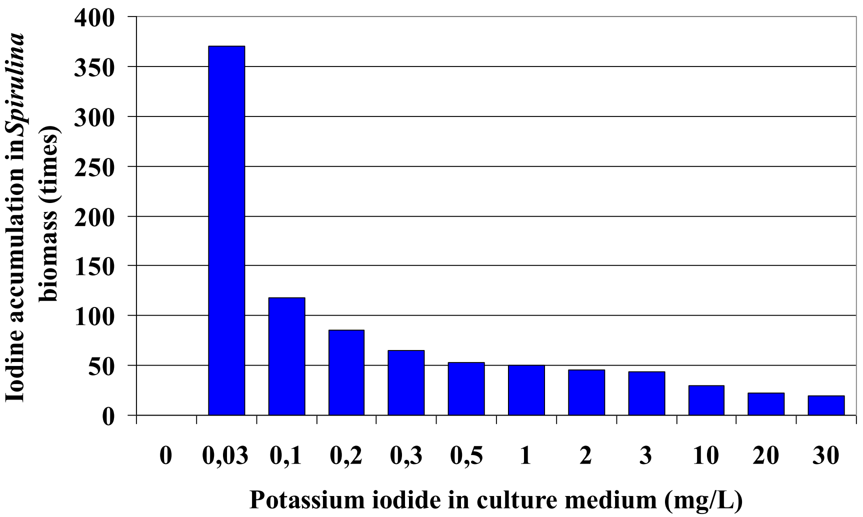
Trials with microalgae:
I. Accumulation of trace minerals

- The **purpose** of this research was to investigate the accumulation of selected trace minerals by *S. platensis*.
- *Spirulina* was grown for 8 d in artificial media containing KI, $ZnCl_2$, or $Na_2SeO_3 \cdot 5H_2O$ at 0.03 to 30 mg/L.
- I, Zn, or Se levels in the dried biomass were determined.



Iodine content of *Spirulina platensis* biomasses after 8-d cultivation of cyanobacteria in growth media containing potassium iodide at various concentrations

Accumulation of iodine in *Spirulina platensis* biomasses after 8-d cultivation of cyanobacteria in growth media containing potassium iodide at various concentrations



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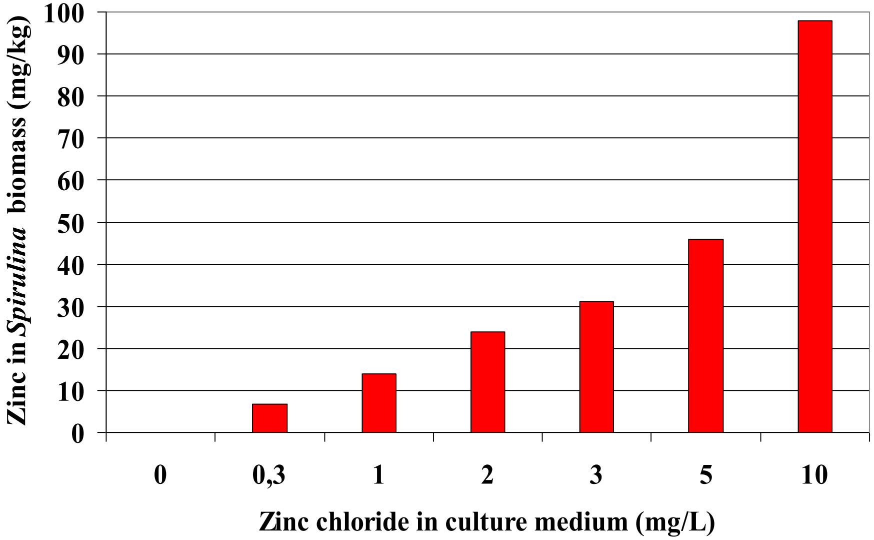
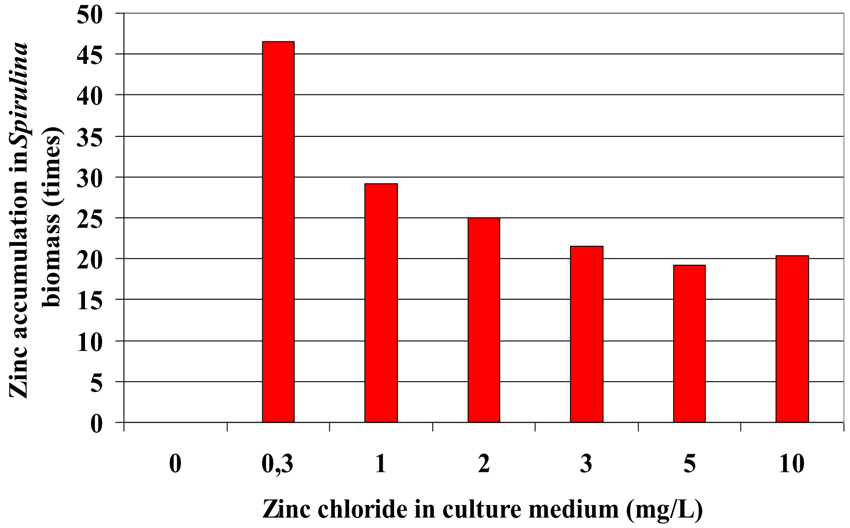


