DOCTORAL (Ph.D.) DISSERTATION

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MOSONMAGYARÓVÁR

1999

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EFFECT OF A CYANOBACTERIAL BIOMASS ENRICHED WITH TRACE ELEMENTS ON THERMOPHILIC DAIRY STARTER CULTURES

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MOSONMAGYARÓVÁR
1999

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1 INTRODUCTION

Fermented dairy products, which are manufactured with starter cultures, are often referred to as the queens of dairy products. If they are consumed on a regular basis, a wide range of beneficial effects may be expected, in humans. The latest statistics show that the average Hungarian consumer purchases about 50-60% less fermented dairy product than his/her Western European counterpart (Szakály *et al.* 1998). It is worth recording, however, that sales of fermented dairy products are growing steadily in Hungary, despite the fact that there has been a decline in the purchasing power of people in the past few years, which is well reflected in the overall consumption of milk and dairy products.

The consumption of fermented dairy products shows an upward tendency worldwide, not only in Hungary. This phenomenon can be accounted for by the fact that people have been aware of the high nutritional value and excellent sensory properties of these products. Dairy companies endeavor to maintain and further stimulate these favorable tendencies by improving quality continuously, widening the assortment of products and packaging goods in an increasingly practical and attractive way.

Several authors, including Blanc (1974), Foissy (1983), Renner (1983), Desmazeaud (1988), Spolaor *et al.* (1988), Kneifel (1989), Rohm and Lechner (1989), Szakály (1989), Kim (1990), Mayer (1990), Szakály (1991), Wynckel *et al.* (1991), Bouhnik (1993), Szakály (1994), Varga (1994), Szigeti (1995), Tamime *et al.* (1995), Szakály (1996), Szakály and Zsinkó (1997), Zsinkó *et al.* (1997) and Szakály *et al.* (1998) point out that fermented dairy products possess a wide range of beneficial properties. These beneficial properties, which are largely due to the presence of starter bacteria in high counts and the change in the composition of milk, can be summarized as follows.

- The characteristic micro-organisms in the product secrete lactic acid and sometimes also acetic acid, which may show specific toxicity against certain species of yeasts or bacteria or, by lowering the pH of the intestinal contents, inhibit the growth of putrefactive organisms in general.

- The starter culture bacteria of human origin can colonize the human digestive tract, thereby replacing the protective effect that was seriously depleted by treatment with antibiotics against pathogens.
- The bifidogenic growth-promoting factors in the product help bifidobacteria and lactobacilli colonize the intestinal tract.
- Owing to proteolytic activity and precipitation of casein, both protein digestion and absorption improve as compared with milk.
- Digestion and absorption of milk fat and resorption rates of calcium, phosphorous and iron also improve.
- By lowering the cholesterol level of blood, they play a key role in the prevention of coronary heart diseases.
- Fermentation substantially improves the nutritional properties (e.g. non-protein nitrogen content, free amino nitrogen content, specific protein utilization, "half-period" of vitamin C etc.) of milk.
- They act as stimulants of the immune system.
- Owing to the β-D-galactosidase activity of certain micro-organisms, these products can be consumed by the majority of people who suffer from lactose maldigestion because the lactose content of milk is decomposed and changed into organic acids.
- Certain starter cultures may possess antitumorigenic properties.
- The fermented dairy products may be superior to milk as regards vitamin content.
- Because of the low pH of the final product, the supplements of beneficial physiological effect (mineral complex compounds, vitamins etc.) become highly stable.
- The ingestion of fermented dairy products contributes to an increased secretion of saliva, bile salts and gastric juices.
- A large variety of fermented dairy products can be manufactured with respect to taste and flavor if the available species of thermophilic starter bacteria are combined with one another and various additives are also employed.
- By total or partial ultrafiltration of the milk, addition of milk protein concentrates and standardization of the fat content, dietary products and roborants of almost any

composition can be manufactured.

- Extremely long shelf-life and stability can be ensured by heat treatment or freeze-drying (lyophilization) of the final product, in accordance with market demands.

The importance of probiotic (therapeutic) micro-organisms has recently come into prominence in several countries which have highly developed dairy production. The probiotic properties of various starter culture strains are further detailed in subchapter 2.2. There is a wide range of fermented dairy products on the market all over the world. Table 1 shows the major types of fermented milks manufactured with starter cultures containing micro-organisms that are indigenous to the intestinal tract.

Table 1 Fermented milk products manufactured with starter cultures containing micro-organisms indigenous to the human intestinal tract (Szakály 1981 and 1992; Tamime *et al.* 1995)

Starter Culture Bacteria (SCB) in the Product		Product or Trade Name
SCB of Intestinal Origin	Other SCB	
Lactobacillus acidophilus	—	Acidophilus Milk
	Streptococcus thermophilus	Bioghurt®
	Lactobacillus bulgaricus + *Streptococcus thermophilus*	Acidophilus Yogurt
		ACO Yogurt
Bifidobacterium bifidum	—	Bifidus Milk
	Streptococcus thermophilus	Bifighurt®
	Lactobacillus bulgaricus + *Streptococcus thermophilus*	Bifidus Yogurt
Bifidobacterium bifidum + *Lactobacillus acidophilus*	—	Fermented AB Milk
		Cultura®
	Streptococcus thermophilus	Fermented ABT Milk
		Biogarde®
	Lactobacillus bulgaricus + *Streptococcus thermophilus*	Acidophilus Bifidus Yogurt

Only few of the above fermented milks are available in Hungary. It is most desirable that consumers should include them in their diet because these products possess specific health benefits. They are expected to become increasingly available and gain widespread acceptance in the long term if the purchasing power of consumers increases. In several of Europe's highly developed countries (e.g. Denmark, France, the

Netherlands, Germany etc.) the market of probiotic products is characterized by an annual growth of 15% or more and, in some cases, this is despite a 70% price premium over regular yogurts (Heasman and Mellentin 1998). However, high quality and attractive packaging do not ensure success automatically. In any market, popular brands are built through heavy investment in marketing communications. As for the probiotic fermented dairy products, advertisements should be based on a scientifically established and documented health claim.

Even the current consumption level of fermented dairy products could easily contribute to consumers' ingesting fewer artificially produced vitamin and trace element preparations and less medicine if fermented dairy products 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 *Spirulina platensis* biomass enriched with trace elements for the manufacture of fermented milk products. The presence of bioactive substances in this cyanobacterial biomass is of great importance because it further improves the high nutritional value of fermented milks.

Productivity is one of the main concerns of dairy technologists and scientists. According to German and Japanese authors, acid development by and growth rate of certain lactic acid bacteria can be stimulated by addition of extracts of green algae (Shirota *et al.* 1964; Stengel 1970; Zielke *et al.* 1978; Kurita *et al.* 1979; Webb 1982).

This work aims to find out whether stimulation of single- and multiple-strain type thermophilic dairy starter cultures can be brought about by a cyanobacterial biomass and identify the substances responsible for the effects observed.

The effect of 3 g l^{-1} *Spirulina platensis* biomass enriched with trace elements (iodine, zinc, selenium) on the rate of acid development by and growth rate of pure and synchronized mixed cultures of *Streptococcus salivarius* subsp. *thermophilus* CH-1, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2, *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 was evaluated in a model milk medium. The components of the cyanobacterial biomass responsible for the stimulation caused were also identified in laboratory simulations wherein trace elements (iodine, zinc, selenium),

vitamins (B-complex, C, A, E) and nitrogenous compounds (peptone, adenine, hypoxanthine) were tested.

This Dissertation meets a long-felt want since reports on similar experiments carried out with this cyanobacterium species, these dairy starter cultures and such a large variety of bioactive substances were not to be found in the literature.

Thereafter storage experiments were conducted to reveal the changes in the characteristic and undesirable microbial flora of cyanobacterial and control yogurts produced according to regular technology of manufacture. The cyanobacterial yogurt was richer in vitamins, trace elements and further bioactive substances than the "normal" fermented milk products and thus it possessed functional properties.

The experiments required milk in large quantities. About 100 l of UHT milk was purchased and deep-frozen for the researches done in a model milk medium and for the preliminary experiments. As to the products manufactured for the storage experiments, 50 l of pasteurized market milk was used as raw material.

2 LITERATURE REVIEW

2.1 Characterization of thermophilic dairy starter cultures

The characteristic microflora of yogurt consist of the spherical or ovoid *Streptococcus salivarius* subsp. *thermophilus* (*Strep. thermophilus*) and the rod-shaped *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lact. bulgaricus*) (Rašić and Kurmann 1978; Deeth and Tamime 1981; Gurr 1987). These two species have a symbiotic relationship when grown in milk. *Strep. thermophilus* is an aerotolerant species, that is the reason why it grows faster at the beginning of fermentation than the microaerophilic *Lact. bulgaricus*. *Strep. thermophilus* can release carbon dioxide from urea and build it into formic acid, thereby lowering the redox potential. All these factors stimulate the growth and acid production of *Lact. bulgaricus*. The lactic acid thus formed, however, begins to retard the growth of *Strep. thermophilus* after a while (Shankar and Davies 1977; Driessen *et al.* 1982; Scherer 1995). As for proteolysis, *Lact. bulgaricus* possesses strong protease activity whereby it breaks down proteins to peptides. However, since *Lact. bulgaricus* lacks peptidase, the peptides formed will be further broken down by *Strep. thermophilus* to amino acids (240-700 mg l^{-1}), which stimulate the growth of both species (Pette and Lolkema 1950; Lee *et al.* 1974; Higashio *et al.* 1977; Desmazeaud 1988; Scherer 1995).

As both micro-organisms require organic carbon and nitrogen sources, trace elements and vitamins for growth, the question comes up as to whether yogurt is of lower or higher nutritional value than milk.

The literature differs on the changes in vitamin content of fermented dairy products. It is a fact that some lactic acid bacterial strains do require certain vitamins for growth and reproduction. Vitamin utilization is especially pronounced in the exponential (log) phase of growth. A review by the International Dairy Federation (1983) reports that fermentation of milk decreases the level of almost all B-vitamins except folic acid while the data summarized by Deeth and Tamime (1981) show that the level of many of the B-vitamins is higher in yogurt compared to milk. The data presented by Shahani and

Chandan (1979) also indicate that lactic cultures increase the B-vitamin content in general. Several authors (Hartman and Dryden 1974; Rašić and Kurmann 1978; Shahani and Chandan 1979; Ayebo and Shahani 1980; Speck and Katz 1980; Deeth and Tamime 1981; International Dairy Federation 1983; Renner 1983; Shahani 1983; National Dairy Council 1984; Rao and Shahani 1987) stress the point that although yogurt definitely is a rich source of vitamins, the biosynthesis and utilization of vitamins are greatly dependent on strain selection and parameters of technological processes. This is particularly true of B-vitamins. Some other factors may also influence the vitamin content of fermented dairy products during manufacture and storage. These include composition of the raw milk mix, cultural conditions such as the rate of inoculation, incubation time and incubation temperature and storage conditions (Shahani and Chandan 1979; Deeth and Tamime 1981; Rao *et al.* 1984).

Many papers and reports indicate a decrease of over 50% in the levels of vitamin B_6 and B_{12}, which may be partly offset by an increase of more than 100% in folic acid content (Glass and Hedrick 1976; Reddy *et al.* 1976; Rašić and Kurmann 1978; Shahani and Chandan 1979; Alm 1982; Friend *et al.* 1983; Rao *et al.* 1984; Gurr 1987; Bourlioux and Pochart 1988). As opposed to this, according to Černá *et al.* (1972), Alm (1982) and Kneifel *et al.* (1989) yogurt culture appears to increase pyridoxine (vitamin B_6) concentration. The ratio of cocci to rods is of great importance to folic acid content because *Strep. thermophilus* synthesizes significant quantities of folic acid while *Lact. bulgaricus* utilizes it (Friend *et al.* 1983). Thiamin and riboflavin are generally reported to be only slightly affected by fermentation (Černá *et al.* 1972; Alm 1982; Hewitt and Bancroft 1985; Kneifel *et al.* 1989). In contrast, Glass and Hedrick (1976) have found that yogurt culture significantly decreases the levels of thiamin and riboflavin. Similarly, yogurt culture decreases the content of niacin (nicotinic acid), which is, however, highly affected by the temperature of heat treatment. As for the effect of fermentation on pantothenic acid, the available data vary widely among different studies, although the majority of reports indicate that the level of pantothenic acid decreases during fermentation.

The significant increase of more than 100% in choline content during the manufacture of cultured dairy products containing lactic streptococci (lactococci) may have a beneficial effect on fat oxidation and on regulation of cholesterol metabolism (Rašić and Kurmann 1978).

The considerable decrease of about 80% in vitamin C content is of little practical importance since this vitamin is present in milk in small amounts and it suffers marked losses during handling, processing and exposure to light. As far as other vitamins are concerned there are comparatively small changes during the manufacture of fermented dairy products.

Most vitamins are stable during storage of the product except for vitamin A, vitamin B_{12}, folic acid and vitamin C (Rašić and Kurmann 1978).

The fortification of yogurt with vitamin C is considered to be a useful method of improving the vitamin value of the product. Vitamins A and D may also be added to milk according to prescriptions (Kon 1962 and 1964). However, it must be noted that the levels of fat-soluble vitamins are greatly influenced by the fat content of the milk (Buttriss 1997). The supplementation of fermented dairy products with fruits or vegetables rich in particular vitamins is also a simple way of increasing the vitamin content (Rašić 1972).

On the whole, when the vitamin levels of milk and yogurt are compared, it is difficult to find any advantage of the fermented product over fresh milk (Kneifel *et al.* 1989).

Another important micro-organism which constitutes, wholly or in part, the characteristic microflora of several fermented dairy products is *Lactobacillus acidophilus* (*Lact. acidophilus*). This species belongs to the Gram-positive, non-spore-forming, anaerobic or facultatively anaerobic rods and is a natural inhabitant of the normal healthy gut flora (Marshall *et al.* 1982; Dellaglio *et al.* 1992; Özbaş 1993). The homofermentation pathway results in 2 mol of lactate for every 1 mol of glucose metabolized, no gas is produced. The metabolites of *Lact. acidophilus* make the environment less favorable for the growth of potentially pathogenic micro-organisms (Gorbach 1990). The use of this species as a starter culture component is of great

significance from a probiotic point of view (Davis 1970; Vedamuthu 1974). The probiotic effect can partly be accounted for by the production of bacteriocins and antibiotics. The major bacteriocins produced by various strains of *Lact. acidophilus* include lactocidin, acidolin, acidophilin and acidophilucin (Gilliland 1975; Marshall and Tamime 1997). Besides these antimicrobial substances, B-vitamins and ascorbic acid are also synthesized by several strains (Shirota 1971; Rašić and Kurmann 1978). For instance, *Lact. acidophilus* produces vitamin B_{12} both in single and mixed cultures (Mitić *et al.* 1973; Rauch and Králová 1986; Kneifel *et al.* 1989).

Lact. acidophilus grows slowly in milk, but its growth rate can be increased by addition of sucrose (Agrawal *et al.* 1986), tomato juice (Miller and Puhan 1980), manganese and magnesium ions (Ahmed *et al.* 1990). If *Lact. acidophilus* is employed in combination with other lactic acid bacteria, the time needed for the manufacture of fermented dairy products can be shortened substantially (Speck 1980). Owing to its production of vitamin B_{12}, *Lact. acidophilus* has a beneficial effect on *Lact. bulgaricus* and *Strep. thermophilus* in mixed cultures (Baril and Cardwell 1984).

Bifidobacteria are also micro-organisms of enteric origin used extensively for the manufacture of fermented dairy products (Kurmann 1980; Marshall 1984; Johnson *et al.* 1987; Kurmann and Rašić 1988; Tamime and Robinson 1988; Driessen and DeBoer 1989; Gilliland 1989; Hunger 1989; Gutknecht 1992; Özbaş 1993; Halfhide 1994; Samona and Robinson 1994; Badran and Reichart 1995; Bylund 1995; Tamime *et al.* 1995; Marshall and Tamime 1997; Shah 1997a and 1997b). They are Gram-positive, non-motile, anaerobic bacteria which take a variety of shapes. Lactobacilli and bifidobacteria play a significant role in the regulation of intestinal pH by the production of lactic and acetic acids, thereby causing the intestinal pH to drop, which in turn restricts or prohibits the growth of many potentially pathogenic and putrefactive bacteria. In the presence of lactobacilli and bifidobacteria, bacterial fermentation of intestinal contents produces short chain fatty acids (acetic, lactic, butyric, propionic acids) and other predigested nutrients, which can easily be absorbed and utilized even by elderly people (Alm *et al.* 1993). Five species of *Bifidobacterium* have attracted attention in the dairy industry for the manufacture of therapeutic fermented milk

products — *Bifid. adolescentis*, *Bifid. bifidum*, *Bifid. breve*, *Bifid. infantis* and *Bifid. longum* (Tamime *et al.* 1995; Shah 1997a). *Bifid. bifidum* is the species most commonly used, followed by *Bifid. longum* and *Bifid. breve*.

The work of Tamime *et al.* (1995) indicates that *Bifidobacterium* strains of human origin seem to require thiamin, pyridoxine, folic acid and cyanocobalamin, although in certain cases the same organisms are also capable of synthesizing some B group vitamins. Riboflavin is not synthesized by the above-mentioned species and this vitamin has been reported by Scardovi (1986) to be a growth factor. The synthesis of ascorbic acid and biotin by *Bifid. longum* and *Bifid. infantis* respectively has been observed by Ballongue (1993). *Bifid. breve*, *Bifid. bifidum* and *Bifid. adolescentis* are also capable of producing these vitamins but at lower concentrations. The production of such vitamins by bifidobacteria may improve the nutritional properties of the fermented milk product or the bioavailability of these vitamins in the human gut.

Bifid. bifidum has also been found to exhibit certain antiviral activity (Rašić and Kurmann 1978).

While *Strep. thermophilus*, *Lact. bulgaricus* and *Lact. acidophilus* ferment the glucose moiety of lactose by homofermentation, bifidobacteria produce acetic acid and lactic acid at a molar ratio of 3:2 and a small amount of formic acid, ethanol and succinic acid by a heterofermentation pathway. Acetic acid acidity has a harshness which is undesirable for milk products. A product with a high acetic acid content will be described as "vinegary". Carbon dioxide may also be produced, which will disrupt the gelling capacity (Tamime *et al.* 1995; Marshall and Tamime 1997; Shah 1997a).

The optical activity of the lactic acid produced varies with the genus or even the species of bacterium. Thus *Streptococcus* species produce L(+)-lactic acid, some *Lactobacillus* species produce D(–)-lactic acid and there are also bacteria that produce a racemic mixture of the two acids. Sometimes the same strain of organism may produce different isomers of lactic acid depending upon the growth conditions. (Wilkinson and Rose 1963; Rašić and Kurmann 1978; Kovács 1997).

Two factors seem to determine the type of lactic acid produced.

1 The stereospecificity of the lactate dehydrogenase involved — Enzymes exist which are specific for a reaction in which either L(+)- or D(–)-lactic acid are produced. However, no enzyme is known which will catalyze the formation or dehydrogenation of both isomers of lactic acid. Thus *Lact. acidophilus*, which produces DL-lactic acid, can be shown to contain two different lactate dehydrogenases, each specific for one of the lactic acid isomers.
2 The presence or absence of a lactate racemase — Although the stereospecificity of the lactate dehydrogenase may determine the type of lactic acid produced by most bacteria, in some instances of organisms producing a racemic mixture, an enzyme is present which catalyzes the racemization of the two isomers of lactic acid. In this event, a racemic mixture will be formed irrespective of the specificity of the dehydrogenase (Wilkinson and Rose 1963; Kovács 1997).

Both L(+) and D(–) isomers of lactic acid are absorbed from the intestinal tract, but there is some difference in their transformation. L(+)-lactic acid, often referred to as physiological lactic acid, is completely transformed either in the respiratory process or in the glucose or glycogen synthesis, while D(–)-lactic acid is transformed to a limited degree and at a slower rate (Rašić and Kurmann 1978; Kovács 1997).

As to *Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus* and *Bifid. bifidum*, these species produce 91-95%, 1-5%, 46-54% and 94-97% L(+)-lactic acid respectively (Szakály 1992).

Since milk is poor in peptides and amino acids and some strains of *Bifidobacterium* do not possess sufficient proteolytic activity for growth in milk, casein hydrolysate, a nutrient rich broth, L-cysteine·HCl or yeast extract must be added to milk in order to accelerate growth (Goh *et al.* 1987; Roy *et al.* 1990; Klaver *et al.* 1993; Proulx *et al.* 1994; Tamime *et al.* 1995; Shah 1997b). The species reported to grow well in milk may be stimulated by naturally occurring growth promoters in milk (Marshall and Tamime 1997). Bifidus factors contained in milk were first described by György *et al.* (1954a and 1954b) as glycoproteins or peptides possibly derived from casein. More recent works have shown that oligosaccharides containing fructose or galactose are particularly effective in helping population development of bifidobacteria (McKellar

and Modler 1989; Ito *et al.* 1990; Modler *et al.* 1990; Yamazaki and Matsumoto 1994; Tamime *et al.* 1995). Although there has been considerable interest in the development of bifidobacteria for milk fermentation, little work has been carried out on their proteinases and peptidases. Because of the inconsistency in growth in milk, mixed starters are often used for the production of fermented milks.

Marshall and Tamime (1997) have pointed out that the probiotic starters, besides being able to grow in milk, must have additional characteristics, i.e. they must be able to resist stomach acid and its proteolytic enzymes, withstand quite rapid changes in pH and resist bile. Through the action of acids, bile and enzymes, most of the digestion in the human gastrointestinal tract is carried out in the stomach and the small intestine. Any important bacteria delivered with or as part of a meal must remain viable for 4 h or so in a changing and hostile environment. There are strains of both *Lact. acidophilus* and *Bifidobacterium* species that resist stomach acid (with many strains surviving and some multiplying in media acidified to pH 3 by means of HCl) (Hood and Zottola 1989; Lankaputhra and Shah 1995) and bile (at 3%). Some strains deconjugate bile and this is thought to be significant as deconjugated bile salts co-precipitate cholesterol in acidic environments (Klaver and Van der Meer 1993; De Smet *et al.* 1994; Bateup *et al.* 1995; Marshall and Taylor 1995; Taylor 1995). Klaver *et al.* (1993) used low oxygen content milk to co-culture *Lact. acidophilus* with bifidobacteria successfully. Proteolytic strains of lactobacilli were also selected as partners for weakly proteolytic bifidobacteria. In the case of mixed culture formulations, however, it is necessary to know how the individual species/strains behave toward each other, how they behave in milk and whether the probiotic organisms are robust enough to withstand processing and the later chill storage at point of sale (Marshall and Tamime 1997).