Zinc content of *Spirulina platensis* biomasses after 8-d cultivation of cyanobacteria in growth media containing zinc chloride at various concentrations



Accumulation of zinc in *Spirulina platensis* biomasses after 8-d cultivation of cyanobacteria in growth media containing zinc chloride at various concentrations

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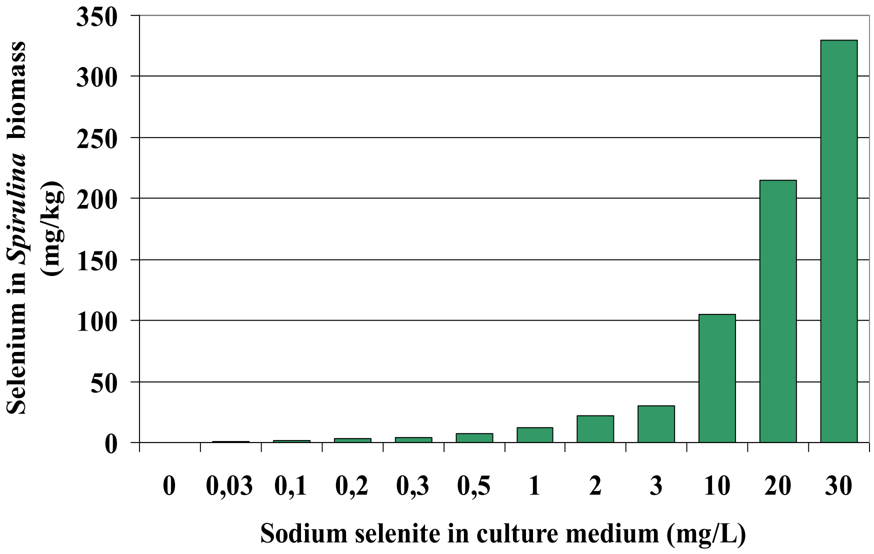
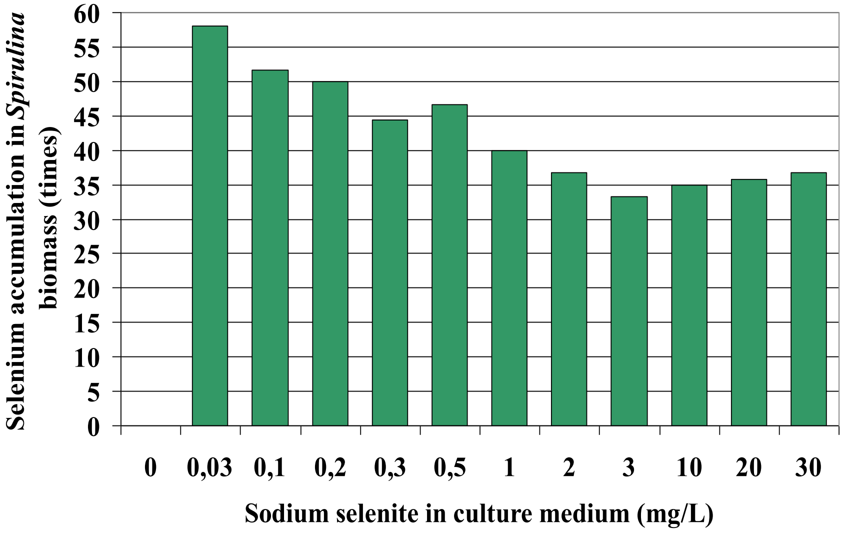


Selenium content of *Spirulina platensis* biomasses after 8-d cultivation of cyanobacteria in growth media containing sodium selenite at various concentrations

Accumulation of selenium in *Spirulina platensis* biomasses after 8-d cultivation of cyanobacteria in growth media containing sodium selenite at various concentrations



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In **summary**, the cyanobacteria accumulating I, Zn, and Se in their cells are highly suitable for human consumption because minerals are present in **organic or complex bonds** in the *Spirulina* cells and, thus, trace elements have an **increased absorption rate** and reduced **toxicity**, and their beneficial effects are further improved by the proteins, vitamins, and other **bioactive substances** found in the *Spirulina* biomass.

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Trials with microalgae:
II. Effects on acid production of dairy starters

- The effects of a dried *Spirulina platensis* biomass enriched with trace elements on the rate of acid development by **single and mixed cultures** of *Streptococcus thermophilus* CH-1, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2, *Lactobacillus acidophilus* La-5, and *Bifidobacterium lactis* Bb-12 were evaluated in milk.

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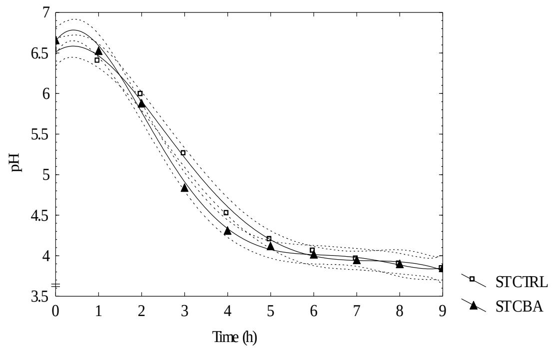

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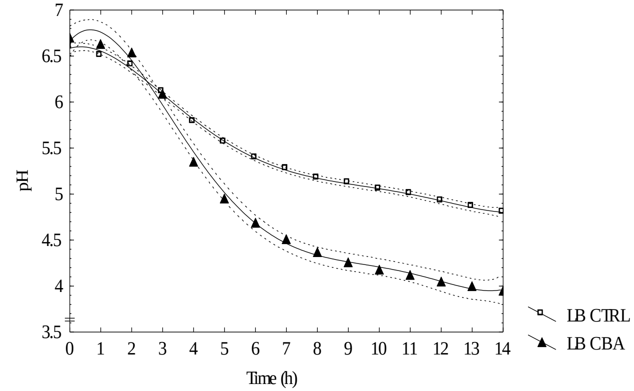

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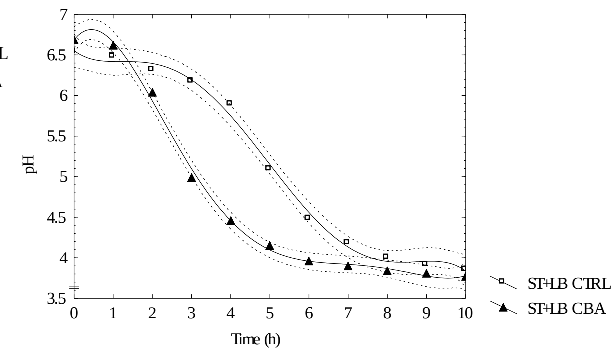

Effect of 3 g/L cyanobacterial (CBA) biomass on acid production by *Streptococcus thermophilus* (**A**), *Lactobacillus delbrueckii* subsp. *bulgaricus* (**B**) and their mixed culture (**C**) in milk (CTRL: control)

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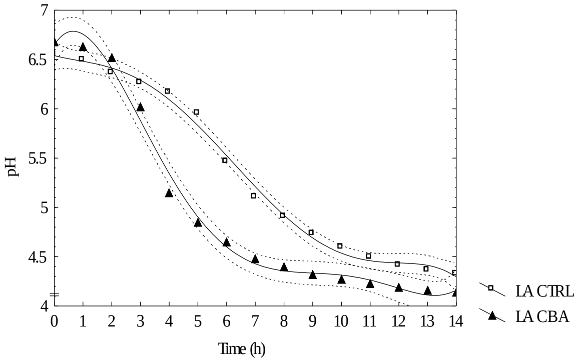