2.2 Probiotic properties of the starter culture strains tested

All the bacteria that enter the intestine via food arrive in an already very complex system. To be potentially influential in the gut, bacteria need to be able to survive passage through the stomach with its extremely low pH and to survive the actions of bile

acids and the immune system. The classic yogurt bacteria (*Strep. thermophilus* and *Lact. bulgaricus*) are likely to have little or no probiotic potential as they are probably killed in the intestine (Hamilton-Miller 1996; Buttriss 1997). In recent years there has been increased interest in the possible health benefits of fermented milks produced with cultures containing *Lact. acidophilus* and bifidobacteria, both of which are found naturally in the human gut. In this subchapter, probiotic properties of the Chr. Hansen's cultures employed in our experiments are reviewed.

Prevention of traveler's diarrhea — It is well established that traveler's diarrhea can be prevented by prophylactic intake of antimicrobial chemotherapeutics to a great extent (Black *et al.* 1983; DuPont *et al.* 1986; Sack 1986). However, the use of such agents may cause adverse reactions and may lead to emergence of resistant strains of enteric pathogens. Testing a group of Danish tourists, Black *et al.* (1989) found that one capsule containing a freeze-dried mixture of *Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus* La-5 and *Bifid. bifidum* Bb-12, administered 3 times a day, gave a protection rate of about 40% against traveler's diarrhea in Egypt. All four strains have also been found to be effective against colonization and subsequent proliferation of foodborne pathogens (Alm 1991; Yaeshima 1996). The regimen can without risk of adverse effects be recommended to all travelers except those belonging to the high-risk groups, where the more effective antimicrobial chemotherapeutics should be used.

Reduction of diarrhea and rotavirus infections in infants — A study of hospitalized infants, between the ages 5-24 months, with acute diarrhea reveals that the supplementation of infant milk formula with *Strep. thermophilus* and *Bifid. bifidum* can reduce the incidence of acute diarrhea and rotavirus shedding (Saavedra *et al.* 1994; Salminen and Tanaka 1996).

Prevention of constipation in elderly people — Alm (1991) and Alm *et al.* (1993) have pointed out that many people, especially the elderly, may have problems with constipation. A trial with 23 severely immobilized patients aged 68-99 years who suffered from chronic constipation has shown that natural bowel evacuation is to be improved by the administration of Cultura®, a fermented AB product containing *Lact. acidophilus* and bifidobacteria. A therapeutic dairy product can only exert an influence

on gastrointestinal function and especially on bowel movement rate in aged individuals if it contains viable and metabolically active micro-organisms that are present in high counts. It is also required that micro-organisms should be able to survive during storage and after consumption, all the way down the lower parts of the colon (Klaenhammer 1982).

Faster recolonization of the intestinal microflora after administration of antibiotics — Intake of broad spectrum antibiotics often causes diarrheal problems. The experiments carried out by Black *et al.* (1991) and Lidbeck *et al.* (1995) indicate that *Lact. acidophilus* La-5 and *Bifid. bifidum* Bb-12 can re-establish the intestinal microflora quicker than normal after an ampicillin treatment.

Improved lactose intolerance — Large groups of people lack lactase, an enzyme which can cleave lactose. This deficiency is very widespread in certain countries. The symptoms of lactose maldigestion include stomach pains, gas formation in the stomach and diarrhea. Some of the lactic acid bacteria tested by Lin *et al.* (1991) (*Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus* La-5) produce considerable amounts of lactase. By intake of dairy products that contain these bacteria and small amounts of the enzyme, the tolerance toward lactose will increase.

Cholesterol reduction — A high blood cholesterol concentration is considered to be one of the major risk factors for coronary heart disease (Buttriss 1997). Assumptions that some fermented dairy products may facilitate a reduction in blood cholesterol level, specifically the low density lipoprotein (LDL) fraction, caused a smaller non-published trial with a fermented ABT (*Lact. acidophilus*, bifidobacteria, *Strep. thermophilus*) product in patients with high cholesterol levels in the blood. After a three-week intake of this fermented ABT product, the level of cholesterol and that of triglycerides decreased. Agerbaek *et al.* (1995) have reported 10% reduction in LDL-cholesterol after six weeks' consumption of a fermented product carrying a specific bacterial culture. Other studies reviewed by the International Dairy Federation (1991b) and Schaafsma (1996) have also demonstrated reductions of similar magnitude (i.e. 4.2% and 5-6% respectively) in LDL-cholesterol. Gilliland and Walker (1990) have tested 12 cultures of *Lact. acidophilus* for ability to assimilate cholesterol. They have pointed out that a culture of *Lact.*

acidophilus of human origin which assimilates cholesterol, grows well in presence of bile and produces bacteriocins can be selected for use as a dietary adjunct for humans. A culture of *Lact. acidophilus* that possesses all these characteristics definitely has an advantage over another one that does not in establishing and functioning in the intestinal tract to assimilate cholesterol.

Stimulation of the immune system — Macrophages are cells in the immune defense system whose function is to take up inert particles from the body and break them down. Laboratory trials have shown that *Lact. acidophilus* La-5 and *Bifid. longum* can stimulate the activity of mouse macrophages (Hatcher and Lambrecht 1993). The process used by macrophages to take up inert particles is called phagocytosis. Trials on humans have shown that a fermented dairy product supplemented with *Lact. acidophilus* La-5 and *Bifid. bifidum* Bb-12 can enhance the phagocytosis of *Escherichia coli* (Schiffrin *et al.* 1995). In another trial, a fermented milk containing *Lact. acidophilus* La-5 and bifidobacteria was given to volunteers simultaneously with *Salmonella typhi* Ty21a. A control group received only *Salm. typhi* Ty21a. A significantly higher specific serum IgA titer was observed in the group that was given the fermented milk compared to the control group. The results indicate that lactic acid bacteria being able to persist in the gastrointestinal tract can act as adjuvants to the humoral immune response (Link-Amster *et al.* 1994).

Improved defense against cancer — There are reports suggesting that fermented dairy products may protect against certain types of cancer, although the data are inconsistent (International Dairy Federation 1991b). Trials with mice have revealed that *Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus* La-5 and *Bifid. bifidum* Bb-12 can reduce the size of artificially induced tumors by 27-63% (Kim *et al.* 1991). Various mechanisms have been suggested, including the potential of some strains of lactic acid bacteria to reduce the activity of fecal (bacterial) enzymes known to promote the synthesis of carcinogens or stimulate the host's immune system. However, there is little or no evidence to show that these mechanisms operate in humans (Buttriss 1997).

2.3 Characterization of *Spirulina platensis*

Cyanobacteria (formerly known as blue-green algae) belonging to prokaryotic algae are more closely related to bacteria than to other (eukaryotic) algae. They differ from photosynthetic bacteria in their photosynthetic pigments and in their ability to produce oxygen. While photosynthetic bacteria contain bacteriochlorophyll, cyanobacteria have chlorophyll *a* in their cells. The average cell size of cyanobacteria is 5-10 times larger than that of bacteria. Cyanobacteria reproduce asexually by fission, their sexual reproduction is unknown as yet (Ördög 1998).

Spir. platensis is a planktonic cyanobacterium that forms massive populations in tropical and subtropical water bodies characterized by high levels of carbonate and bicarbonate and high pH (up to 11). It is a widely distributed species, mainly found in Africa, but also in Asia and South America (Tomaselli 1997).

Spir. platensis is a filamentous cyanobacterium recognizable by the main morphological feature of the genus: the arrangement of the multicellular cylindrical trichomes in an open left-hand helix along the entire length (Figs 1 and 2).

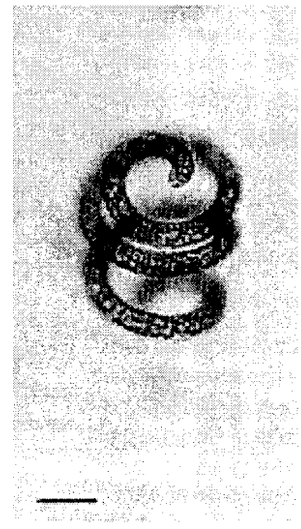


Fig. 1 Light micrograph of *Spirulina platensis* (bar represents 20 μm)

Fig. 2 Scanning electron micrograph of *Spirulina platensis* with part of trichome in continuous helical coil (bar represents 10 μm)

Spir. platensis is characterized by short trichomes, usually with 5-7 coils. Its cell organization, observed by electron microscopy, is typical of that of prokaryotic organisms, being devoid of a morphologically limited nucleus and of plastids and displaying an outer Gram-negative type envelope, the cell wall. Trichomes are surrounded by a thin, diffluent sheath which has a fibrillar, net-like structure. The multilayered cell wall is 40-60 nm thin and has an easily-detectable electron-dense layer corresponding to the peptidoglycan (murein). Regularly spaced cross-walls divide the trichome into cells. Just below the cell wall there is the plasma membrane, enclosing the cytoplasm, which is rich in subcellular inclusions typical of cyanobacteria. The peripheral region of the cell is characterized by a low electron-dense cytoplasm mainly filled with polyglucan granules and gas vacuoles. There are also small osmiophilic granules, fibrils and lipid droplets. The thylakoid membranes, located between the

peripheral and the central cytoplasm, are arranged in parallel and have associated electron-opaque phycobilisomes. The thylakoids, formed by two closely appressed unit membranes, appear as straight or sinuous bundles running parallel to the longitudinal wall and transversely to cross-walls. Low electron-dense thylakoid-free areas are filled with ribosomes and fibrils of DNA. Sometimes, some spherical, highly osmiophilic polyphosphate granules and large structured cyanophycin granules can be observed. The central electron-dense cytoplasmic region contains carboxysomes, recognizable by their polyhedral profile and pseudocrystalline appearance (Balloni *et al.* 1980; Tomaselli 1997).

The dried biomass of *Spir. platensis* typically contains 3-7% moisture, 55-60% protein, 6-8% lipids, 12-20% carbohydrate, 7-10% ash, 8-10% fiber, 1-1.5% chlorophyll *a* and a wide range of vitamins (Belay 1997; Cohen 1997; Vonshak 1997a).

Spir. platensis is especially rich in proteins. The proteins having the highest economic potential are the biliproteins. *Spir. platensis* contains two biliproteins, c-phycocyanin and allophycocyanin, which are water-soluble blue pigments. The protein fraction may have a phycocyanin content of up to 20% (Cohen 1997).

As for fatty acid composition, it is largely influenced by environmental conditions. *Spir. platensis* can be characterized by about 45-50% saturated and 50-55% unsaturated fatty acids. 10-30% of fatty acids is γ-linolenic acid (GLA), a rare polyunsaturated fatty acid, which is claimed to have medicinal properties. Good *Spirulina* strains and a good processing procedure should yield biomass with at least 1% of GLA (Cohen 1997, Vonshak 1997a).

Spirulina-containing foods have been consumed for decades by African tribes and no toxic effects have ever been encountered (Helmeczi 1994; Vonshak 1997c).

Today, the commercial production of *Spirulina* biomass is carried out exclusively in open systems. Closed photobioreactors have so far been employed for research and in small field installations. Recently, the uses and mass cultivation of *Spir. platensis* have risen substantially world-wide owing to an increased understanding of its biological systems. More than 70% of the current *Spirulina* market is for human consumption, mainly as health food. The total annual production of food-grade *Spirulina* biomass is

estimated to be about 1,000-1,500 tonnes (Belay 1997; Tredici and Zittelli 1997; Vonshak 1997c and 1997 d).

2.4 Physiological roles of trace elements

The finding that addition of manganese and magnesium ions to milk has a stimulatory effect on certain lactic acid bacteria was referred to in subchapter 2.1. Attention should also be given to the human aspects of the enrichment of fermented dairy products with trace elements.

In recent years thousands of scientific papers have analyzed the correlation between the trace element supply of humans and the incidence of diseases peculiar to civilized communities. The findings make it clear that there is a strong connection between the trace element supply of the population and the incidence of circulatory and cardiac disorders. There is also growing evidence of a role for trace elements in prevention of tumorous diseases and in stimulation of the immune system (Pais 1992 and 1993).

The trace elements of nutritional significance can be divided into four groups such as elements of general essentiality (I, Zn, Mn, Mo, Fe, Co, Ni, B), elements of partial essentiality (Se, F, Cr etc.), elements of physiologically beneficial effect (Sr, Cs, Au etc.) and little known elements and elements of conflicting physiological role (Cd, Ag, Al etc.).

Underwood and Mertz (1987) and Mertz (1993 and 1995) claim that the former division into toxic and essential elements is being replaced gradually by the acceptance of the "total dose-response curve" of elements on the basis of overwhelming evidence that even the most essential substrates for life become toxic in excess. They stress the point that essentiality does not exclude toxicity, nor is toxicity inconsistent with proof of essentiality. No reliable information can be obtained on the bioavailability (i.e. proportion available for absorption and utilization within the body) of elements essential for life by the measurement of their absolute quantity because the total amount can completely be absorbed only very rarely. Furthermore, certain foods contain various

organic substances (e.g. phytate) that form such firmly bound complexes with the majority of essential elements that absorption will partly or completely be inhibited. On the other hand, some less firmly bound complex compounds containing trace elements may be of beneficial effect in that they provide prolonged absorption for these trace elements. The question is further complicated by the fact that some elements can be repressed or substituted for by others in the intermediary cell metabolism. A considerable part of toxic elements is excreted from the body in this way (Mertz 1993; Szigeti 1995). The physiological effect of the utilizable quantity of elements changes according to a bell-shaped curve (Pais 1992).

Nowadays, there is a wide variety of paramedicinal preparations on the market. These products contain trace elements as inorganic salts. However, the absorption of minerals is generally rather limited in this form. Janzsó *et al.* (1995) and Hegóczki *et al.* (1997) enriched *Saccharomyces*, *Candida* and *Schizosaccharomyces* species with various trace elements, including zinc, selenium and iodine. The yeasts which accumulated trace elements in their cells were found to be highly suitable for human and animal feeding purposes because the majority of minerals were present in the yeast cells in organic or complex bonds; thus trace elements had a higher absorption rate, they were less toxic and their beneficial effect was further improved by the proteins and vitamins of yeasts.

Comparing the trace element content of milk (Lampert 1970; Souci *et al.* 1973; Szakály 1996) with the US Recommended Dietary Allowance (RDA) values of trace elements (National Research Council, Food and Nutrition Board 1989), one can reach the conclusion that not even cow's milk, which is considered to be relatively rich in minerals, provides a sufficent supply of trace elements (Table 2).

Table 2 Trace element content of cow's milk in proportion to RDA

Trace Element	RDA (mg/day)	Trace Element Content of Milk (mg/100g)	RDA Proportion Found in 100 g of Milk (%)
I	0.12-0.15	0.004-0.006	2.7-5
Fe	10-15	0.1	0.7-1
Zn	12-20	0.3-0.6	1.5-5
Se	0.05-0.075	0.0025	3.3-5
Cu	1-2.5	0.01-0.02	0.4-2
Mn	2-3	0.002-0.01	0.1-0.5
F	1.5-4	0.01-0.05	0.3-1.3

Vandierendounck (1971) has pointed out that the trace element content of raw milk does not undergo any remarkable change during manufacture of fermented dairy products. However, owing to the absorbing capacity of lactic acid, the absorption rates of calcium, phosphorous and iron do improve substantially.

Buttriss (1997) also underlines that the fermentation process has little effect on the mineral content of milk. Yogurt is an excellent source of a number of minerals, especially calcium, zinc, phosphorous and magnesium. Not only are the concentrations of these minerals high, but the bioavailability is also generally good.

In contrast, a review by the International Dairy Federation (1991b) indicates that although some researchers speculate that bioavailability of minerals from yogurt might be enhanced, compared with milk, because of the lower gastric pH induced by yogurt, scientific support for this is inconclusive and, generally, findings do not support superior bioavailability.

Rigó (1992) reports that the average hospital diet in Hungary covers only 64% of selenium, 62% of iron, 55% of zinc, 32% of copper and only 28% of manganese RDA values. All these trace elements are of primary importance because, in addition to playing a key role in metabolism, they exert a protective effect against three of the most dangerous toxic heavy metals, as shown in Fig. 3.

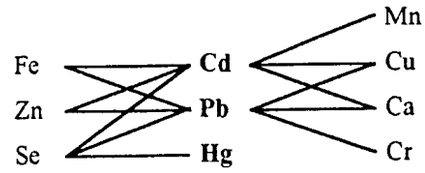


Fig. 3 Interactions between essential and toxic elements (Pais 1992)

Competition for absorption and transport at the intestinal mucosa is the probable mechanism for antagonistic interactions among trace elements, as has been suggested for zinc-copper or zinc-cadmium antagonism (Hooper *et al.* 1980; Chandra *et al.* 1993). The protection by selenium against excess of cadmium and mercury is well documented in experimental animals (Whanger 1981).

2.4.1 Physiological roles of zinc

Large regions of soils deficient in zinc exist in many countries. Normal soils contain 10-300 ppm zinc, the average is about 50 ppm. Zinc is essential for all living organisms. It is the second most abundant trace element after iron. The human body has a Zn content of about 2.2 g with an overall concentration of roughly 30 ppm. In contrast to most trace elements, zinc is fairly evenly distributed throughout the tissues. The highest concentrations are found in epidermal tissues such as skin and hair. The large amounts found in muscle and bone do not seem to form a readily exchangeable pool. Well over 90% of the body's zinc is intracellular while in whole blood 80% of the zinc is within the erythrocytes, 3% within leukocytes and about 15% in the plasma. Here it is bound mainly to albumin and to a lesser extent to α-2-macroglobulin such that only a minute fraction is unbound and therefore biologically active. Normally approximately 10-40% of ingested zinc is absorbed (Turnbull and Thompson 1989; McDowell 1992; Aggett and Comerford 1995).

As for its physiological functions, zinc is associated with enzymes, both as part of molecules and as an activator. Zinc is known to be an essential component of more than 200 metalloenzymes, including alkaline phosphatase, alcohol dehydrogenase,

carboxypeptidase, lactate dehydrogenase, superoxide dismutase etc. (Vallee 1982; Mertz 1987; Hambidge 1988; Turnbull and Thompson 1989; Kieffer 1991). Zn proteins are involved in the transcription and translation of genetic material, perhaps accounting for its essentiality to all forms of life. In its structural role, zinc mostly stabilizes the quaternary structure of the enzymes. Substantial quantities of firmly bound Zn stabilize the structures of RNA, DNA and ribosomes. In recent years several biological roles for zinc have been clarified, including those related to cell replication and differentiation. Zn deficiency greatly reduces the synthesis of DNA, RNA and proteins and hence impairs cellular division, growth and repair (Turnbull and Thompson 1989; McDowell 1992; Aggett and Comerford 1995).

Zinc has many biologically significant interactions with hormones. Among the most notable effects of zinc deficiency on hormone production and secretion are those related to testosterone, insulin and adrenal corticosteroids. Spermatogenesis and the development of the primary and secondary sex organs in the male and all phases of the reproductive process in the female can be adversely affected by zinc deficiency (Kirchgessner and Roth 1983; McDowell 1992).

Aggett (1989), Golden (1989) and Hambidge (1989) have pointed out that zinc deficiency can resemble deficiencies of other essential nutrients such as amino acids, fatty acids and some vitamins. Indeed, features of zinc deficiency can be ameliorated or exacerbated by the supply of these other nutrients. Zinc deficiency results in impaired amino acid utilization or protein synthesis. Owing to impairment of nucleic acid biosynthesis, growth retardation is universally observed in zinc deficiency. Zinc is essential to the integrity of the immune system (Tucker 1995). Furthermore, zinc maintains normal concentrations of vitamin A in plasma and is necessary for the normal functioning of the general epithelium of the ovary. Synthesis of the retinol-binding protein, the carrier of vitamin A in the blood, is decreased in Zn deficiency, resulting in inadequate vitamin A mobilization from the liver, thus causing impaired vision (Keeling *et al.* 1982; Turnbull and Thompson 1989). Zinc is found in the membrane fraction of many cells. Its function in membranes probably is to maintain their integrity by preventing membrane lipid peroxidation by free radicals (Turnbull and Thompson

1989). Moreover, metabolites of prostaglandins are affected by Zn deficiency; and in Zn deficient animals, glucose incorporation into fatty acids is also greatly reduced. Various organisms require zinc for growth, including micro-organisms in the rumen (McDowell 1992).

Fiber and phytate are thought to impair zinc bioavailability while some amino acids, notably histidine and cysteine may facilitate absorption (Reinhold *et al.* 1976; Kirchgessner and Roth 1983; Navert *et al.* 1985; Turnbull and Thompson 1989; Aggett and Comerford 1995).

Methods of assessment of Zn status are relatively insensitive. Plasma Zn is a widely used, though unreliable index for zinc status evaluation for humans (Campbell 1988; Aggett and Comerford 1995). Turnbull and Thompson (1989) have pointed out that the zinc content of blood leukocytes seems to be the best index of tissue zinc, although the assay is technically difficult and gives a wide range of normal values. Estimated human Zn requirements range from 5 to 10 mg/day for children and 12 to 15 mg/day for adults. Pregnancy and lactation requirements range from 15 to 19 mg/day (National Research Council, Food and Nutrition Board 1989). For humans, zinc has been administered to patients in a tenfold excess of the dietary allowances for months and years without adverse reactions. However, there is increasing evidence that excessive intakes of Zn may aggravate marginal Cu deficiency (National Research Council, Food and Nutrition Board 1989; Turnbull and Thompson 1989; McDowell 1992).

2.4.2 Physiological roles of selenium

Few, if any, of the major trace elements have gone through as dramatic a role metamorphosis as selenium. During the mid 1930s Se was first identified as the toxic element in some forages that caused animals to lose hair, nails and hooves. In the 1940s Se was implicated as causing cancer in laboratory animals, but now it is thought to modify the cancer risk in humans. Selenium is now known to be required by laboratory animals, food animals and humans. The total areas of the world affected by Se

deficiency are far greater and the consequences are more economically important than those afflicted with Se excess (McDowell 1992).