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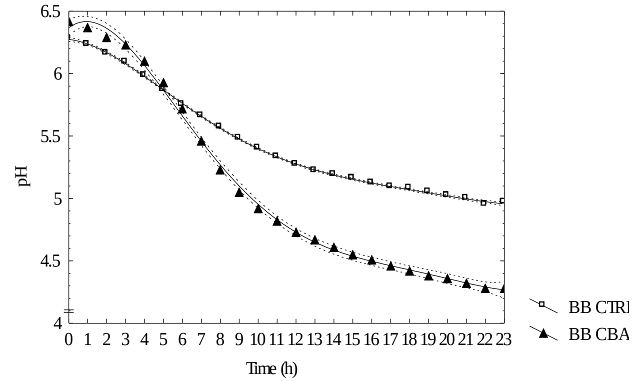

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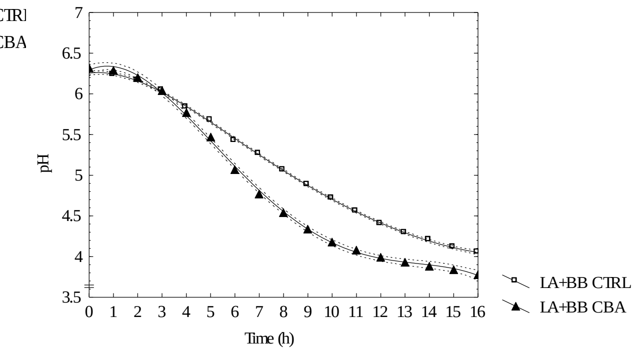

Effect of 3 g/L cyanobacterial (CBA) biomass on acid production by *Lactobacillus acidophilus* La-5 (A), *Bifidobacterium lactis* Bb-12 (B) and their mixed culture (C) in milk (CTRL: control)

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- The **components** of the *Spirulina* biomass *probably* responsible for the stimulation were tested in laboratory simulations, wherein the effects of **trace elements** (iodine, zinc, selenium), **vitamins** (B complex, C, A, E) and **nitrogenous compounds** (peptone, adenine, hypoxanthine) were investigated.

- Stimulation of the strains by the *Spirulina* biomass was, for the most part, due to **peptone**, **adenine** and **hypoxanthine**.

- The *Spirulina* biomass, that was rich in trace elements, vitamins, sulfur-containing amino acids and unsaturated fatty acids, also had a highly beneficial effect on the **nutritional value of milk**, thus providing a new opportunity for manufacture of **functional dairy products**.

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Trials with microalgae:
III. Effects on microbiota during storage

- **Yogurts** and fermented **ABT milks** fortified with *Spirulina* were produced and stored at either 4°C or 15°C*.*
- Microbiological analyses were performed at regular intervals.

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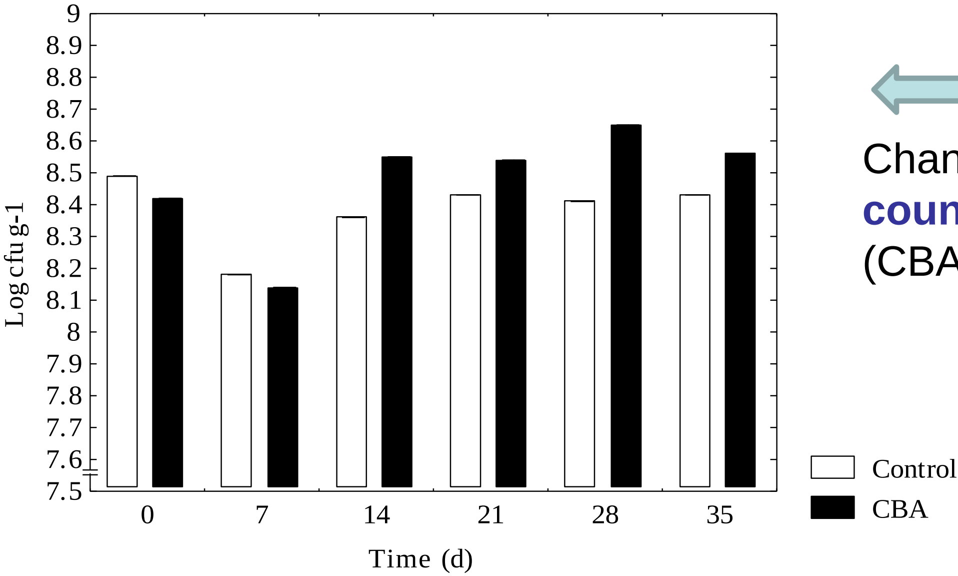
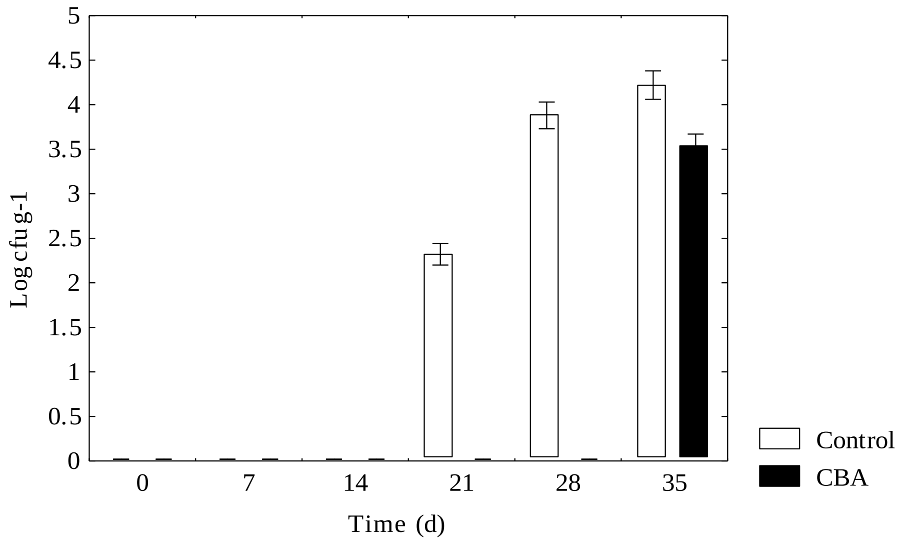


Changes in the characteristic **viable cell counts** of control and *Spirulina*-enriched (CBA) **yogurts** during storage at 4°C



Changes in **yeast and mold counts** in control and *Spirulina*-enriched (CBA) **yogurts** during storage at 4°C