As far as physiological functions are concerned selenium is closely linked to vitamin E since both of them protect biological membranes from oxidative degeneration. Lack of these nutrients results in tissue breakdown. Selenium is an essential constituent of glutathione peroxidase (GSH-Px). Among other functions, this enzyme aids in protecting cellular and subcellular membranes from oxidative damage. Vitamin E in cell membranes is the first line of defense against peroxidation of vital phospholipids. Even with adequate vitamin E supply, however, some peroxides are formed. Selenium, as part of the enzyme GSH-Px in the cytosol and mitochondrial matrix, is a second line of defense, destroying these peroxides before they could damage the membranes. Vitamin E, Se and sulfur-containing amino acids, through various biochemical mechanisms, prevent some of the same nutritional diseases. Vitamin E prevents fatty acid hydroperoxide formation, S-containing amino acids are precursors of GSH-Px and Se is a component of GSH-Px (National Research Council 1983; Casey 1988; McDowell 1992; Mertz 1993).

The sparing effect of Se and vitamin E on each other can be summarized as follows.

Selenium spares vitamin E in at least three ways.

1 Preserves the integrity of pancreas, which allows normal fat digestion and thus normal vitamin E absorption.
2 Reduces the amount of vitamin E required to maintain the integrity of lipid membranes via GSH-Px.
3 Aids in the retention of vitamin E in the blood plasma.

Vitamin E reduces Se requirement in at least two ways.

1 Maintains selenium content of the body in an active form.
2 Prevents destruction of membrane lipids within the membrane, thereby inhibiting the production of hydroperoxides and reducing the amount of the Se-dependent enzyme needed to destroy the peroxides formed in the cell (Schwarz 1954; McDowell 1992).

Vitamin E and selenium are mutually replaceable, to some extent, but there are lower limits below which substitution is ineffective. Levels of Se and vitamin E above the generally accepted requirements enhance the immune response. Both vitamin E and selenium protect leukocytes and macrophages during phagocytosis, the mechanism whereby mammals immunologically kill invading bacteria. Vitamin E and Se nutrition is also important for preservation of the organelles (mitochondria and microsomes) responsible for building the defense mechanisms against disease, radiation and other stresses (McDowell 1992).

As was mentioned earlier, selenium also has a strong tendency to complex with heavy metals, including Cd, Hg and Pb, thus exerting protective effect against them (Sarudi *et al.* 1992b).

Both vitamin E and Se provide protection against heavy metal toxicity with three classes of heavy metals.

1 Those like Cd and Hg, with which Se is highly effective in altering toxicities, but vitamin E has little influence.
2 Those like Ag and As, with which vitamin E is highly effective and selenium is also effective but at relatively high levels.
3 Those like Pb, which are counteracted by vitamin E, but on which Se has little effect (McDowell 1992).

Selenium also influences the metabolism and toxicity of a variety of drugs and chemicals.

The recommended dietary allowance for adults is 50-75 μg/day, with correspondingly lower intakes for younger age groups (National Research Council, Food and Nutrition Board 1989).

Some laboratories have been able to make limited correlations of GSH-Px activity with blood Se concentrations, but the enzyme assay has not been standardized well enough for routine use in Se assessment in humans. For many species, Se concentrations in liver, renal cortex, blood and other tissues adequately portray Se status (McDowell 1992). However, Casey (1988) has pointed out that in humans, only the amount found in

the blood can be measured routinely and it is not known how this relates to activity in other tissues.

The concentration of selenium in milk reflects dietary selenium intake. McDowell (1992) reports that cow's milk from a low-Se area in Oregon contains less than 20 μg l^{-1}, compared with 50 μg l^{-1} from a high-Se area in South Dakota.

The studies by Sarudi *et al.* (1992a) show that the average selenium content of milk in Hungary is 8.95 μg l^{-1}, ranging from 3.5 μg l^{-1} (western part of the country) to 18.0 μg l^{-1} (eastern part).

During the latter 1970s two human diseases were found to be associated with a severe Se deficiency. Either of them is a cardiomyopathy known as Keshan disease. It affects primarily children and women of child-bearing age and is found almost exclusively among people living in the mountainous regions of China. The peasants are thought to be susceptible because their diet consists solely of home-grown foods produced on Se-deficient soils. Sudden acute insufficiency of heart function characterizes acute cases whereas chronic cases usually exhibit moderate to severe heart enlargement with varying degrees of insufficient heart function. A second Se-responsive disease in China is referred to as Kaschin-Beck disease (big-joint disease). This is a chronic, disabling, degenerative, generalized osteoarthrosis involving both the peripheral joints and the spine. Unlike the cardiomyopathy, which is most prevalent among children aged 2-7 years, the big joint disease appears to be more prevalent among older children. Other disease conditions are likely to occur in the more severely Se-deficient areas of the world (Keshan Disease Research Group 1979; Levander 1985; Yang 1985; McDowell 1992; Mertz 1993 and 1995; Yang *et al.* 1993).

Ecological comparisons have suggested an inverse association between Se intake and the risk of cancer. Selenium has also been found to reduce the incidence of cancer in some animal models (Ip and Sinha 1981). However, most of these studies involved the administration of amounts of Se above the nutritional requirement and are difficult to evaluate.

Se appears to act at the promotional stage of carcinogenesis but the actual mechanism is unknown as yet. There is evidence that the antitumorigenic action of Se is

not mediated through its antioxidant activity, but possibly through a more direct interaction of Se with metabolites of tumor-inducing chemicals.

In contrast, Casey (1988) reports that if Se has an effect on the population rates of human cancer mortality, it is not strong.

Self-medication with Se cannot be regarded as harmless because Se has a narrower range of safety than many other trace elements and there have been a number of cases of toxicity reported in the USA in individuals who were taking high levels of supplemental selenium (Poirer and Milner 1983; Ip 1985; Levander 1985; Casey 1988; McDowell 1992).

2.4.3 Physiological roles of iodine

McDowell (1992) has pointed out that iodine deficiency in humans is one of the most prevalent deficiency diseases and it occurs in almost every country in the world. More than one billion people are at risk for iodine deficiency world-wide. Many soils are low in iodine; goiter regions are located on every populated continent.

The only known role of iodine is in the synthesis of the thyroid hormones, triiodothyronine and thyroxine, the latter of which contains about 65% iodine. Thyroid hormones have an active role in thermoregulation, intermediary metabolism, reproduction, growth and development, circulation and muscle function; they control the oxidation rate of all cells. The conversion of carotene to vitamin A also appears to be regulated by thyroid hormones (McDowell 1992).

In foods and water, iodine occurs largely as inorganic iodide. In this form, it is absorbed throughout the gastrointestinal tract, transported by loose attachment to plasma proteins and is quickly distributed to plasma where it enters an iodide pool. From the plasma, iodine is transported to the thyroid, with over 90% administered I accounted for by thyroid uptake and urinary excretions. In the thyroid, iodine is trapped, concentrated, rapidly oxidized and converted to organic I by combining with tyrosine. Iodine is present in the thyroid as inorganic I, monoiodotyrosine, diiodotyrosine, triiodothyronine (T_3), tetraiodothyronine (thyroxine, T_4) and other iodinated organic compounds.

Thyroglobulin, an iodinated glycoprotein in the thyroid, is the storage form of the hormones and represents 90% of the total thyroid I (McDowell 1992; Anke *et al.* 1993).

Lactating animals secrete large amounts of iodine in the milk. Milk I concentration generally increases with advancing stages of lactation. Colostrum is 4-5 times higher in iodine than later milk. Moderate changes in dietary iodine are quickly reflected in milk. Teodoru *et al.* (1976 and 1985) had given rations supplemented with marine algae meal (220 g/cow/day on average) to a test group of cows in an iodine-deficient area in Romania. After 45 days, their protein-bound iodine levels rose by 74% in blood and 88% in milk, reaching values similar to those commonly found in regions with adequate iodine supply.

Prior to about 1960, milk was considered to be a rather poor source of iodine. Recent surveys of market milk in the United States indicate that milk is now a good source of the mineral. Surveys of I intake have also revealed that dairy products contribute 38-50% of the I for adults and 56-85% for young children (McDowell 1992).

Recommended daily I intake for both sexes in humans is 40 μg for children aged 0-6 months, 50 μg from 6 to 12 months, 70-120 μg from 1 to 10 years and 120-150 μg from 11 years onward. The requirements during pregnancy and lactation are 175 μg and 200 μg respectively (National Research Council, Food and Nutrition Board 1989).

Severe iodine deficiency and thus status can be diagnosed on the clinical evidence of goiter alone. Less severe forms of goiter or iodine deficiency are more difficult to diagnose and thus weight and histological structure of the thyroid gland as well as serum iodine (predominantly thyroxine) are used in diagnosis (McDowell 1992).

Iodized salt is the most widely used and simplest source of supplemental iodine for humans. Iodization of salt as a method of preventing goiter was first suggested by the French scientist Boussingault in 1831; however mass prophylaxis was first attempted in Michigan, USA in 1924. In only five years, goiter rate fell by 30%. Other methods include the addition of I as iodide or iodate to various foods. Iodination of municipal water supplies is a relatively effective method of I prophylaxis. However, in industrialized countries, excessive I intake by humans, rather than I deficiency, has become a health concern. This change may stem from the presence of iodates in baked

goods, milk and dairy products and from increased consumption of I supplements and seafoods. For humans, iodine intakes of 2 mg/day should be regarded as harmful (McDowell 1992).

2.5 Use of various substances for increasing acid development by and growth rate of lactic acid bacteria

The growth and acid production of lactic acid bacteria can be stimulated by addition of various extracts of micro-organisms, higher plants and animal tissues. Some of the substances responsible for the stimulation of lactic acid bacteria have been identified and extensively studied whereas others are unknown as yet (Nath and Wagner 1973; Selby Smith *et al.* 1975; Sugihara and Kline 1975; Glass and Hedrick 1976).

The work of Sprince and Woolley (1945) has focused attention on the importance of peptides for the growth of many bacteria. Subsequent investigations have revealed the presence of peptides and nucleic acid derivatives in protein hydrolysates (Anderson and Elliker 1953), in pancreas extract (Sandine *et al.* 1956; Koburger *et al.* 1963), in liver and in yeast extract (Anderson and Elliker 1953; Huhtanen and Williams 1963; Selby Smith *et al.* 1975; Sugihara and Kline 1975) and in algal extracts (Shirota *et al.* 1964; Kurita *et al.* 1979; Zielke *et al.* 1978; Webb 1982), which are responsible for the stimulatory property of these materials on the growth and acid production of lactic acid bacteria in milk.

The studies by Huhtanen and Williams (1963) have shown that milk is deficient in the purine and pyrimidine bases of RNA and DNA and it contains low levels of free amino acids and oligopeptides which are insufficient for the optimal growth of several lactic acid bacteria (Desmazeaud and Juge 1976; Thomas and Mills 1981). Consequently, proteolysis is needed to provide additional amino acids and small peptides from milk proteins (Pritchard and Coolbear 1993). *Strep. thermophilus*, for instance, has complex requirements for nutritional factors and its growth in milk is influenced by the availability of simple and assimilable nitrogen sources (Carminati *et al.* 1994; Desmazeaud 1994) because this species is weakly proteolytic, although several

peptidase activities have been detected (Tsakalidou and Kalantzopoulos 1992; Carminati *et al.* 1993; Oberg and Broadbent 1993; Pritchard and Coolbear 1993; Coolbear *et al.* 1994; Crow *et al.* 1994; Midwinter and Pritchard 1994; Monnet and Grippon 1994; Neviani *et al.* 1995).

The growth-promoting effect of extracts of plants and micro-organisms has been measured in a number of ways, e.g. turbidimetrically (Kennedy *et al.* 1955; Sandine *et al.* 1956), by titrimetric measurement of lactic acid production (Selby Smith *et al.* 1975), by determination of pH shifts (Koburger *et al.* 1963; Gilliland and Speck 1969), by enumeration of bacterial counts (Nath and Wagner 1973) and with bioautographical methods (Kennedy *et al.* 1955).

2.5.1 Use of other substances than algal extracts for increasing acid development by and growth rate of lactic acid bacteria

Kennedy and Speck (1955), Kennedy *et al.* (1955) and Huhtanen and Williams (1963) have reported that corn steep liquor is stimulatory to acid development by some lactic acid bacteria. This substance found considerable use as a source of nutrients for certain molds and bacteria employed in industrial fermentations. Liggett and Koffler (1948) have revealed that corn steep liquor alone has a sufficient supply of nutrients for the growth and development of many bacteria. Kennedy and Speck (1954) have pointed out that corn steep contains a growth stimulant for a number of lactic acid bacteria grown either in milk or in synthetic media that are complete nutritionally. Kennedy *et al.* (1955) studied the stimulatory effect of corn steep on *Lact. casei* but all of the known stimulants failed to replace the corn steep factor(s). The results of the experiments carried out by Zuraw *et al.* (1960) have shown that corn steep liquor contains phenylalanine and possibly a nucleoside, which contribute to the growth-promoting properties of this material.

Huhtanen and Williams (1963) studied the stimulation caused by yeast extract of acid development in milk by various lactobacilli. It has been found that supplementation

of milk with purine and pyrimidine bases and amino acids allows nearly maximal acid development by certain *Lact. bulgaricus* and *Lact. acidophilus* strains.

Addition of salts (particularly trace elements), purines, pyrimidines and optimization of casein hydrolysate and Tween 80 levels result in markedly improved growth of *Lact. sanfrancisco* but do not eliminate the need for freshly prepared yeast extractives (Sugihara and Kline 1975).

Selby Smith *et al.* (1975) fractionated yeast extract into seven fractions. The fraction most stimulatory to the growth of *Lactococcus lactis* subsp. *lactis* (*Lactoc. lactis*) contained more than 70% of the amino nitrogen found in yeast extract and consisted of a wide range of free amino acids and a small amount of peptide material. Further investigations have revealed that amino acids, purine and pyrimidine bases and inorganic constituents are responsible for the stimulation of this species. *Lactoc. lactis* is dependent upon its proteolytic enzymes for release of nitrogenous compounds needed for growth (Citti *et al.* 1965). When milk is supplemented with simple nitrogenous compounds, e.g. tryptic digest of casein, growth is promoted because the need for the production of proteolytic enzymes is bypassed, especially when the cultures possess only poor proteolytic activity (Harriman and Hammer 1931). In addition, yeast extract has been found to contain a component capable of decomposing H_2O_2.

Growth of and rate of acid development by six cultures of lactic acid bacteria were increased in the presence of *Micrococcus* isolate F4. The existence of two mechanisms for micrococcal stimulation of the lactic acid bacteria has been postulated by Nath and Wagner (1973). Either of them involves removal of hydrogen peroxide while the other mechanism has not been further characterized. Addition of ferrous ions and catalase to milk also stimulates acid development by certain lactic acid bacteria, apparently due to the destruction of metabolically produced peroxide, which is inhibitory (Gilliland and Speck 1969). Stimulation of acid production and growth of lactobacilli by capsular material from micrococci has been demonstrated by Nath and Ledford (1971).

Studying slow and fast acid-producing strains of *Lactoc. lactis*, Citti *et al.* (1965) have found a direct relationship between available nitrogen and total growth in milk, reflected by the final amount of acid produced in the cultures.

Koburger *et al.* (1963) have reported that the growth of *Lactoc. lactis* in milk is accelerated by pancreas extract. The active compounds have been identified as nucleic acid derivatives such as inosine, hypoxanthine and adenine. Sandine *et al.* (1956) have also pointed out that pancreas tissue contains two stimulants for *Lact. casei* and *Lactoc. lactis*. Either of the stimulants shows characteristics of a peptide.

Anderson and Elliker (1953) have revealed that addition of liver fraction, trypsinized skim milk and peptonized milk produces stimulation in the growth of mixed starter cultures and individual strains of *Lactoc. lactis* and *Lactoc. cremoris* (*Lactococcus lactis* subsp. *cremoris*). The results suggest that the material responsible for the increased growth rate is a peptide or peptide-like in nature and that an enzymatic digestion of milk tends to release these substances from the milk proteins in a readily available form for the starter culture organisms.

2.5.2 Use of algal extracts for increasing acid development by and growth rate of lactic acid bacteria

Extracts of the green microalga *Chlorella* are widely used in biotechnological processes as a unique source of growth factors for reducing the production time of fermented dairy products involving lactic acid bacteria. Shirota *et al.* (1964) have discovered that *Chlorella* species contain substances which significantly promote the growth of lactobacilli. A medium that contained 0.5-2.0% *Chlorella* powder produced 7 times more lactobacilli after 24 h, 3 times more after 48 h and 1.8 times more after 72 h than controls; another one that had a *Chlorella* content of 1% produced as much lactic acid during 48 h as the medium without *Chlorella* supplementation during 144 h. Having been extracted the effective component was found to promote the growth of all kinds of lactobacilli, thereby increasing the amount of lactic acid produced. Kurita *et al.* (1979) carried out investigations to identify the substances in *Chlorella* extract that promoted the growth of *Lactobacillus* species. Two nucleosides (adenosine and guanosine) have been found responsible for the stimulation observed.

Zielke *et al.* (1978) have pointed out that the hot-water extract of the green alga *Scenedesmus acutus* enhances acid development by *Lact. casei* subsp. *casei* biovar. *shirota*, *Lactoc. lactis* and *Strep. thermophilus*. Peptone, adenine and hypoxanthine have been identified as growth factors in the algal extract, contributing to the stimulation of the above-mentioned micro-organisms. The results have shown that a mixture of peptone, hypoxanthine and adenine can stimulate the souring of milk by *Lactoc. lactis* and this stimulation is comparable to that achieved with the algal extract.

The effect of yeast extract, casein peptone, meat peptone and an aqueous extract of *Scen. obliquus* on acid development by four species of lactic acid bacteria (*Strep. thermophilus*, *Lact. bulgaricus*, *Lact. casei*, *Lactoc. lactis*) was tested by Webb (1982). The algal and yeast extracts stimulated all bacteria whereas casein peptone had no effect on *Lactoc. lactis*. All the substances tested retarded acid production of *Strep. thermophilus* to some extent during the first 5 h of incubation but were stimulatory thereafter. The algal extract was fractionated and two fractions with stimulatory activity were found by means of *Lactoc. lactis*. The results have suggested that the hypoxanthine content of the algal extract is largely responsible for shortening the lag phase. However, the stimulatory effect of the algal extract is not due to hypoxanthine alone because tests with pure hypoxanthine have shown that the maximum stimulation achievable is less than that caused by the complete extract. The other stimulatory compounds could not be identified but are probably not amino acids.

2.6 Products manufactured with addition of vitamins, trace elements and algal extracts

Several patents suggest that vitamins and trace elements should be used as additives for the manufacture of certain foods and drinks. The raw material to be supplemented is mostly milk, whey or fruit juice but no fermented dairy product is recommended for this purpose.

Other patents describe the use of marine algal extracts as stabilizers for yogurts and as additives contributing to the improvement of fatty acid composition of butter.

Products synthesized by microalgae are employed as coloring agents in butter and margarine production. Food additives are also produced from the biomass of marine algae.

2.6.1 Products manufactured with addition of vitamins and trace elements

Zhao (1994) made whole milk powder supplemented with zinc, vitamins A, D and E and organic germanium extracted from plants. The product can be characterized by a wide range of beneficial effects such as stimulation of the immune system, improvement in senility resistance and prevention of diseases, including cancer.

Another patent by Wiesenberg *et al.* (1984) outlines a conditioning drink that contains 30-90% (w/w) fruit juice, 1.2-5.0% (w/w) whey proteins and/or partially hydrolyzed soy bean proteins, as well as mineral salts and added vitamins. Lactose content is enzymatically cleaved in the product, which has a pH of 4.2-4.4 preferably, thereby ensuring that vitamins and trace elements have a higher stability.

A product made from semi-skimmed milk and a concentrate containing vitamins, mineral salts and trace elements has been patented recently by Gerome *et al.* (1994). The drink contains Na-selenite, ZnO, Fe-gluconate, folic acid and vitamins C, A, D_3 and E. A daily intake of 0.5 liter ensures consumers at least 50% of essential nutrients.

A UHT drink and powder consisting of milk, egg white (both of them added preferably in powder form), liquid from medicinal herbs, B-complex vitamins and even algae has been patented by Philippe (1994). The product has a refreshing effect, it helps to do without sleeping pills and provides tonic elements and protecting agents for the brain and the heart.

2.6.2 Products manufactured with addition of algal extracts

A German patent suggests that a mixture of several components, including a red algal extract called carrageenan should be used as a stabilizer in the manufacture of natural and flavored yogurts (Weseloh J.H. OHG 1972).

An emulsion stabilizer that is capable of extending the shelf-life of milk drinks is outlined in a Japanese patent by Kawaguchi *et al.* (1989). The product consists of κ-carrageenan extracted from red algae with water, sodium caseinate and a food emulsifier.

Schirmann (1985) has employed *Fucus vesiculosus* juice concentrate and fruit syrups and concentrates for yogurt manufacture. The low calorie product has curative and diuretic properties.

Shino and Tanaka (1981) reveal, in another patent, that the consumption of butter supplemented with β-1,3-glucan extracted from *Chlorella*, *Scenedesmus* or *Spirulina* species can contribute to the reduction of cholesterol, phospholipid and neutral lipid levels of blood.

A French company (USSI Ingeniérie 1990) has developed a bioreactor for microalgae. A major product synthesized by *Dunaliella* species in this system is carotene, which can be used as a coloring agent in butter and margarine production. Other products synthesized include vitamins and polyunsaturated fatty acids.

Iizuka *et al.* (1994) suggest that various foods, including dairy products should be supplemented with dried *Crypthecodinium cohnii* marine microalgae. The algal biomass contains at least 2% docosahexaenoic acid and has a protein content of over 80%.

Egli (1980) has patented a method of producing shelf-stable and sterile yogurt. After homogenization and pasteurization of raw milk, a modifying additive consisting essentially of red algae, starch, gelatin and sugar is added and yogurt of long shelf-life is subsequently made.

No patent outlining a method of manufacturing fermented dairy products supplemented with cyanobacterial biomass, with or without addition of trace elements, is available.

2.7 Changes in yogurts during storage

Owing to post-acidification, spoilage by microbial contaminants and protein or fat degradation resulting in smell and taste defects, regular yogurts, which contain viable

micro-organisms, have a limited shelf-life (Rohm and Lechner 1989; Mayer 1990). Even if stored at a temperature of below 10°C (at 4-6°C), yogurts have a shelf-life of 2-3 weeks (up to 4-6 weeks in the case of aseptic packaging).