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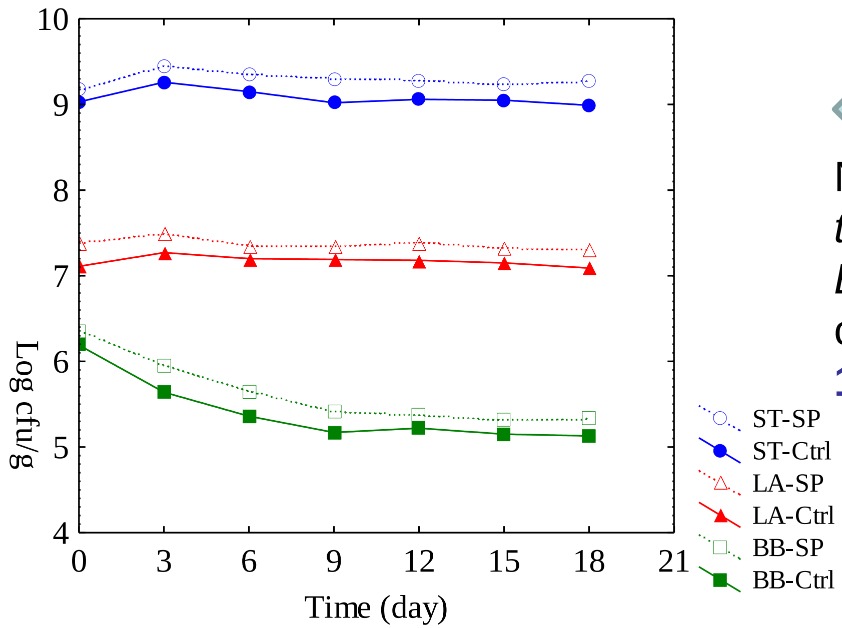
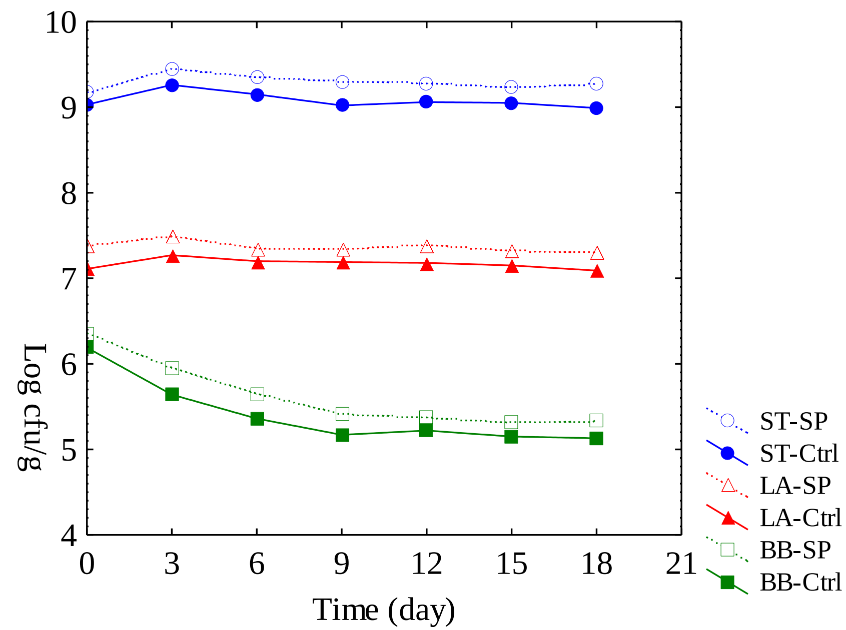


Numbers of surviving *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* spp. in *Spirulina*-enriched and control fermented **ABT milks** during storage at **15°C**



Numbers of surviving *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* spp. in *Spirulina*-enriched and control fermented **ABT milks** during storage at **4°C**

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Trials with microalgae:
IV. Effects of *Chlorella vulgaris* on growth of probiotics used in feedingstuffs

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- The influence of a spray-dried ***Chlorella vulgaris*** biomass, added at a concentration of 3 g/L, on growth of ***Lactobacillus plantarum*** and ***Enterococcus faecium*** strains primarily used for feed fermentation purposes was evaluated in milk.

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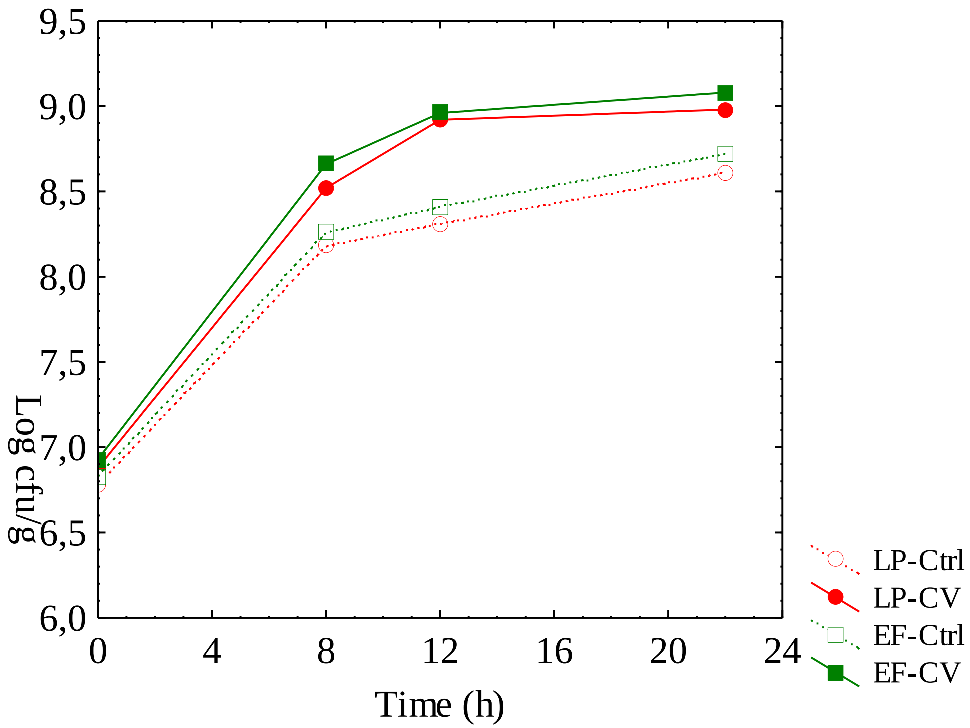