Some countries have developed standards with respect to the characteristic viable cell count of yogurt. The values range between 10^6 and 10^8 cfu g^{-1} (Rašić and Kurmann 1978; Gläser 1992). Heat treatment of fermented dairy products is contrary to the regulations in Austria and both natural and flavored yogurts with a characteristic viable population of less than 10^6 cfu g^{-1} are considered to be imitations (Bundeskanzleramt 1990). A draft version of Codex Alimentarius Hungaricus (Magyar Élelmiszerkönyv Bizottság 1996) suggests that yogurts should contain at least 10^7 g^{-1} viable bacteria of starter culture origin. This is in conformity with the draft of an International Dairy Federation Standard suggesting that fermented dairy products should contain at least 10^7 g^{-1} characteristic viable starter bacteria until they reach the sell-by date. Rohm and Lechner (1988 and 1989) are of the same opinion. According to Gläser (1992) yogurts of high quality can be characterized by $\geq 10^8$ g^{-1} lactic acid bacteria; he suggests that a *Strep. thermophilus* / *Lact. bulgaricus* ratio of 1:1 to 3:1 and a defined level of lactase activity should also be required at the time of sale in the European Union.

Rohm *et al.* (1990) investigated the storage-induced changes in the populations of viable yogurt starter organisms and microbial contaminants in randomly selected Austrian natural set yogurts. Counts of *Strep. thermophilus* and *Lact. bulgaricus* remained above 10^8 cfu g^{-1} in yogurts stored at 10°C until the sell-by-date (15-20 days after manufacture). Both increased storage temperatures and prolonged storage periods markedly reduced the survival of yogurt starter bacteria. Depending on sample origin, the yeast populations increased from less than 10^1 g^{-1} to above 10^6 g^{-1} when yogurts were stored at 10°C until the sell-by-date.

3 MATERIALS AND METHODS

3.1 Researches done in a model milk medium

3.1.1 Raw material

UHT milk free of inhibitory substances, tested by means of Delvotest® SP MINI, was used. It was deep-frozen (in Ultra Low Freezer U 41085, New Brunswick Scientific) and stored at -75°C so that all the experiments would be done with raw material from the same lot of production and thus the results could be compared. It contained 28 g l^{-1} fat, 34 g l^{-1} protein, 47 g l^{-1} lactose and 7 g l^{-1} ash. Although having been treated at an ultra high temperature, milk was heated to 90°C and held for 5 min before being cooled to inoculation temperature so that the levels of thermal denaturation of whey proteins would be increased (Kessler 1988a and 1988b). The experiments were carried out in the Institute of Food Science at Pannon University of Agricultural Sciences, Faculty of Agricultural Sciences at Mosonmagyaróvár.

3.1.2 Starter culture strains

Four freeze-dried strains, manufactured by Chr. Hansen A/S, were kindly supplied by the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár. The strains were subcultured twice at 37°C for 12-72 h before being employed for the experiments. The single strains and the combinations of strains used for the fermentation experiments are listed in Table 3.

Table 3 Single strains and combinations of strains employed in the fermentation experiments

Starter Culture Bacteria (SCB)		Name of product manufactured with the given starter culture
SCB of Intestinal Origin	Other SCB	
—	*Strep. thermophilus*	—
	Lact. bulgaricus	—
	Strep. thermophilus + *Lact. bulgaricus*	Yogurt
Lact. acidophilus	—	Acidophilus Milk
	Strep. thermophilus	Bioghurt®
	Strep. thermophilus + *Lact. bulgaricus**	Acidophilus Yogurt
		ACO Yogurt
Bifid. bifidum	—	Bifidus Milk
	Strep. thermophilus	Bifighurt®
Lact. acidophilus + *Bifid. bifidum*	—	Fermented AB Milk (Cultura®)
	Strep. thermophilus	Fermented ABT Milk
		Biogarde®

Strep. thermophilus, *Streptococcus salivarius* subsp. *thermophilus* CH-1; *Lact. bulgaricus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2; *Lact. acidophilus*, *Lactobacillus acidophilus* La-5; *Bifid. bifidum*, *Bifidobacterium bifidum* Bb-12; *, Since *Lact. bulgaricus* is added to such products in order to ensure more pronounced acid development, no experiment was performed with this combination

3.1.3 Spirulina platensis biomass

The *Spir. platensis* biomass, which is a licensed food supplement in Germany, was obtained from the Institute of Cereal Processing Inc. (Institut für Getreideverarbeitung GmbH) at Bergholz-Rehbrücke. The composition of this product is shown in Table 4.

Table 4 Composition of the *Spirulina platensis* biomass

Component	Quantity (in 1 kg of biomass)
Original component	
Dry matter	941 g
Protein	576 g
Total lipids	111 g
Ash	114 g
Zn	515 mg
Cu	7 mg
Cd	<0.01 mg
Pb	0.05 mg
Enriched component	
KI	0.131 g
$ZnCl_2$	2.052 g
$Na_2SeO_3 \cdot 5H_2O$	0.333 g

Since the product was added to the milk at a concentration of 3 g l^{-1}, 0.393 mg l^{-1} KI, 6.156 mg l^{-1} $ZnCl_2$ and 0.999 mg l^{-1} $Na_2SeO_3 \cdot 5H_2O$ were used when the individual effect of trace elements on acid development by the starter culture strains was tested.

The idea of employing the cyanobacterial (CBA) biomass at a concentration of 3 g l^{-1} was borrowed from a previous work of ours wherein the effective and economic concentration of CBA biomass resulting in good sensory properties was determined (Springer *et al.* 1998).

3.1.4 Further components tested

In addition to iodine, zinc and selenium, the following vitamins and nitrogenous substances were tested separately. Vitamin B_1 (0.5 mg l^{-1}), vitamin B_2 (2.0 mg l^{-1}), nicotinic acid (1.0 mg l^{-1}), pantothenic acid (4.0 mg l^{-1}), vitamin B_6 (0.6 mg l^{-1}), vitamin B_{12} (5.0 μg l^{-1}), vitamin C (50 mg l^{-1}), vitamin A (1.0 mg l^{-1}), vitamin E (3000 IU l^{-1}), peptone (1.0 g l^{-1}), adenine (2.0 mg l^{-1}), hypoxanthine (3.5 mg l^{-1}). The effect of peptone + adenine and that of peptone + hypoxanthine were also investigated. All these substances, including iodine, zinc and selenium, were purchased from Merck Kft.

3.1.5 Experimental conditions

The heat-treated and cooled milk was portioned out into 250 ml Erlenmeyer flasks and supplemented with the substrate(s) to be tested (CBA biomass or its components). As for single strains, the rate of inoculation was 1% (v/v) except for *Bifid. bifidum* (6%, v/v). *Strep. thermophilus* and *Lact. bulgaricus* were incubated at 42.5°C whereas *Lact. acidophilus* and *Bifid. bifidum* at 37.5°C. As regards combinations of strains, the rate of inoculation employed was between 0.1% (v/v) and 6.0% (v/v) with respect to the single strains. The mixed culture of *Strep. thermophilus* and *Lact. bulgaricus* was incubated at 42.5°C whereas the combinations of strains containing *Lact. acidophilus* and/or *Bifid. bifidum* at 37.5°C. Yeast extract (0.25 g l^{-1} from Merck Kft.) was added to the samples containing *Bifid. bifidum* so that the special nutritional requirements of this species would be satisfied. Acid production was determined by hourly pH measurements and growth was checked by enumeration of viable cells. All the experimental results contained in figures and tables are means of 3 replicates (n=3).

3.1.6 pH measurement

The pH of samples was measured at room temperature with a HANNA 8521 pH meter and combined glass electrode.

3.1.7 Enumeration of micro-organisms

The circumstances of the enumeration of micro-organisms are outlined in Table 5.

Table 5 Determination of viable cell counts of mixed culture components

	Mixed Culture Component	Culture Medium	Incubation		
			Conditions	Time (h)	Temp. (°C)
1	*Strep. thermophilus*	M 17 Agar	Aerobic	48	37
	Lact. bulgaricus	MRS Agar*	Anaerobic	72	37
2	*Strep. thermophilus*	M 17 Agar	Aerobic	48	37
	Lact. acidophilus	MRS Agar	Anaerobic	72	37
3	*Strep. thermophilus*	M 17 Agar	Aerobic	48	37
	Bifid. bifidum	MRS Agar	Anaerobic	72	37
4	*Lact. acidophilus*	MRS Agar	Aerobic	72	37
	Bifid. bifidum	MRS+NNL† Agar	Anaerobic	72	37
5	*Strep. thermophilus*	Lee's Agar	Aerobic	120	37
	Lact. acidophilus	MRS-Maltose Agar	Anaerobic	72	37
	Bifid. bifidum	MRS+NNLP‡ Agar	Anaerobic	72	37

*, Acidified to pH 5.4; †, Nalidixic acid + Neomycin sulfate + Lithium chloride; ‡, Nalidixic acid + Neomycin sulfate + Lithium chloride + Paromomycin sulfate

The pour-plate method was used in all cases. Oxoid anaerobic jars were employed, atmospheric oxygen being absorbed by means of ANAERO*GEN*™ AN 25 paper sachets (from Oxoid Kft.). Components of the NNL and NNLP solutions were purchased from Sigma-Aldrich Kft. and all the other components of the culture media from Merck Kft.

3.2 Manufacture of a model product and storage experiments

The cyanobacterial and control yogurts needed for the storage experiments were manufactured in the pilot plant of the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár.

The composition of the raw material (i.e. pasteurized market milk) was identical with that of the UHT milk outlined in subchapter 3.1.1. Homogenization resulted in an average diameter of fat globules of <0.6 μm, without cluster formation (Szakály 1981). Heat treatment took 5 min at 90°C. FYE-43 yogurt starter culture (kindly supplied by the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár) was employed at a concentration of 3% (v/v). Incubation took 2.5 h at 42°C.

In the case of cyanobacterial yogurt the *Spir. platensis* biomass was added to the product when pH dropped to 5.2, at a temperature of 25°C; thereafter the normal

technology of yogurt manufacture was followed. Both the cyanobacterial and the control samples were filled into 40 retail containers apiece, which were then sealed with aluminum foil. After a day's pre-cooling, half of the samples was placed into a cooling and heating thermostat at 15°C while the rest of them was stored in a refrigerator at 4°C. Three (n=3) retail containers apiece were opened after 0, 3, 6, 9, 12 and 15 days of storage in the case of cyanobacterial and control yogurt samples stored at 15°C and after 0, 7, 14, 21, 28 and 35 days of storage in the case of cyanobacterial and control samples stored at 4°C. The viable cell counts of the following micro-organisms and microbial groups were determined according to the standard methods of the International Dairy Federation (1985, 1988, 1990 and 1991a): total microbial count, counts of *Strep. thermophilus*, *Lact. bulgaricus*, yeasts and molds, total enterococci and coliform organisms (Table 6).

Table 6 Enumeration of micro-organisms during the storage experiments

Micro-organism (Group)	Method	Culture Medium*	Incubation	
			Time (h)	Temp. (°C)
Strep. thermophilus	Pour-plate	M 17 Agar	48	37
Lact. bulgaricus	Pour-plate	MRS Agar†	72‡	37
Total Microbial Count	Addition∇	—	—	—
Yeasts and Molds	Pour-plate	YGC Agar	96	25
Coliform Organisms	MPN	BRILA Broth	24-48	37
Enterococci	Pour-plate	KEA Agar♦	72	37

*, All culture media were purchased from Merck Kft.; †, Acidified to pH 5.4; ‡, Incubated anaerobically; ∇, *Strep. thermophilus* count + *Lact. bulgaricus* count (calculated values); ♦, Kanamycin Esculin Azide Agar.

Along with the microbiological investigations, the pH of samples was measured so that data concerning the degree of post-acidification would also be gained.

3.3 Mathematical-statistical evaluation

As was mentioned in subchapter 3.1.5, all the experimental results published in chapter 4 are means of three replicates (n=3).

The average pH values and average logarithmic viable cell counts were plotted by means of the STATISTICA 4.5 program package.

The pH values of experimental samples and those of the corresponding controls were tested for significance of difference by means of the MicroCal Origin 3.0 program package. + stands for significant stimulation of acid production at the P=0.05 level, - stands for significant retardation of acid production at the P=0.05 level and 0 means that no significant difference was found between controls and test samples at the P=0.05 level. Throughout the rest of this Dissertation, the terms "significantly (different)" or "significant (difference)" etc. mean that controls and test samples were significantly different at the P=0.05 level. Fifth order polynomials are fitted in the figures illustrating acid development results. Dotted lines, in these figures, represent the 95% confidence bands of curves.

Whiskers in the bar charts showing average logarithmic viable cell counts of combinations of strains and in those illustrating the results of storage experiments indicate standard deviation (±s).

The average growth rate of the individual starter culture strains, during a given interval of time, was calculated by the formula $\mu_{i(y;x)} = (\log N_x - \log N_y) / (x-y) \cdot \log 2$, where $\mu_{i(y;x)}$ is the average growth rate of strain "i" during time interval y–x; N_x is the average viable cell count of strain "i" at time x; N_y is the average viable cell count of strain "i" at time y; x-y is the length of the interval of time studied, expressed in hour.

4 RESULTS

4.1 Effect of the *Spirulina platensis* biomass and that of its active components on acid development by single-strain type thermophilic dairy starter cultures in a model milk medium

The influence of the cyanobacterial biomass and that of its active components on acid development by the four single strains were determined first. On the basis of preliminary experiments, a rate of inoculation of 6% was found to be appropriate in the case of *Bifid. bifidum* while the other three strains were tested at 1% inoculation.

4.1.1 Effect of the Spirulina platensis biomass and that of its active components on acid development by Streptococcus thermophilus CH-1 in a model milk medium

In the case of *Strep. thermophilus*, just as in that of all the other strains, the degree of decrease in pH was indicative of the rate of acid production. The differences in pH between controls and samples supplemented with various substrates, measured hourly, are shown in Table 7. Negative numbers mean that experimental samples had higher pH than controls whereas positive numbers show that the pH of experimental samples was lower than that of controls.

Table 7 Effect of various substances on acid development by *Streptococcus thermophilus* CH-1 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls									
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
CBA*	**-0.13**	**-0.14**	**+0.10**	**+0.41**	**+0.19**	**+0.07**	+0.03	+0.01	-0.01	-0.01
Iodine	0	+0.01	+0.06	+0.08	+0.02	+0.03	+0.01	0	+0.01	+0.01
Zinc	0	**+0.05**	**+0.10**	+0.08	+0.03	+0.03	-0.01	-0.02	+0.01	+0.01
Selenium	0	**+0.05**	+0.07	+0.12	+0.02	+0.02	0	0	0	+0.01
Vitamin B†	0	+0.01	**+0.08**	+0.07	0	0	+0.02	0	+0.01	+0.02
Vitamin C	0	**+0.03**	**+0.14**	**+0.18**	**+0.05**	**+0.02**	+0.04	+0.01	+0.01	+0.02
Vitamin A	0	**+0.02**	**+0.13**	**+0.20**	**+0.03**	0	+0.04	-0.01	0	+0.01
Vitamin E	0	**+0.03**	**+0.11**	**+0.21**	**+0.05**	+0.01	+0.03	0	+0.01	+0.02
Peptone	0	0	**+0.15**	**+0.20**	**+0.10**	**+0.04**	**+0.04**	**+0.03**	+0.01	+0.03
Adenine	0	**+0.02**	**+0.14**	**+0.18**	**+0.08**	+0.02	+0.01	+0.03	0	**+0.03**
Hypoxanthine	0	**+0.03**	**+0.22**	**+0.27**	**+0.12**	+0.04	+0.04	+0.04	+0.02	**+0.03**
Pep.+Ade.	0	**+0.02**	**+0.33**	**+0.36**	**+0.13**	**+0.07**	**+0.06**	**+0.05**	+0.03	**+0.05**
Pep.+Hyp.	0	**+0.02**	**+0.34**	**+0.38**	**+0.15**	**+0.08**	**+0.06**	**+0.05**	**+0.03**	**+0.05**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

The average of the initial pH values of samples supplemented with cyanobacterial biomass was higher than that of controls because the cyanobacterial supplement was of alkaline character (an aqueous solution containing 3 g l^{-1} *Spir. platensis* biomass had a pH of 9.9) and it also had considerable buffering capacity (Fig. 4).

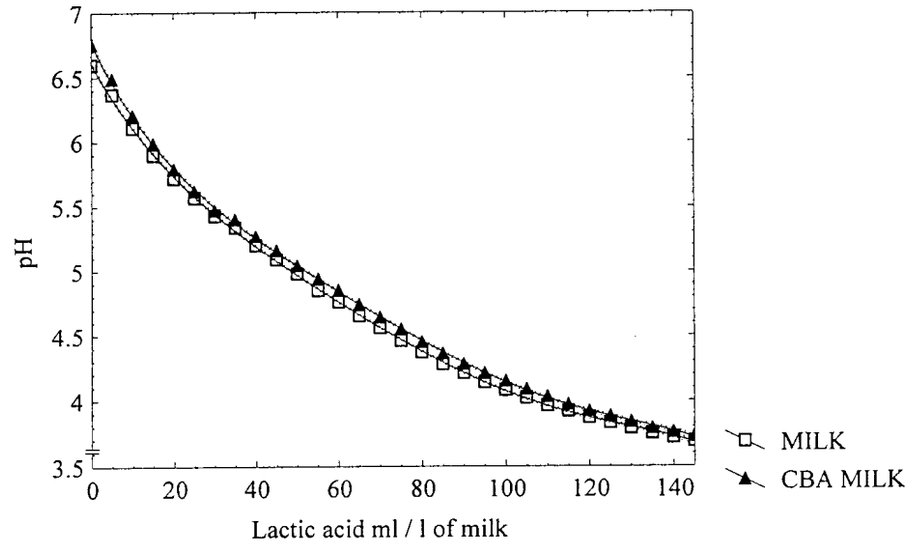


Fig. 4 Buffering capacity curve of milk and that of milk supplemented with 3 g l^{-1} *Spirulina platensis* biomass (titrated with 1 mol l^{-1} lactic acid)

Fig. 5 shows the effect of addition of 3 g l^{-1} *Spir. platensis* biomass on the rate of acid development by *Strep. thermophilus*.

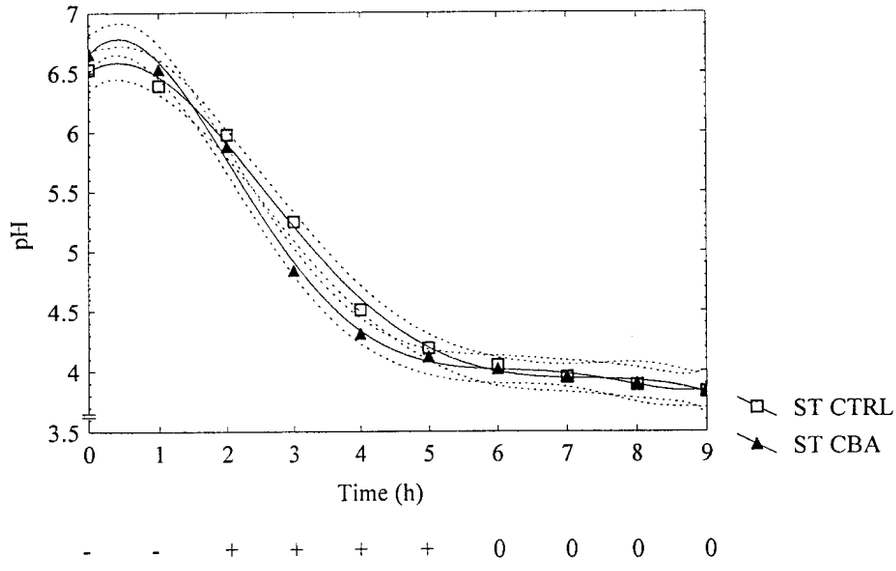


Fig. 5 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by *Streptococcus thermophilus* CH-1 in milk

Table 7 and Fig. 5 reveal that the cyanobacterial biomass enhanced acid development by *Strep. thermophilus* significantly during hours 2-5 of the fermentation process.

Table 7 and Figs 6-8 show the effect of addition of trace elements on the rate of acid development by *Strep. thermophilus*.