Effect of 3 g/L ***Chlorella vulgaris*** biomass on growth of ***Lactobacillus plantarum*** and ***Enterococcus faecium*** in milk

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In **summary**, the powdered *Chlorella* biomass rich in biologically active compounds is potentially suitable for use in manufacture of milk-based **functional fermented feeds**.

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Conclusions

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- **Growth, acid production, and survival** of thermophilic dairy starters during manufacture and subsequent refrigerated storage of fermented milks can be improved, although to varying degrees, by addition of:
  - oligofructose or inulin,
  - honey,
  - *Spirulina* or *Chlorella* biomasses.

- The stimulatory/protective effect of these substances on **bifidobacteria** is probably the most important finding of this study because bifidobacteria do not grow well in milk and they have poor survival rates in conventional fermented milks.

- Some of the substances tested are also capable of improving the **nutritional and sensory properties** of the final product or exerting an **antifungal effect** on spoilage organisms.

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

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Objectives

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- Testing the influence of a *S. platensis* biomass on **growth and acid production** of various ***Lactococcus*** **and** ***Leuconostoc*** **strains** in milk.

- Developing a *Spirulina*-containing **commercial cultured milk** fermented with the mesophilic lactic acid bacteria (LAB) strains selected.

- Running **storage trials** to determine the effect of the *Spirulina* biomass on viability of starter organisms in the refrigerated product.

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Materials and Methods

- Milk samples enriched with *S. platensis* at concentrations up to 0.8% (w/v) inoculated at 1% (v/v) with mesophilic LAB strains.
- Incubation at 30°C.
- pH values and LAB counts of samples measured at regular intervals.
- Sensory tests performed by untrained panelists to optimize organoleptic properties of the final product.
- Storage trials carried out.

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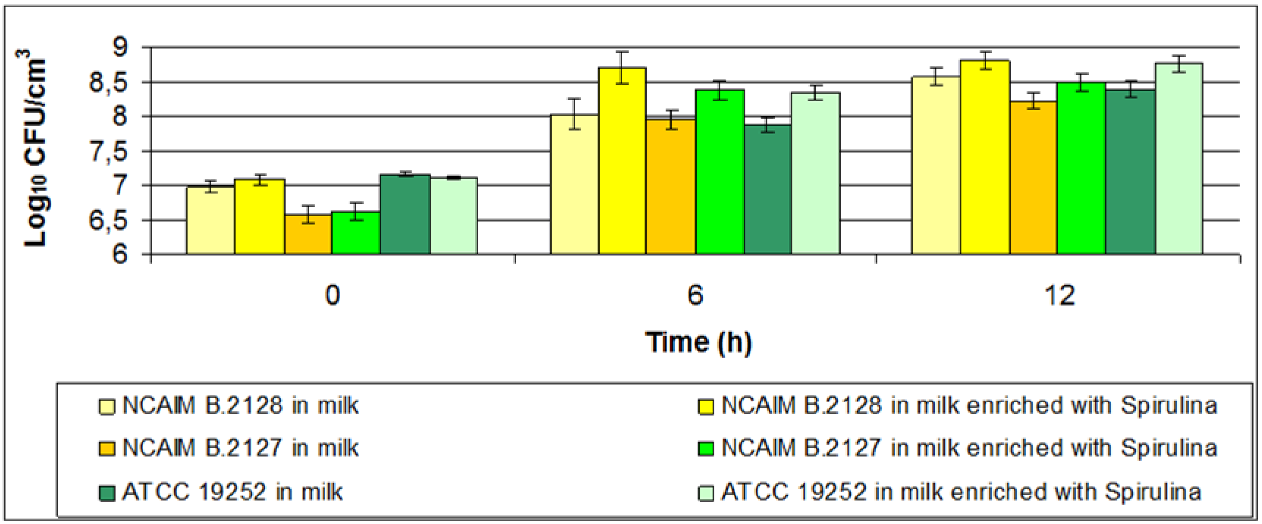
Results