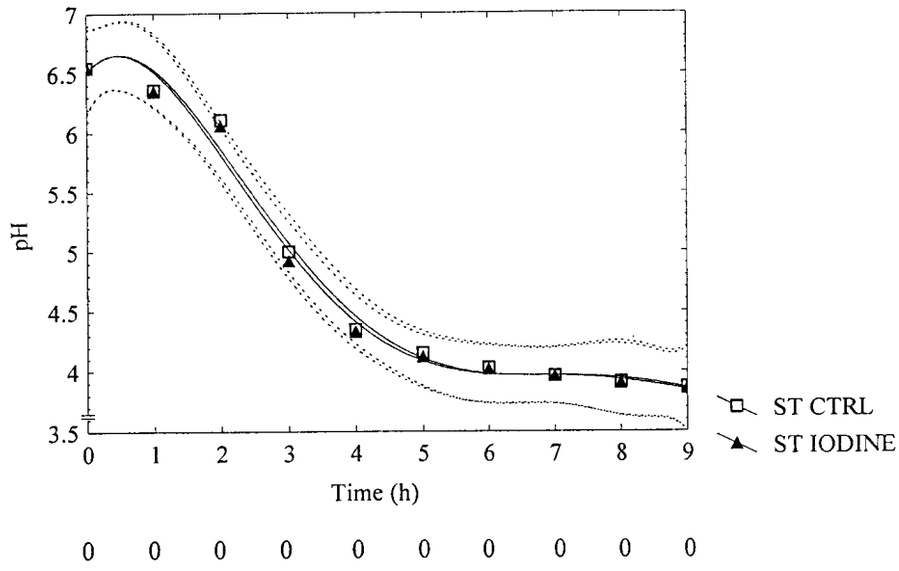


Fig. 6 Effect of iodine on acid development by *Streptococcus thermophilus* CH-1 in milk

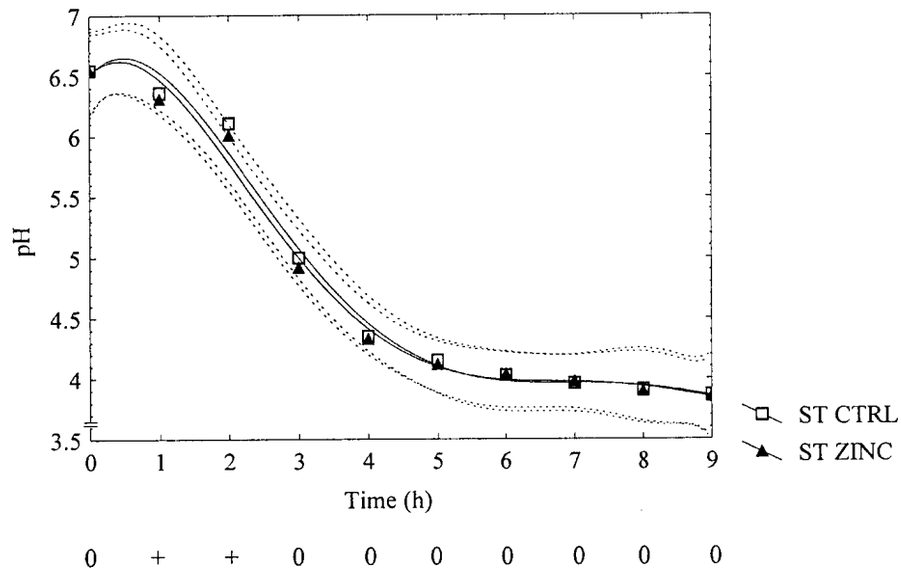


Fig. 7 Effect of zinc on acid development by *Streptococcus thermophilus* CH-1 in milk

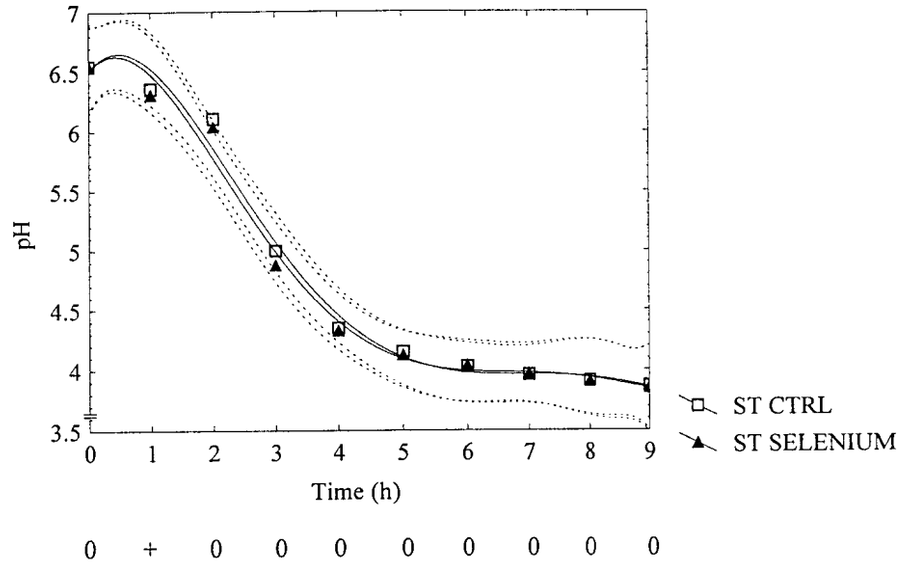


Fig. 8 Effect of selenium on acid development by *Streptococcus thermophilus* CH-1 in milk

Even though all three trace elements stimulated the acid production of *Strep. thermophilus* in the exponential phase to some extent, this stimulation was not significant on the whole. It can be seen that particularly zinc and selenium had some share in the beneficial effect of the cyanobacterial biomass on the rate of acid development by *Strep. thermophilus*.

The effect of addition of vitamins on the rate of acid development by *Strep. thermophilus* is illustrated in Table 7 and Figs 9-12.

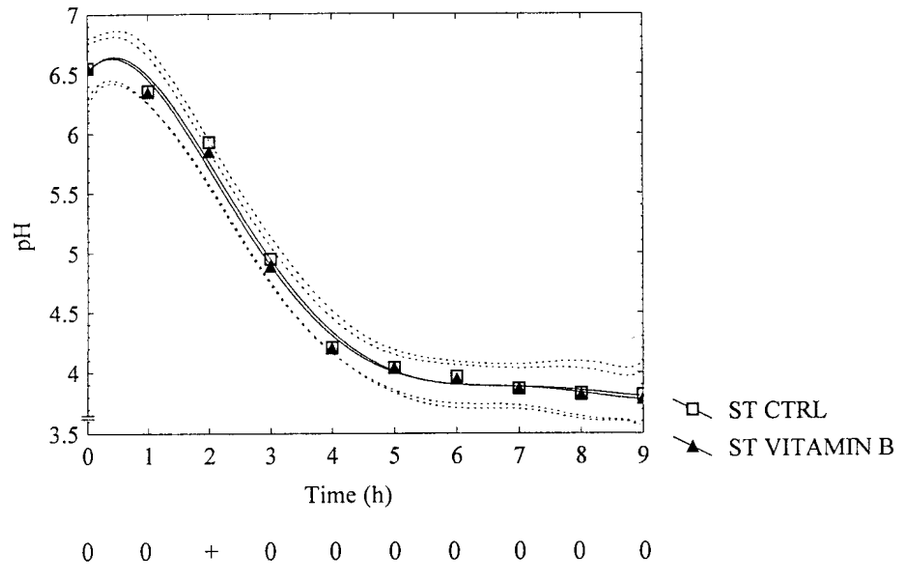


Fig. 9 Effect of B-complex vitamins on acid development by *Streptococcus thermophilus* CH-1 in milk

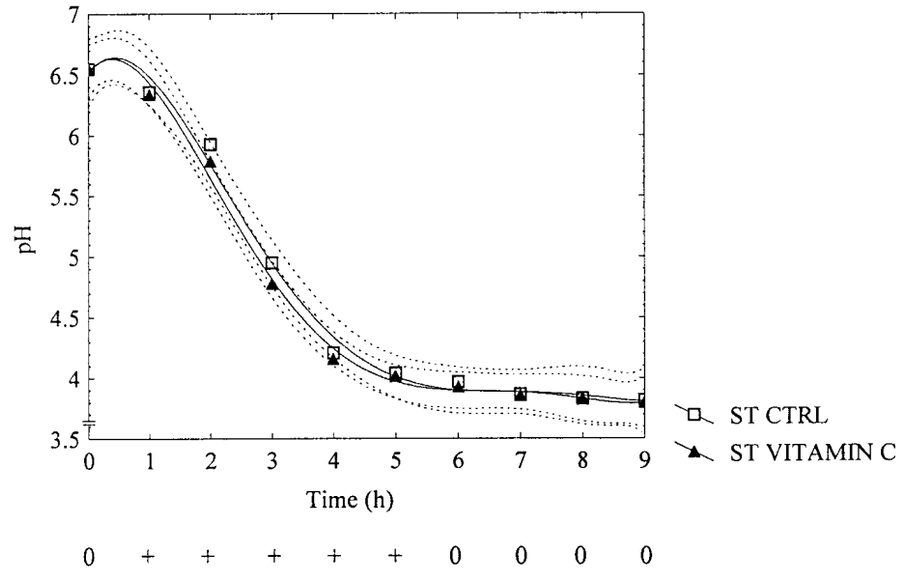


Fig. 10 Effect of vitamin C on acid development by *Streptococcus thermophilus* CH-1 in milk

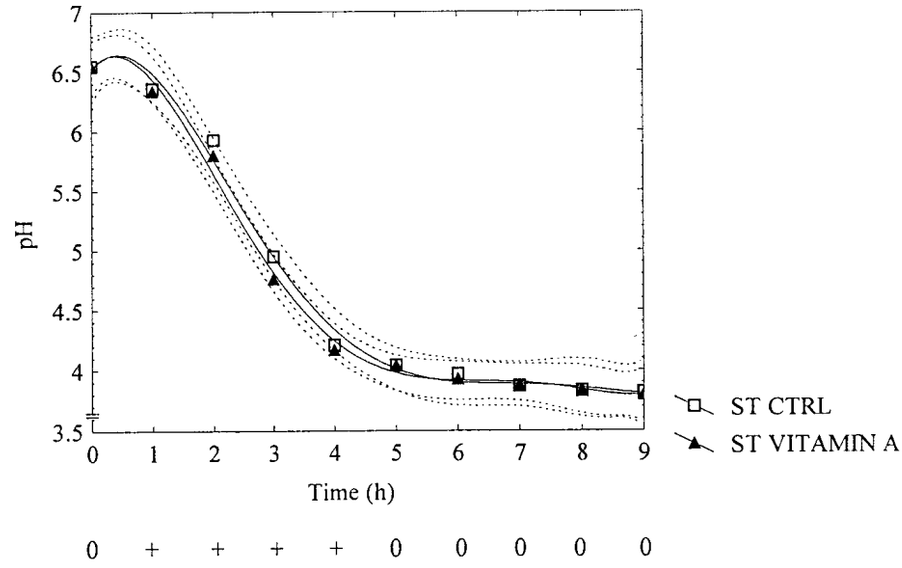


Fig. 11 Effect of vitamin A on acid development by *Streptococcus thermophilus* CH-1 in milk

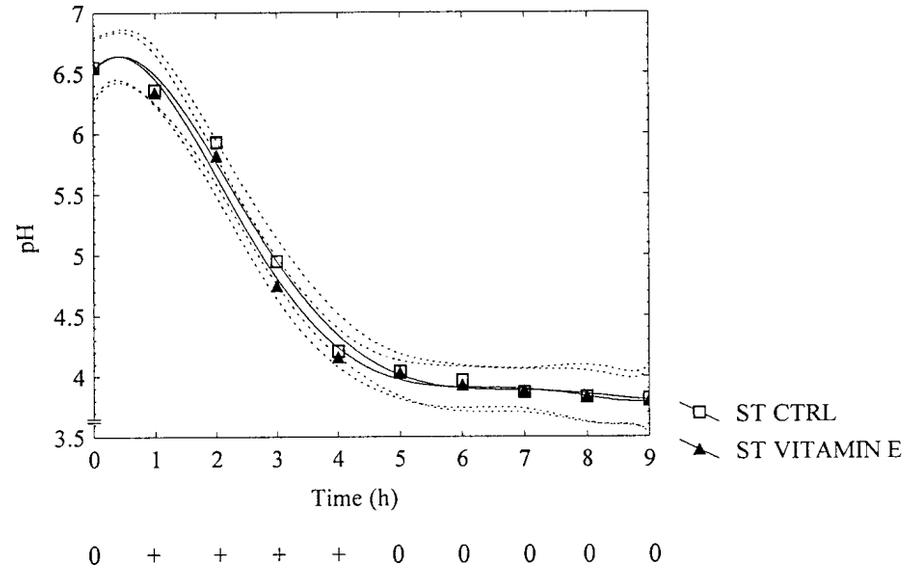


Fig. 12 Effect of vitamin E on acid development by *Streptococcus thermophilus* CH-1 in milk

Although the B-complex vitamins tested (B_1, B_2, nicotinic acid, pantothenic acid, B_6, B_{12}) were beneficial to the acid production of *Strep. thermophilus* to some extent, the stimulation caused by them was not significant. The addition of vitamins C, A and E, in which cyanobacteria are abundant, resulted in a considerable drop in pH in the exponential phase of growth (hours 2-4), which was indicative of the significant contribution of these vitamins to the stimulation of *Strep. thermophilus* by the *Spir. platensis* biomass.

Table 7 and Figs 13-17 display the effect of addition of nitrogenous compounds on the rate of acid development by *Strep. thermophilus*.

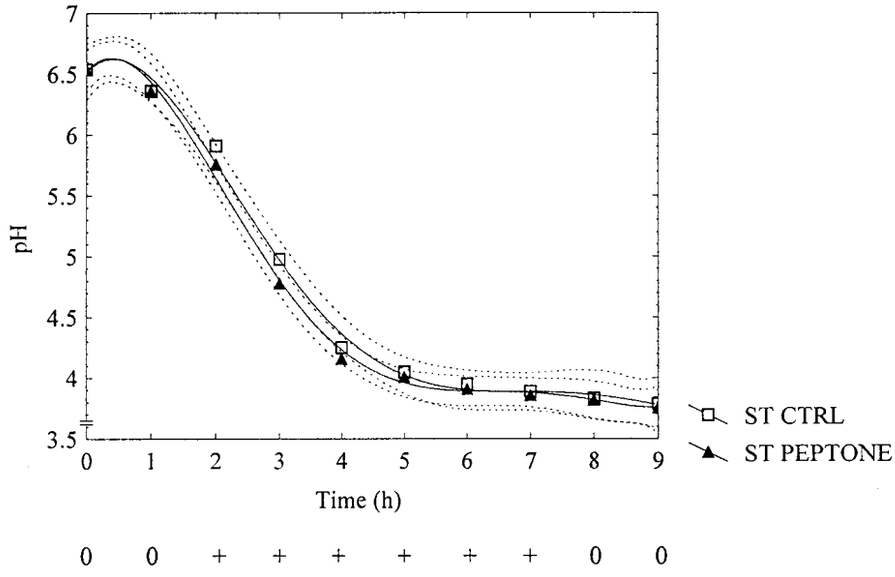


Fig. 13 Effect of peptone on acid development by *Streptococcus thermophilus* CH-1 in milk

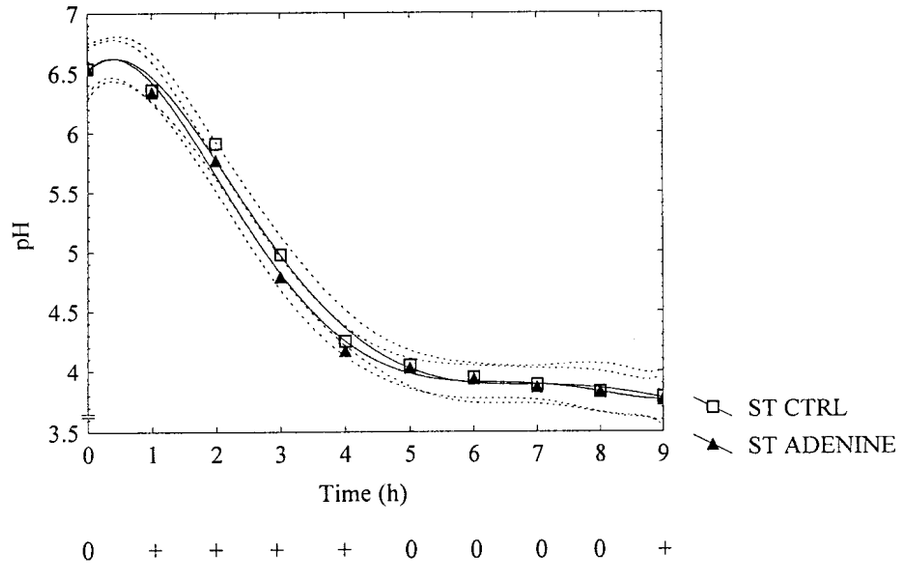


Fig. 14 Effect of adenine on acid development by *Streptococcus thermophilus* CH-1 in milk

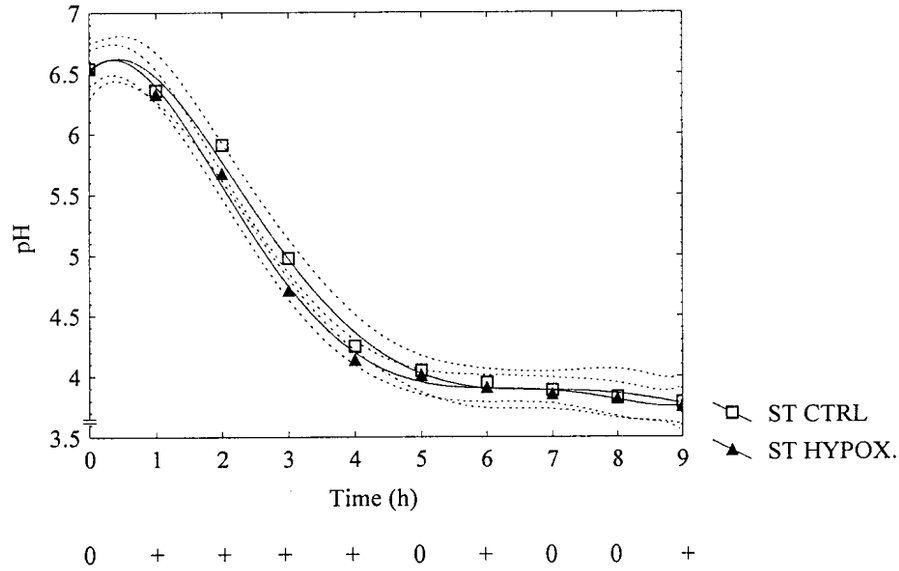


Fig. 15 Effect of hypoxanthine on acid development by *Streptococcus thermophilus* CH-1 in milk

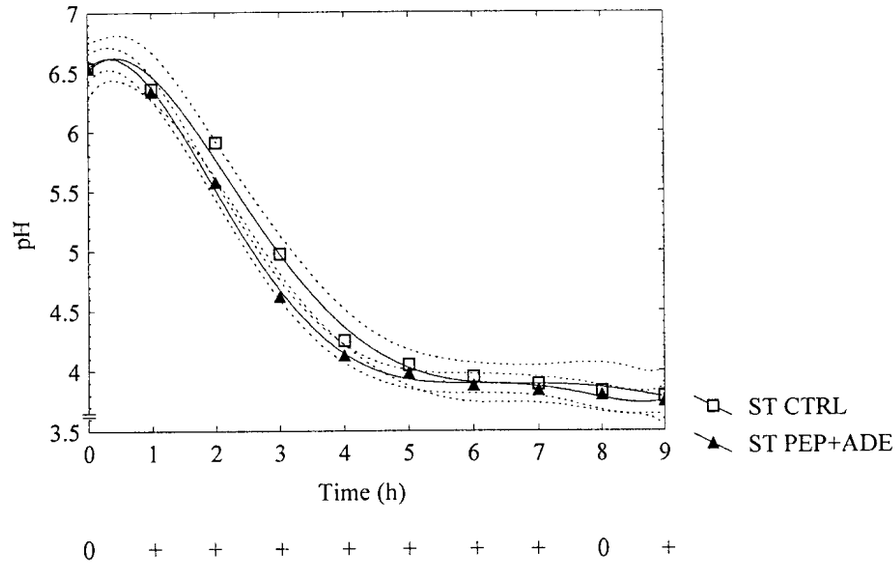


Fig. 16 Effect of peptone and adenine on acid development by *Streptococcus thermophilus* CH-1 in milk

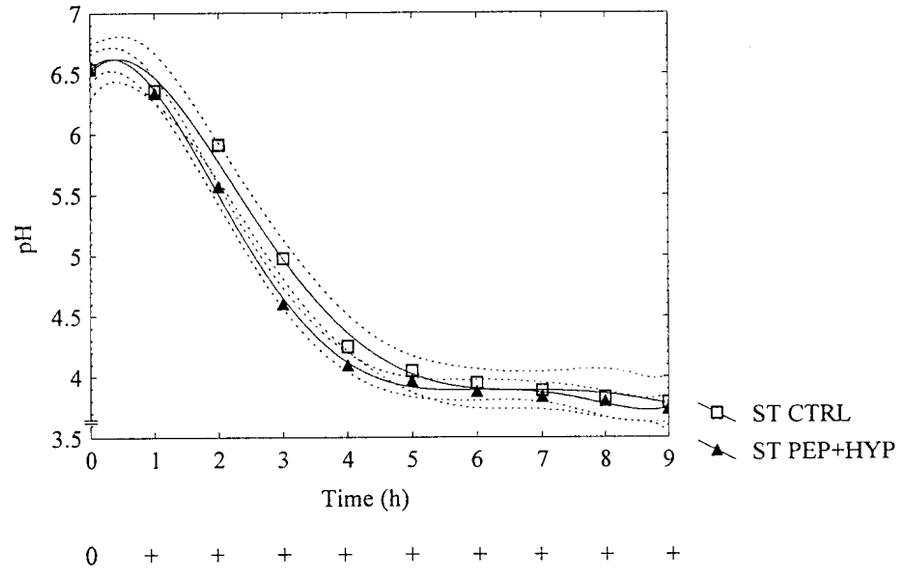


Fig. 17 Effect of peptone and hypoxanthine on acid development by *Streptococcus thermophilus* CH-1 in milk

The results make it clear that it was the nitrogenous compounds (peptone, adenine, hypoxanthine) that had the most beneficial effect of all the substances tested on this starter bacterial strain. They proved to be significantly stimulatory, in the exponential phase, to the acid production of *Strep. thermophilus* in all cases. The mixture of peptone and hypoxanthine, added at a proper concentration, largely stimulated the souring of milk by *Strep. thermophilus* and this stimulation was comparable to that achieved with the cyanobacterial biomass.

Zielke *et al.* (1978) have pointed out that the activity of the stimulatory factors found in the aqueous extract of the green alga *Scenedesmus acutus* can be increased by heat treatment, especially when the algal extract is subjected to heating at low pH. The explanation of this phenomenon is that additional amino acids and small peptides are released from proteins because heat treatment at low pH results in increasing the rate of proteolysis.

4.1.2 Effect of the Spirulina platensis biomass and that of its active components on acid development by Lactobacillus bulgaricus CH-2 in a model milk medium

Tables 8a and 8b show the effect of various substrates added to milk on acid development by *Lact. bulgaricus*.

Table 8a Effect of various substances on acid development by *Lactobacillus bulgaricus* CH-2 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
CBA*	**-0.12**	**-0.14**	**-0.14**	+0.02	**+0.43**	**+0.61**	**+0.70**
Iodine	0	0	0	+0.01	0	-0.01	0
Zinc	0	0	0	+0.01	0	0	-0.02
Selenium	0	-0.01	0	0	**-0.03**	-0.03	-0.03
Vitamin B†	0	-0.01	-0.01	-0.02	-0.02	-0.01	-0.01
Vitamin C	0	+0.01	0	0	-0.01	0	-0.01
Vitamin A	0	-0.01	-0.01	-0.03	-0.02	0	-0.01
Vitamin E	0	-0.01	0	0	0	0	-0.01
Peptone	0	+0.01	+0.01	+0.05	**+0.16**	**+0.19**	**+0.17**
Adenine	0	-0.01	-0.01	+0.07	**+0.11**	**+0.13**	**+0.11**
Hypoxanthine	0	-0.01	-0.01	+0.03	**+0.15**	**+0.19**	**+0.18**
Pep.+Ade.	0	-0.01	-0.01	0	**+0.19**	**+0.23**	**+0.24**
Pep.+Hyp.	0	0	0	+0.01	**+0.25**	**+0.32**	**+0.33**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

Table 8b Effect of various substances on acid development by *Lactobacillus bulgaricus* CH-2 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls						
	7 h	8 h	9 h	10 h	11 h	12 h	13 h
CBA*	**+0.75**	**+0.81**	**+0.85**	**+0.87**	**+0.88**	**+0.88**	**+0.87**
Iodine	-0.01	0	+0.01	-0.01	0	0	-0.01
Zinc	-0.01	-0.01	0	+0.01	+0.02	+0.03	+0.01
Selenium	**-0.06**	**-0.07**	**-0.05**	**-0.05**	**-0.06**	**-0.07**	**-0.06**
Vitamin B†	-0.02	0	0	0	0	0	+0.01
Vitamin C	+0.01	+0.02	+0.04	+0.05	**+0.06**	**+0.09**	**+0.11**
Vitamin A	-0.01	0	-0.01	-0.01	-0.02	-0.01	0
Vitamin E	-0.01	+0.02	0	-0.01	0	0	+0.01
Peptone	**+0.18**	**+0.19**	**+0.23**	**+0.26**	**+0.25**	**+0.27**	**+0.28**
Adenine	**+0.12**	**+0.12**	**+0.13**	**+0.14**	**+0.14**	**+0.17**	**+0.16**
Hypoxanthine	**+0.20**	**+0.22**	**+0.25**	**+0.28**	**+0.29**	**+0.31**	**+0.33**
Pep.+Ade.	**+0.24**	**+0.27**	**+0.31**	**+0.33**	**+0.34**	**+0.36**	**+0.38**
Pep.+Hyp.	**+0.37**	**+0.40**	**+0.44**	**+0.47**	**+0.48**	**+0.51**	**+0.51**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

As is noted from Tables 8a and 8b and Fig. 18, the cyanobacterial biomass stimulated the acid production of *Lact. bulgaricus* to a greater extent than that of *Strep. thermophilus*.

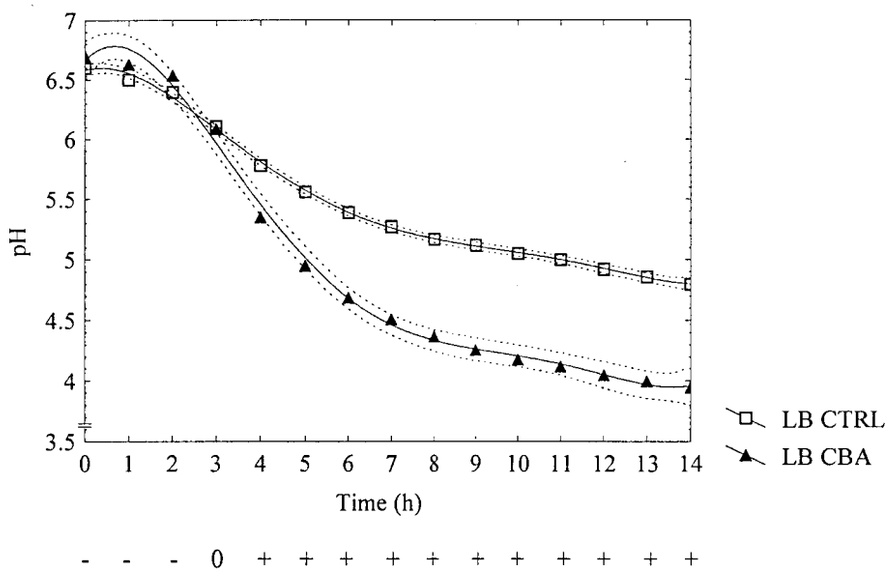


Fig. 18 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by *Lactobacillus bulgaricus* CH-2 in milk

Tables 8a and 8b and Figs 19-21 illustrate the effect of addition of trace elements on the rate of acid development by *Lact. bulgaricus*.

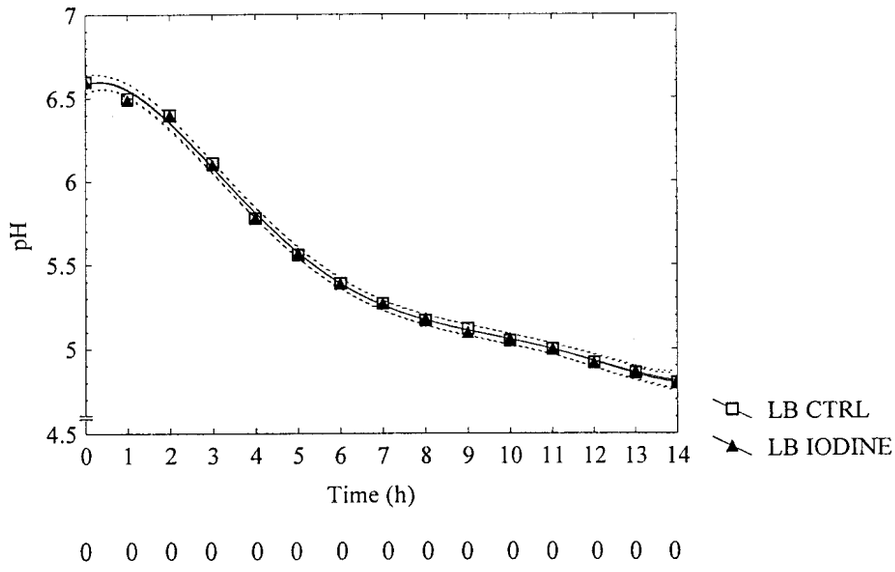


Fig. 19 Effect of iodine on acid development by *Lactobacillus bulgaricus* CH-2 in milk

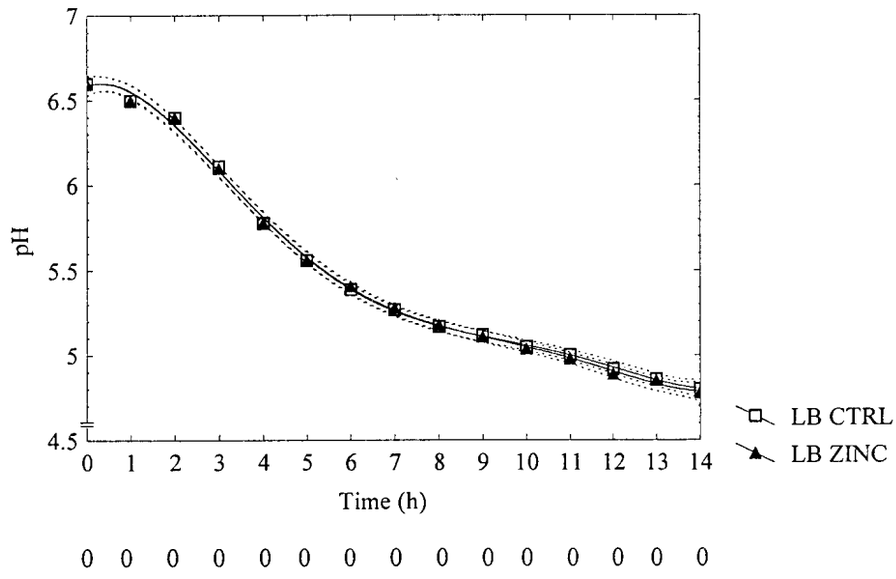


Fig. 20 Effect of zinc on acid development by *Lactobacillus bulgaricus* CH-2 in milk

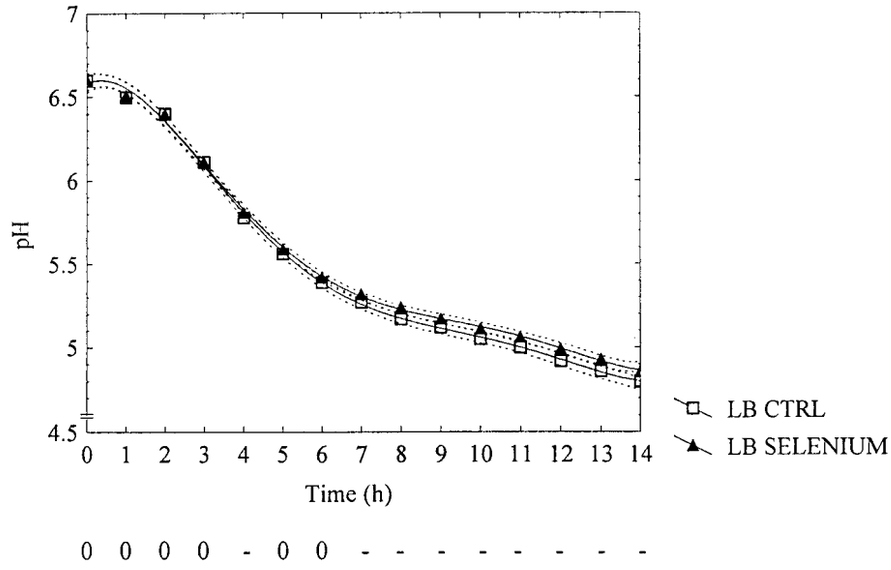


Fig. 21 Effect of selenium on acid development by *Lactobacillus bulgaricus* CH-2 in milk

Neither iodine nor zinc had any influence on the acid production of *Lact. bulgaricus*. However, the addition of inorganic selenium resulted in a small, although significant decrease in the rate of acid development during the stationary phase of fermentation.

The effect of addition of vitamins on the rate of acid development by *Lact. bulgaricus* is shown in Tables 8a and 8b and Figs 22-25.

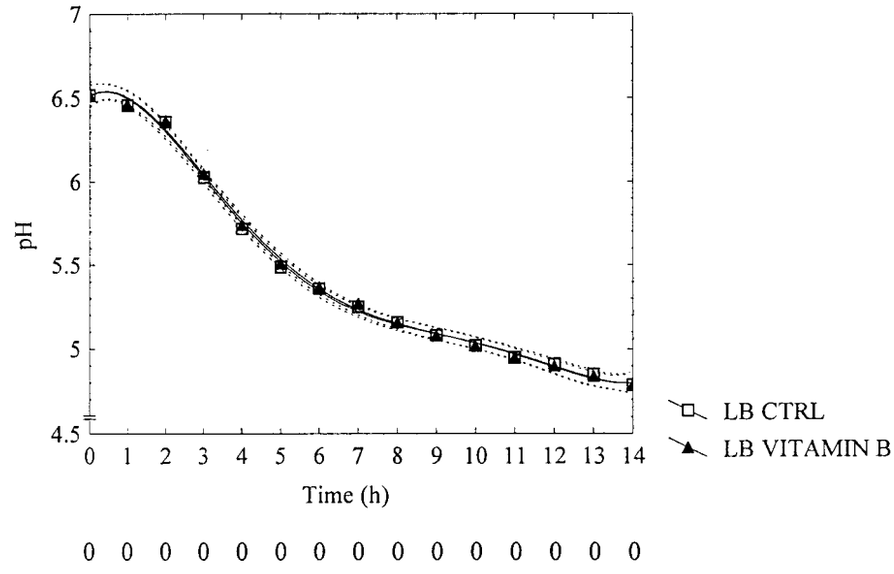


Fig. 22 Effect of B-complex vitamins on acid development by *Lactobacillus bulgaricus* CH-2 in milk

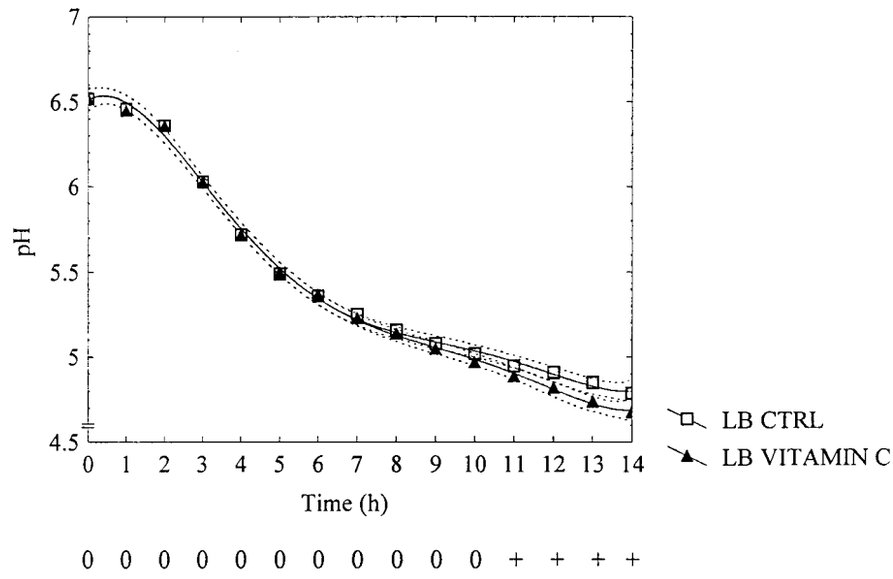


Fig. 23 Effect of vitamin C on acid development by *Lactobacillus bulgaricus* CH-2 in milk

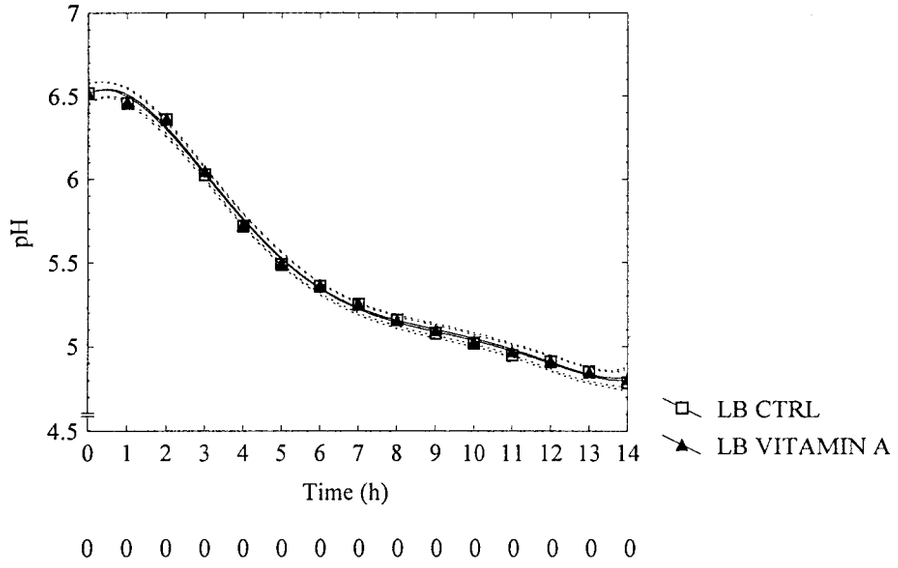


Fig. 24 Effect of vitamin A on acid development by *Lactobacillus bulgaricus* CH-2 in milk

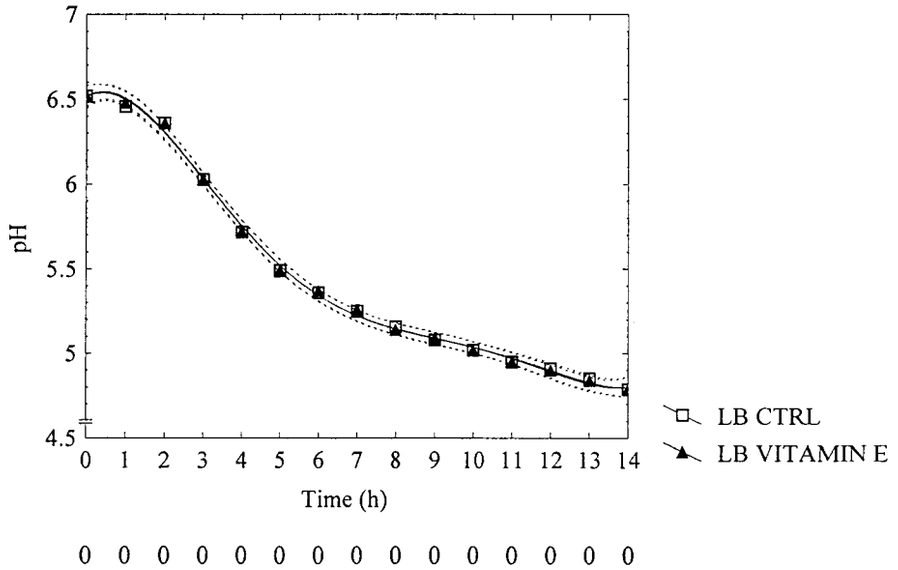


Fig. 25 Effect of vitamin E on acid development by *Lactobacillus bulgaricus* CH-2 in milk

Vitamins B, A and E—similarly to iodine and zinc—had no influence at all on the acid production of *Lact. bulgaricus*. On the evidence of Fig. 23, however, vitamin C contributed to the beneficial effect of the cyanobacterial biomass on acid development by and acid tolerance of this moderate acid-producing strain of *Lact. bulgaricus* during the stationary phase of fermentation.

Tables 8a and 8b and Figs 26-30 display the effect of addition of nitrogenous compounds on the rate of acid development by *Lact. bulgaricus*.

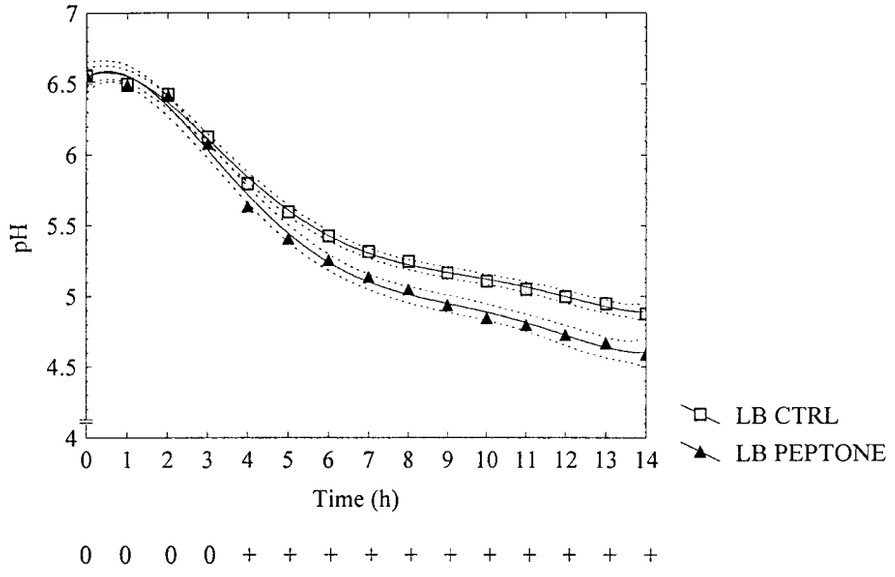


Fig. 26 Effect of peptone on acid development by *Lactobacillus bulgaricus* CH-2 in milk

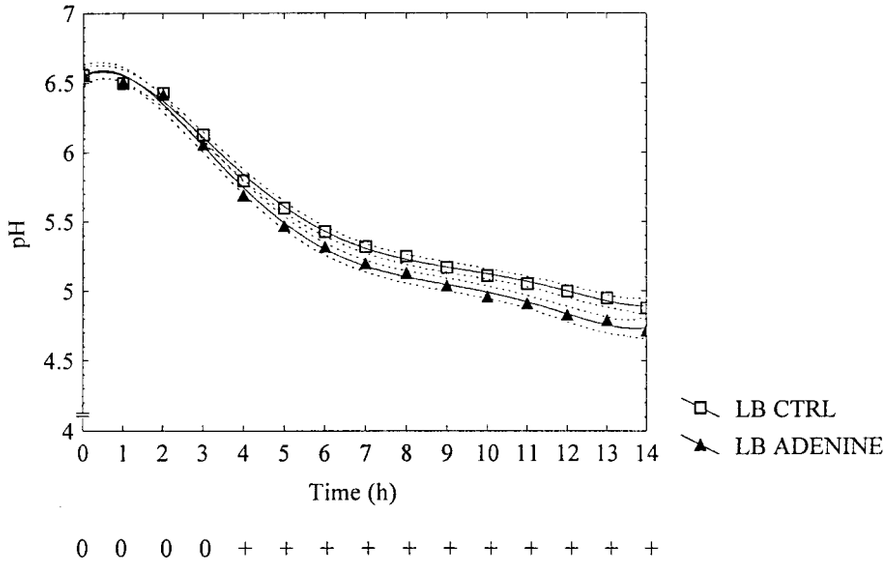


Fig. 27 Effect of adenine on acid development by *Lactobacillus bulgaricus* CH-2 in milk

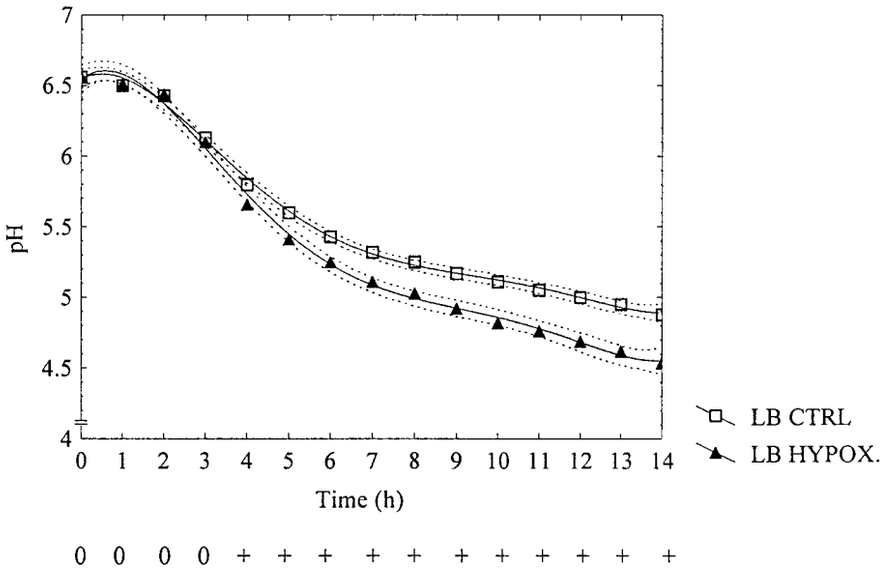


Fig. 28 Effect of hypoxanthine on acid development by *Lactobacillus bulgaricus* CH-2 in milk

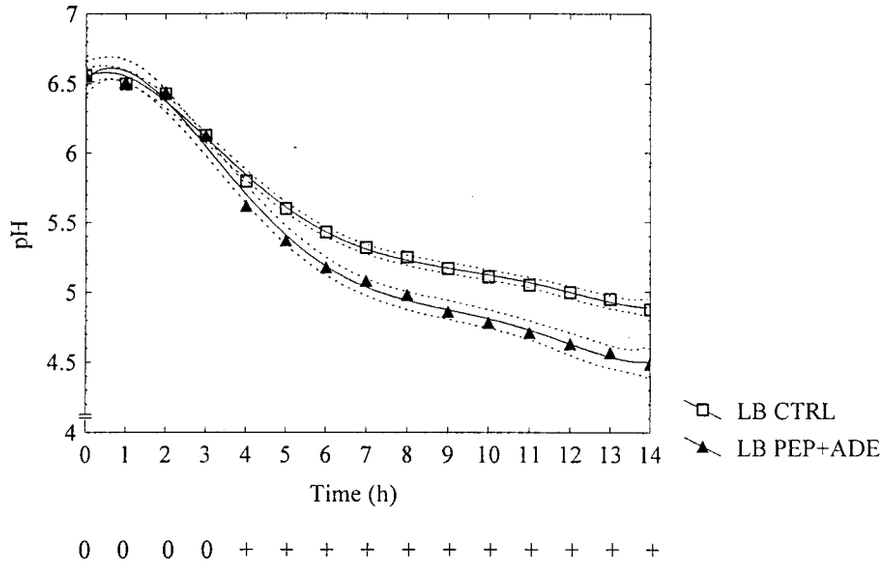


Fig. 29 Effect of peptone and adenine on acid development by *Lactobacillus bulgaricus* CH-2 in milk

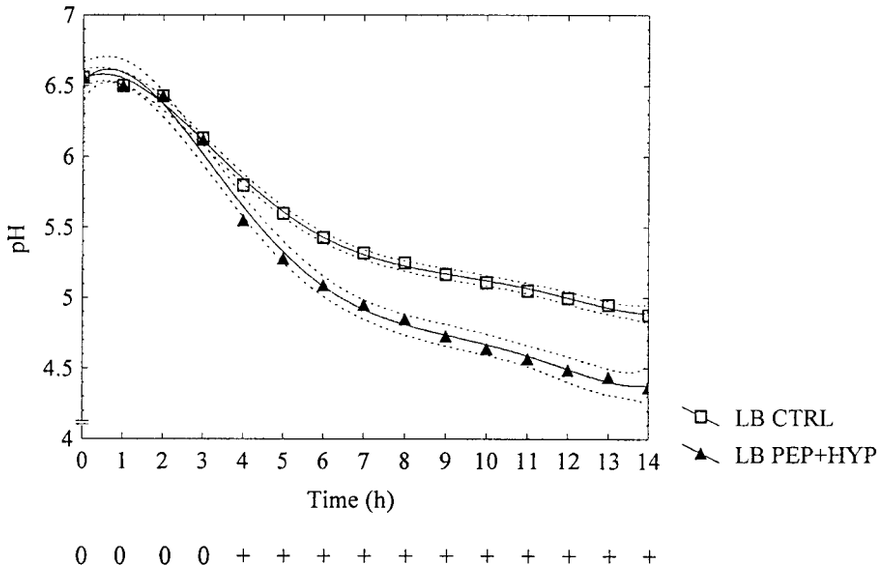


Fig. 30 Effect of peptone and hypoxanthine on acid development by *Lactobacillus bulgaricus* CH-2 in milk

It is clearly visible that the stimulation of *Lact. bulgaricus* by the cyanobacterial biomass, during the main phase of fermentation, could be attributed to the additive effect of nitrogenous substances; the addition of peptone and that of hypoxanthine substantially increased the rate of acid development by *Lact. bulgaricus* and adenine also had a key role in this stimulation.