Used at the rate of 0.3% (w/v), the *Spirulina* biomass significantly stimulated (*P* < 0.05) several of the mesophilic LAB strains screened:



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

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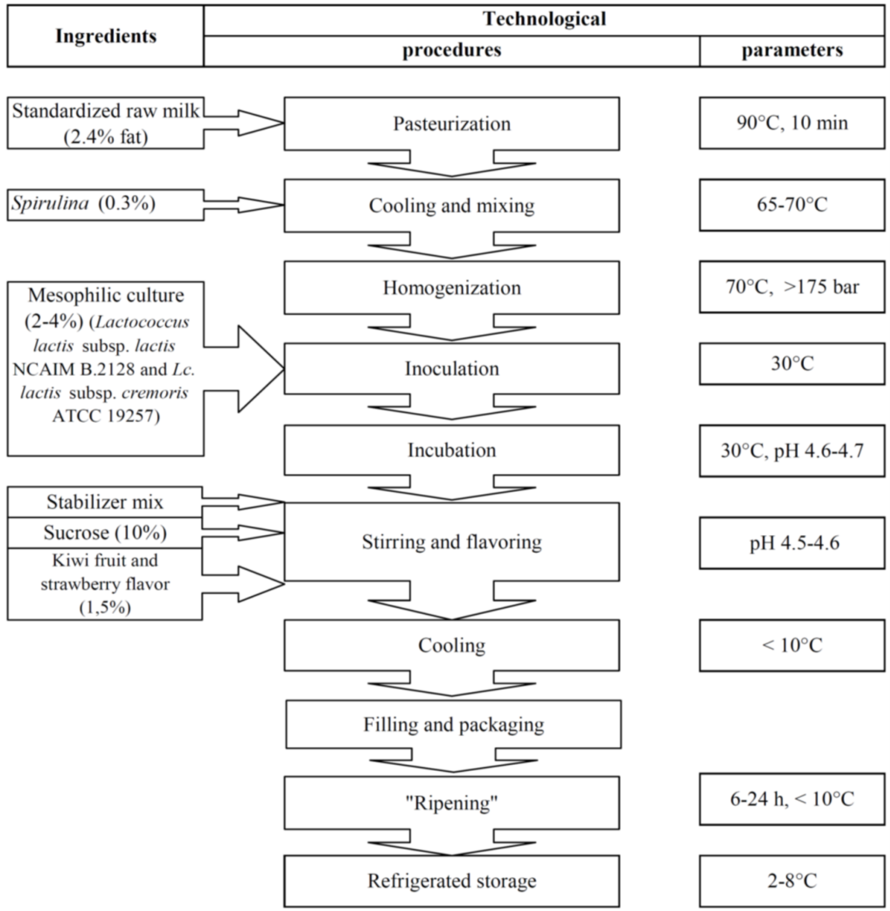
Results

- 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%**.

Results



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

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Results

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During the first 2 wk of storage at 4°C, the *S. platensis* biomass significantly increased ($P < 0.05$) the viability of lactococci in the functional fermented milk developed:

Table 1: 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[a]	**8.65 ± 0.07[b]**	100.00	100.00
7	8.66 ± 0.17[a]	**8.92 ± 0.18[b]**	133.78	186.00
14	8.49 ± 0.17[a]	**8.79 ± 0.23[b]**	91.21	137.03
21	8.47 ± 0.05[a]	8.65 ± 0.16[a]	86.78	100.92
28	8.39 ± 0.10[a]	8.26 ± 0.17[a]	71.83	40.72
35	7.57 ± 0.11[a]	7.57 ± 0.12[a]	11.05	8.34
42	7.44 ± 0.07[a]	7.43 ± 0.05[a]	8.06	6.09

* Values are means ± SD, based on 6 observations (three samples × two replicates); **[a,b] means within a row without a common superscript differ ($P < 0.05$).** ** Values are means calculated from *Lactococcus* count (Log_{10} CFU/cm^3) means.

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