4.1.3 Effect of the Spirulina platensis biomass and that of its active components on acid development by Lactobacillus acidophilus La-5 in a model milk medium

The results of pH measurements shown in Tables 9a and 9b and Fig. 31 make it clear that the cyanobacterial biomass significantly increased the rate of acid development by *Lact. acidophilus*.

Table 9a Effect of various substances on acid development by *Lactobacillus acidophilus* La-5 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
CBA*	**-0.13**	**-0.14**	**-0.17**	**+0.24**	**+1.01**	**+1.10**	**+0.81**
Iodine	0	+0.01	+0.02	+0.01	**+0.03**	+0.03	+0.02
Zinc	0	+0.01	+0.03	0	+0.02	-0.01	-0.01
Selenium	0	+0.01	+0.02	0	+0.01	-0.06	**-0.14**
Vitamin B†	0	0	-0.02	0	**-0.02**	**-0.05**	**-0.18**
Vitamin C	0	+0.02	-0.02	+0.01	**+0.12**	**+0.45**	**+0.46**
Vitamin A	0	0	**-0.04**	0	**-0.01**	**-0.04**	-0.10
Vitamin E	0	0	-0.03	-0.01	**-0.02**	**-0.06**	**-0.22**
Peptone	0	0	+0.02	**+0.19**	**+0.63**	**+0.59**	**+0.41**
Adenine	0	+0.01	+0.02	**+0.08**	**+0.18**	**+0.30**	**+0.19**
Hypoxanthine	0	0	+0.02	**+0.15**	**+0.36**	**+0.42**	**+0.28**
Pep.+Ade.	0	0	+0.02	**+0.24**	**+0.71**	**+0.66**	**+0.46**
Pep.+Hyp.	0	+0.01	+0.04	**+0.30**	**+0.73**	**+0.67**	**+0.47**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

Table 9b Effect of various substances on acid development by *Lactobacillus acidophilus* La-5 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls						
	7 h	8 h	9 h	10 h	11 h	12 h	13 h
CBA*	**+0.62**	**+0.50**	**+0.41**	**+0.33**	**+0.27**	**+0.22**	**+0.20**
Iodine	0	0	0	0	-0.01	-0.01	-0.01
Zinc	-0.03	-0.03	-0.02	-0.02	-0.02	-0.03	-0.02
Selenium	**-0.11**	**-0.10**	-0.08	**-0.08**	**-0.06**	**-0.06**	-0.05
Vitamin B†	-0.16	-0.11	-0.09	-0.08	-0.07	-0.04	-0.04
Vitamin C	**+0.35**	**+0.31**	**+0.29**	**+0.24**	**+0.21**	**+0.17**	**+0.14**
Vitamin A	-0.16	-0.12	-0.08	-0.08	-0.06	-0.03	-0.02
Vitamin E	**-0.18**	**-0.13**	-0.09	**-0.09**	**-0.08**	-0.07	**-0.05**
Peptone	**+0.32**	**+0.27**	**+0.24**	**+0.18**	**+0.15**	**+0.13**	**+0.13**
Adenine	**+0.14**	**+0.11**	**+0.10**	**+0.06**	**+0.05**	**+0.05**	**+0.05**
Hypoxanthine	**+0.22**	**+0.17**	**+0.14**	**+0.11**	**+0.09**	**+0.08**	**+0.09**
Pep.+Ade.	**+0.37**	**+0.29**	**+0.25**	**+0.19**	**+0.16**	**+0.14**	**+0.13**
Pep.+Hyp.	**+0.37**	**+0.30**	**+0.25**	**+0.19**	**+0.16**	**+0.14**	**+0.14**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

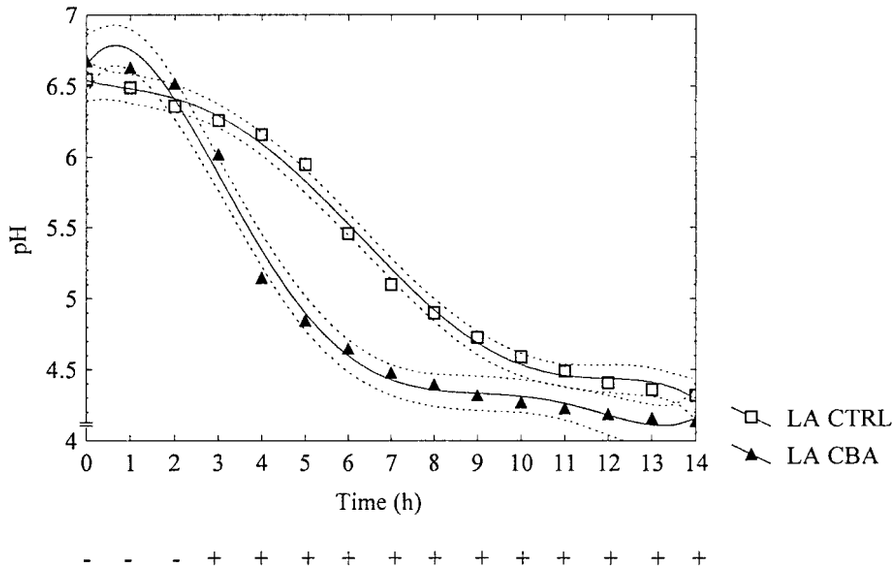


Fig. 31 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by *Lactobacillus acidophilus* La-5 in milk

Tables 9a and 9b and Figs 32-34 show the effect of addition of trace elements on the rate of acid development by *Lact. acidophilus*.

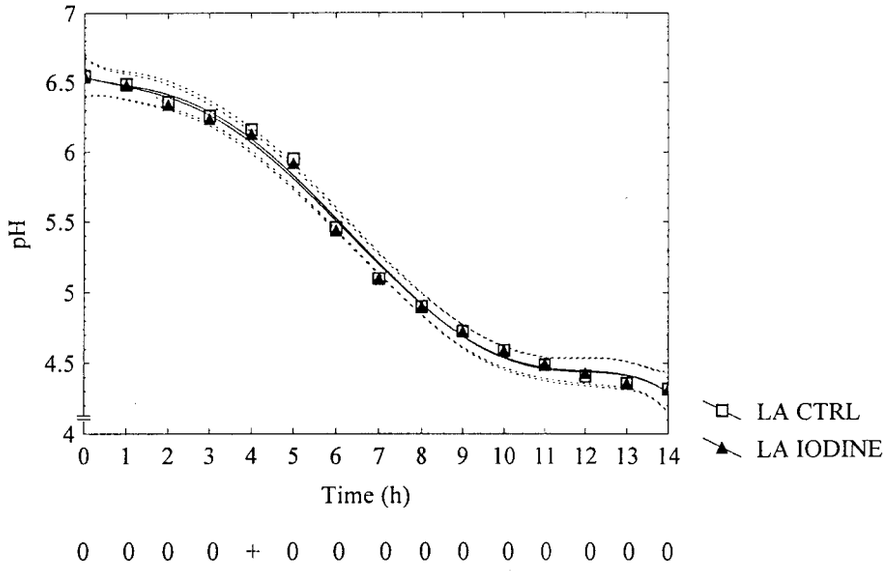


Fig. 32 Effect of iodine on acid development by *Lactobacillus acidophilus* La-5 in milk

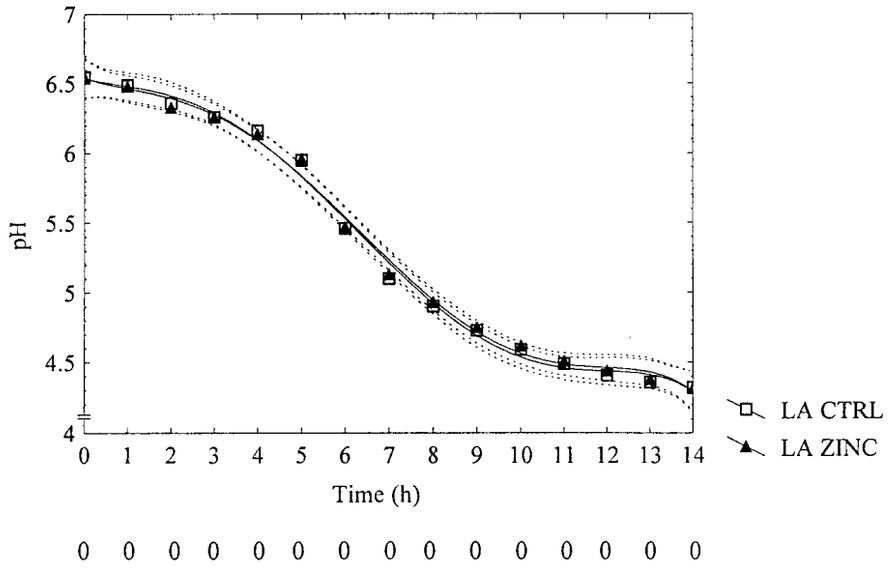


Fig. 33 Effect of zinc on acid development by *Lactobacillus acidophilus* La-5 in milk

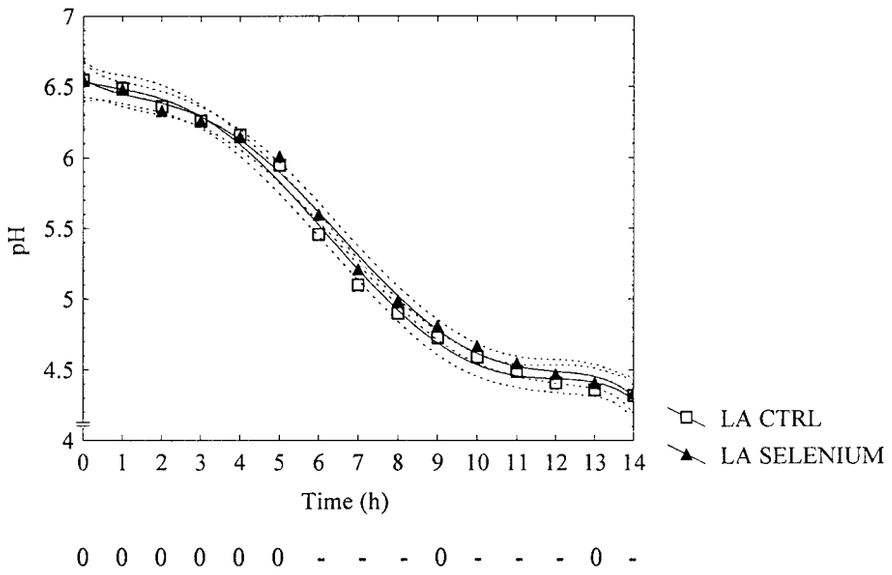


Fig. 34 Effect of selenium on acid development by *Lactobacillus acidophilus* La-5 in milk

The trace elements had the same effect on *Lact. acidophilus* as on *Lact. bulgaricus*, i.e. iodine and zinc neither retarded nor stimulated acid production while the adverse effect of selenium was also observed in the case of *Lact. acidophilus*.

The effect of addition of vitamins on the rate of acid development by *Lact. acidophilus* is illustrated in Tables 9a and 9b and Figs 35-38.

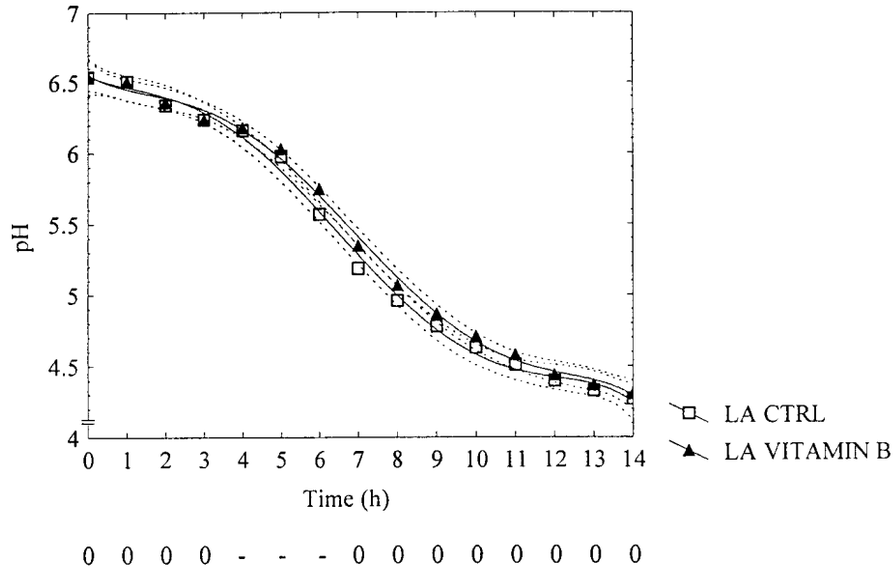


Fig. 35 Effect of B-complex vitamins on acid development by *Lactobacillus acidophilus* La-5 in milk

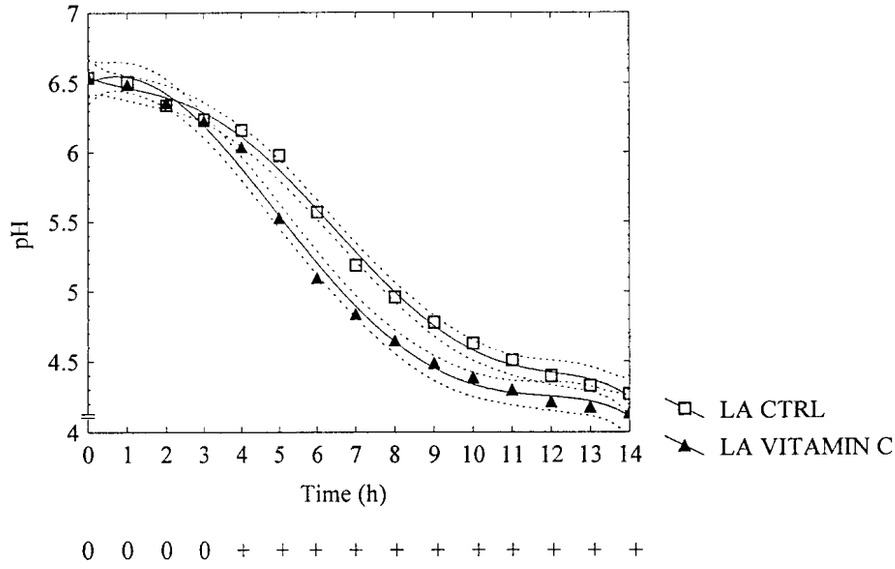


Fig. 36 Effect of vitamin C on acid development by *Lactobacillus acidophilus* La-5 in milk

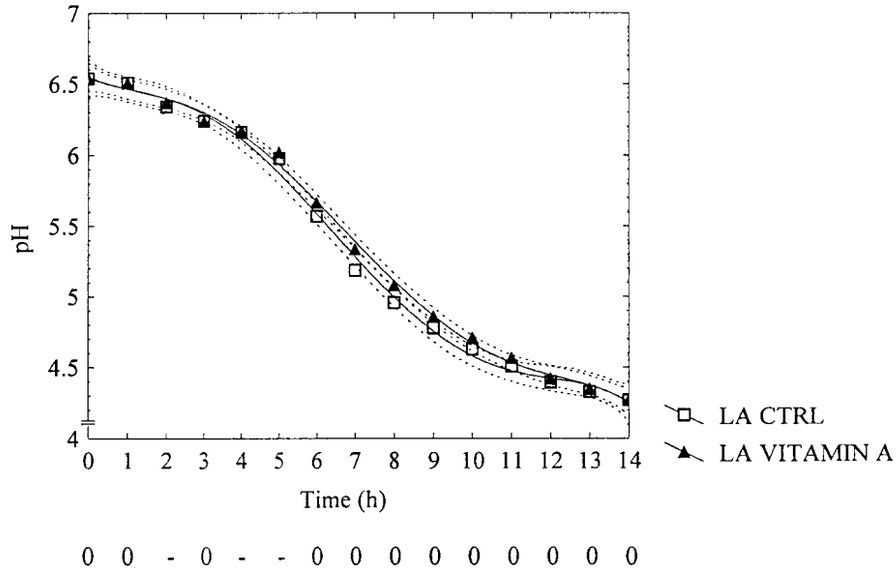


Fig. 37 Effect of vitamin A on acid development by *Lactobacillus acidophilus* La-5 in milk

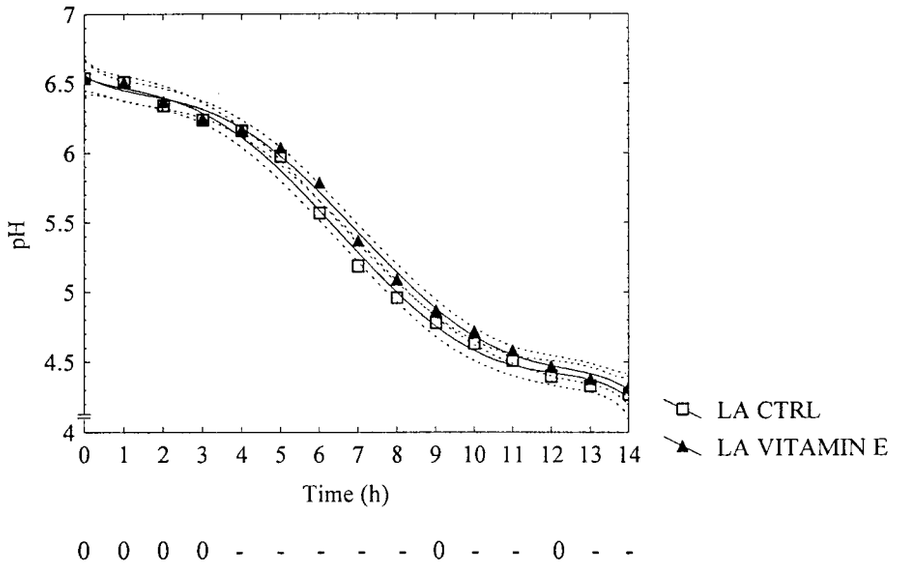


Fig. 38 Effect of vitamin E on acid development by *Lactobacillus acidophilus* La-5 in milk

In contrast to what had been experienced with the trace elements, the vitamins tested had different effects on the two *Lactobacillus* species. Among the antioxidants preventing membrane lipid peroxidation by free radicals, only vitamin C proved to stimulate the acid production of *Lact. acidophilus* while vitamins A and E (similarly to selenium) retarded it to some extent. The B-complex vitamins were also found to reduce the rate of acid production.

Tables 9a and 9b and Figs 39-43 display the effect of addition of nitrogenous compounds on the rate of acid development by *Lact. acidophilus*.

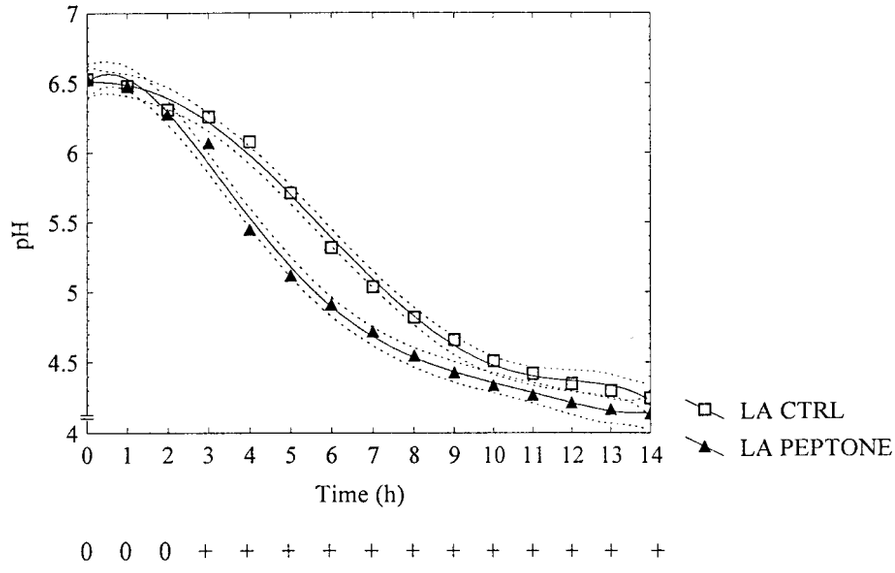


Fig. 39 Effect of peptone on acid development by *Lactobacillus acidophilus* La-5 in milk

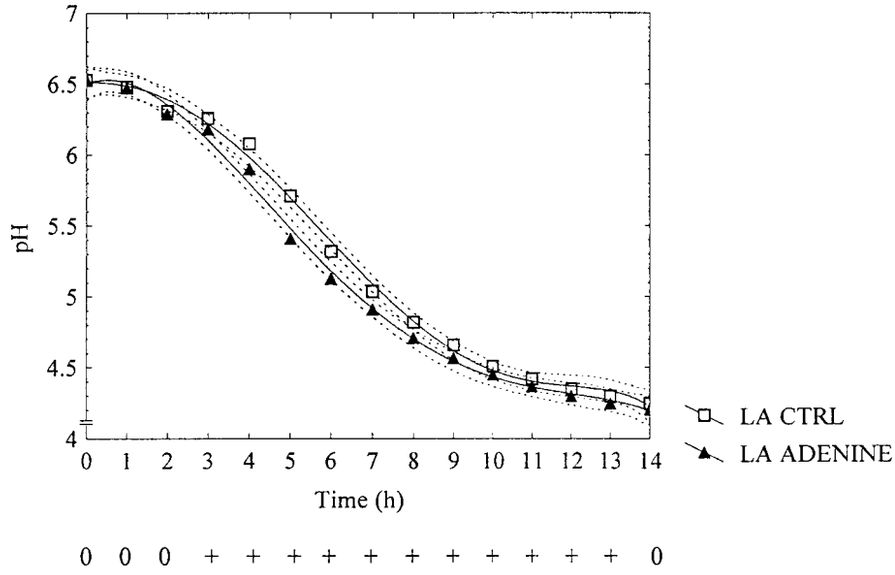


Fig. 40 Effect of adenine on acid development by *Lactobacillus acidophilus* La-5 in milk

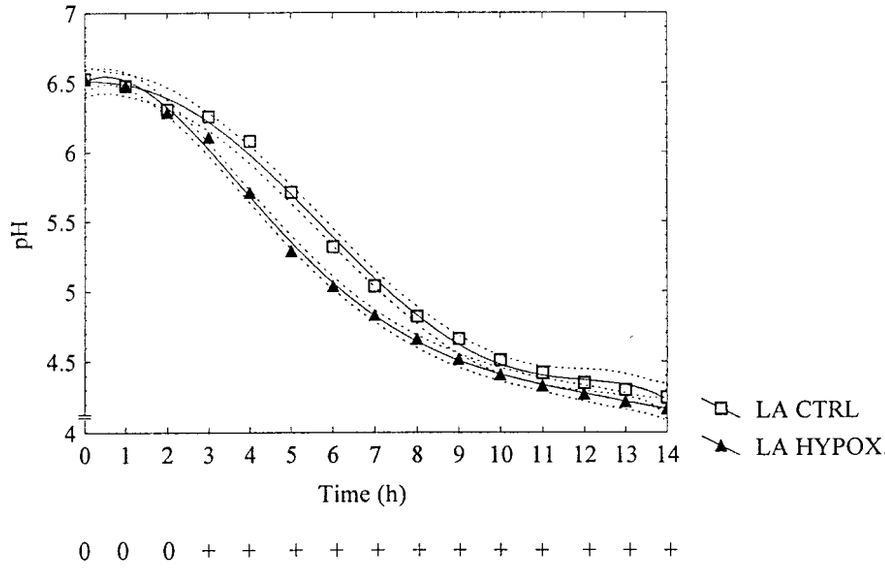


Fig. 41 Effect of hypoxanthine on acid development by *Lactobacillus acidophilus* La-5 in milk

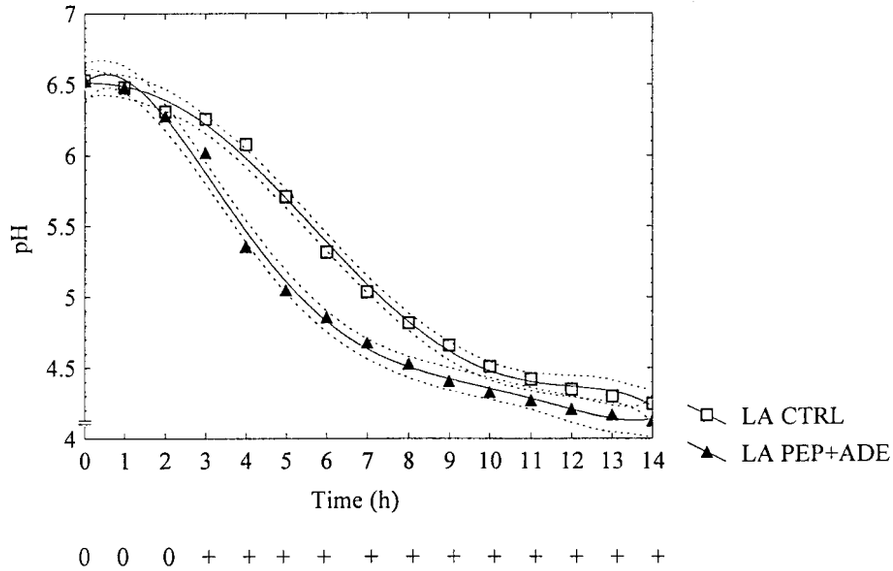


Fig. 42 Effect of peptone and adenine on acid development by *Lactobacillus acidophilus* La-5 in milk

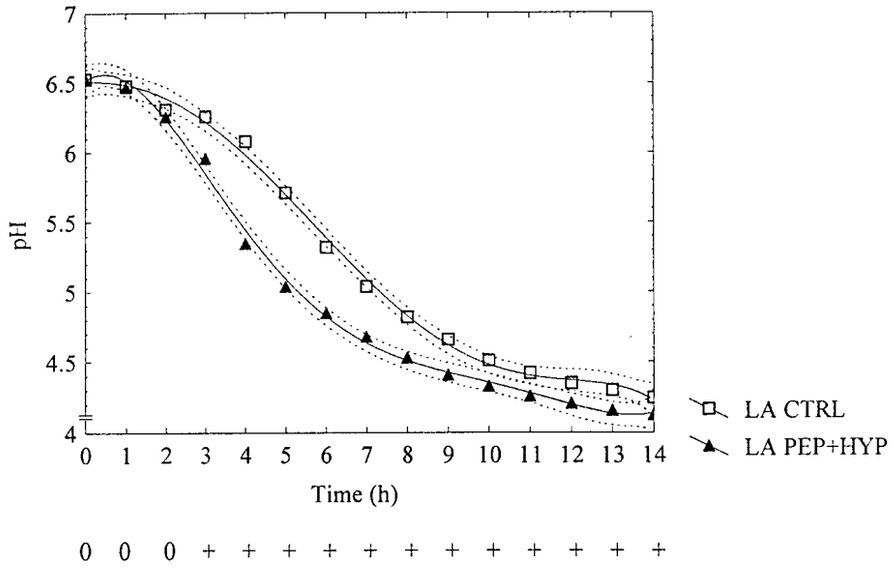


Fig. 43 Effect of peptone and hypoxanthine on acid development by *Lactobacillus acidophilus* La-5 in milk

It is obvious that the beneficial effect of the cyanobacterial biomass on the rate of acid development by *Lact. acidophilus* was due to the presence of nitrogenous compounds, besides that of vitamin C. Both peptone and hypoxanthine were largely responsible for the stimulation observed and adenine also contributed to it significantly.

4.1.4 Effect of the Spirulina platensis biomass and that of its active components on acid development by Bifidobacterium bifidum Bb-12 in a model milk medium

Most of the substrates tested had similar effects on *Bifid. bifidum* and on *Lact. acidophilus*, although the two species differ widely in some of their major characteristics, including their metabolic system (Tables 10a and 10b).

Table 10a Effect of various substances on acid development by *Bifidobacterium bifidum* Bb-12 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls					
	0 h	2 h	4 h	6 h	8 h	10 h
CBA*	**-0.14**	**-0.13**	**-0.12**	+0.03	**+0.34**	**+0.49**
Iodine	0	+0.01	0	**-0.01**	+0.01	+0.02
Zinc	0	+0.01	+0.01	**-0.02**	-0.01	0
Selenium	0	+0.02	0	**-0.02**	-0.02	**-0.05**
Vitamin B†	0	+0.01	0	+0.01	-0.01	-0.03
Vitamin C	0	+0.03	**+0.03**	**+0.06**	**+0.06**	+0.05
Vitamin A	0	+0.01	-0.01	-0.02	-0.03	**-0.06**
Vitamin E	0	+0.01	-0.01	**-0.02**	**-0.03**	**-0.05**
Peptone	0	**+0.04**	+0.02	+0.01	+0.02	**+0.06**
Adenine	0	+0.02	0	-0.02	-0.03	**-0.04**
Hypoxanthine	0	+0.02	0	-0.02	-0.04	-0.06
Pep.+Ade.	0	**+0.05**	+0.03	-0.01	+0.05	**+0.06**
Pep.+Hyp.	0	**+0.04**	+0.02	-0.02	+0.01	0

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

Table 10b Effect of various substances on acid development by *Bifidobacterium bifidum* Bb-12 in milk

Substance added to milk	Average decrease in pH during fermentation compared to controls					
	12 h	14 h	16 h	18 h	20 h	22 h
CBA*	**+0.55**	**+0.58**	**+0.61**	**+0.66**	**+0.66**	**+0.67**
Iodine	+0.03	**+0.04**	+0.04	+0.05	**+0.05**	+0.04
Zinc	0	+0.02	+0.02	+0.03	**+0.06**	+0.07
Selenium	**-0.07**	**-0.06**	**-0.06**	-0.05	**-0.06**	-0.09
Vitamin B†	-0.03	-0.03	-0.01	0	+0.03	+0.03
Vitamin C	+0.03	+0.01	+0.03	+0.02	+0.01	-0.01
Vitamin A	**-0.05**	**-0.05**	**-0.05**	**-0.05**	**-0.04**	**-0.04**
Vitamin E	**-0.05**	**-0.06**	**-0.05**	**-0.06**	**-0.05**	**-0.07**
Peptone	**+0.17**	**+0.34**	**+0.37**	**+0.37**	**+0.42**	**+0.43**
Adenine	-0.05	-0.03	-0.03	-0.03	-0.04	-0.02
Hypoxanthine	**-0.08**	-0.06	-0.06	-0.05	-0.05	-0.06
Pep.+Ade.	**+0.18**	**+0.36**	**+0.42**	**+0.41**	**+0.47**	**+0.49**
Pep.+Hyp.	**+0.10**	**+0.30**	**+0.33**	**+0.34**	**+0.41**	**+0.43**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; †, B-complex vitamins; Concentration of substrates is detailed in subchapters 3.1.3 and 3.1.4; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

As can be seen from Fig. 44, the cyanobacterial biomass increased the rate of acid development by *Bifid. bifidum* to a very high degree.

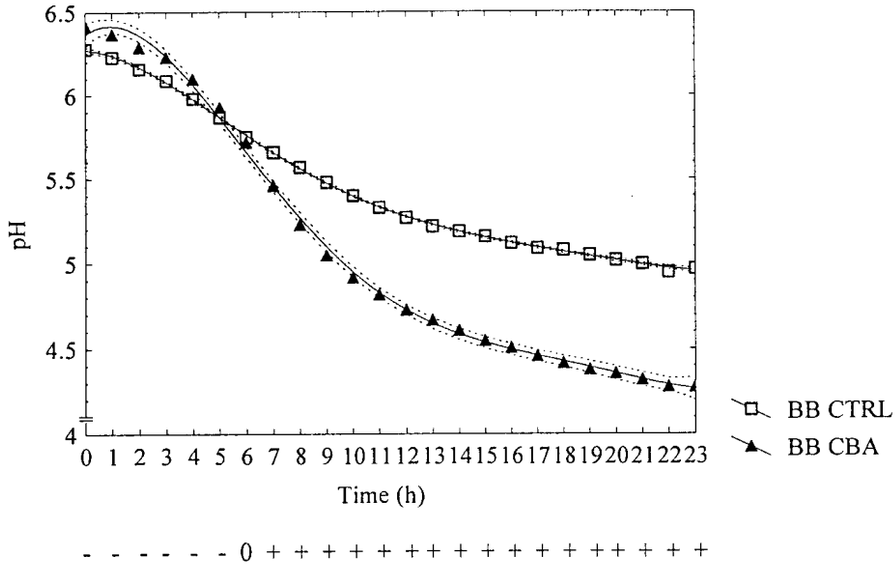


Fig. 44 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by *Bifidobacterium bifidum* Bb-12 in milk

Tables 10a and 10b and Figs 45-47 show the effect of addition of trace elements on the rate of acid development by *Bifid. bifidum*.

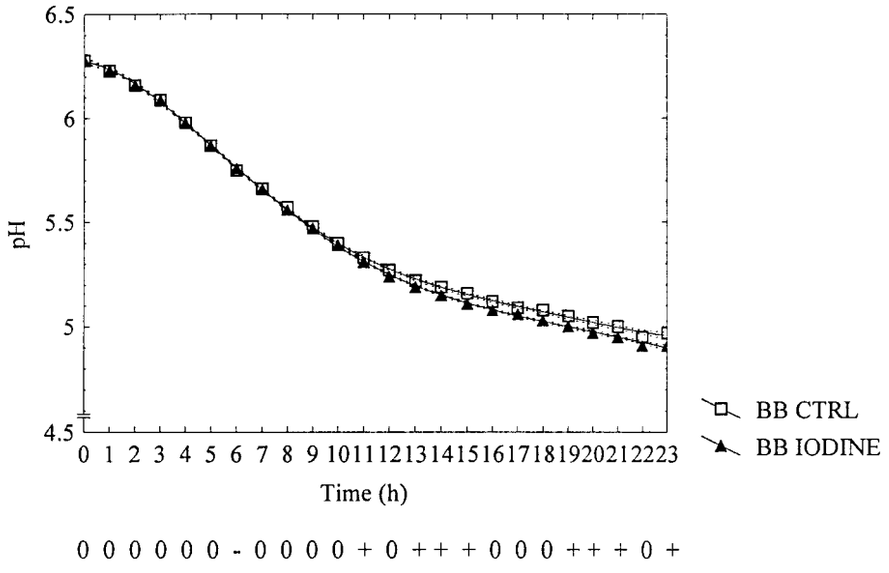


Fig. 45 Effect of iodine on acid development by *Bifidobacterium bifidum* Bb-12 in milk

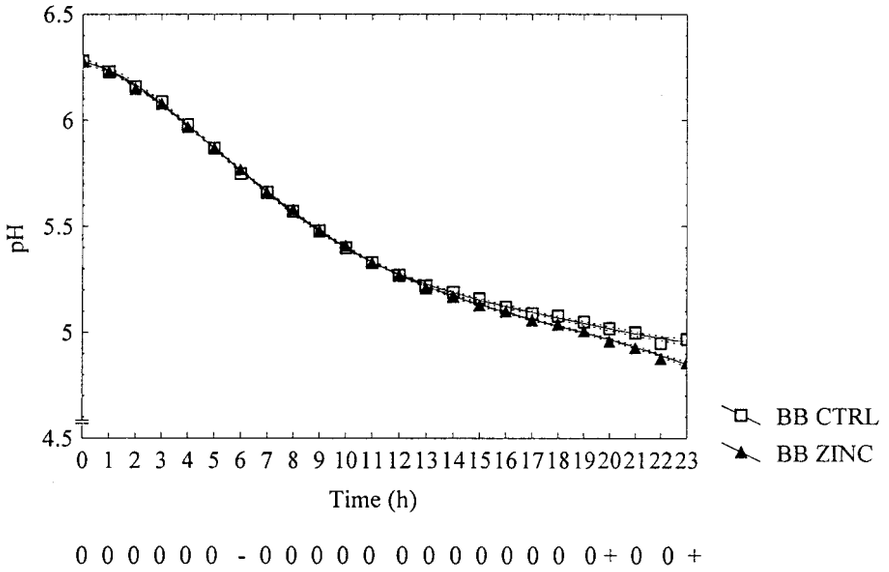
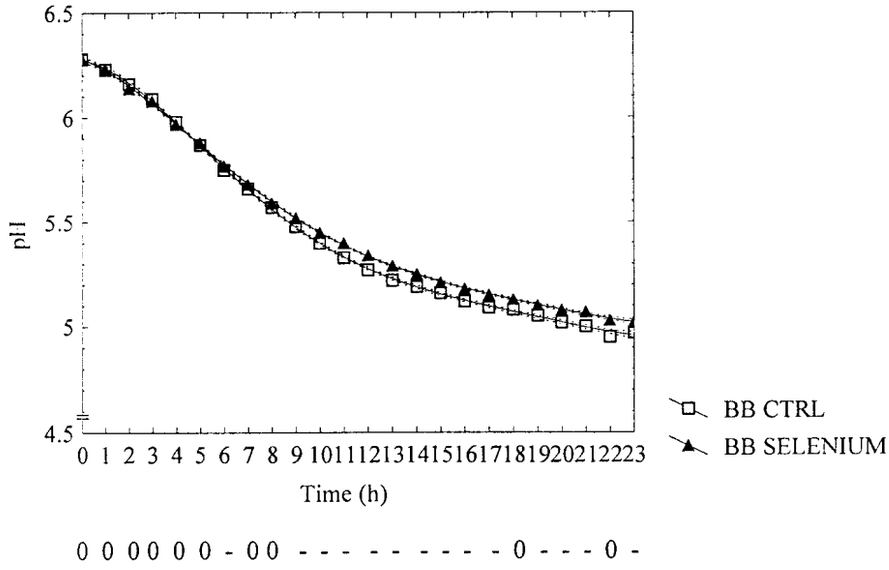


Fig. 46 Effect of zinc on acid development by *Bifidobacterium bifidum* Bb-12 in milk



0 0 0 0 0 0 - 0 0 - - - - - - - - - - 0 - - - 0 -

Fig. 47 Effect of selenium on acid development by *Bifidobacterium bifidum* Bb-12 in milk

The addition of iodine resulted in a small, although significant increase in the rate of acid development by *Bifid. bifidum* whereas the stimulation caused by zinc was not significant. The acid production of *Bifid. bifidum*, similarly to that of the rod-shaped lactic acid bacteria tested, was retarded significantly by the presence of selenium.

The effect of addition of vitamins on the rate of acid development by *Bifid. bifidum* is shown in Tables 10a and 10b and Figs 48-51.

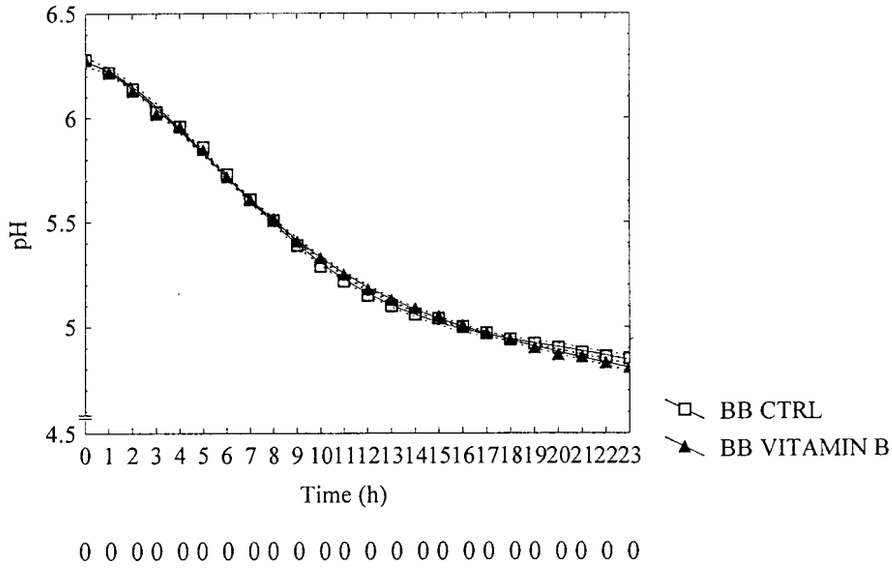


Fig. 48 Effect of B-complex vitamins on acid development by *Bifidobacterium bifidum* Bb-12 in milk

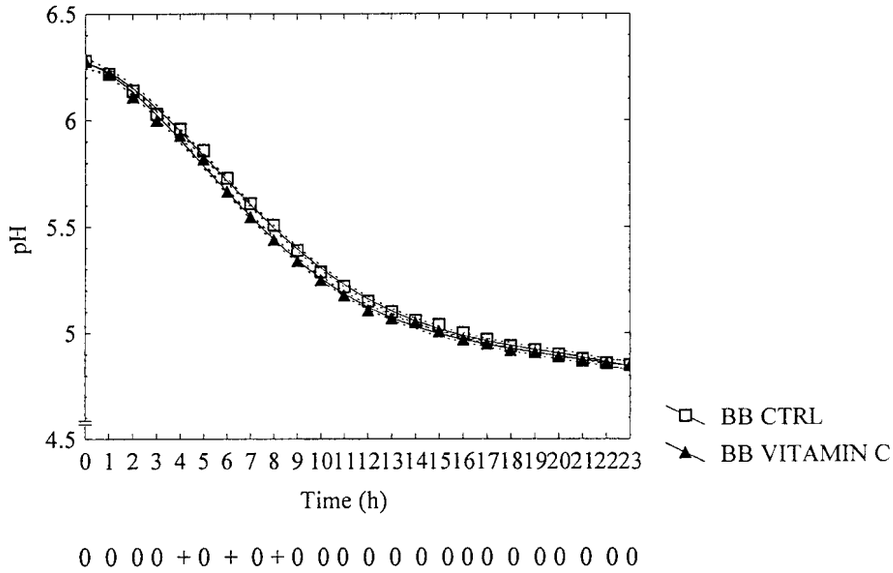


Fig. 49 Effect of vitamin C on acid development by *Bifidobacterium bifidum* Bb-12 in milk

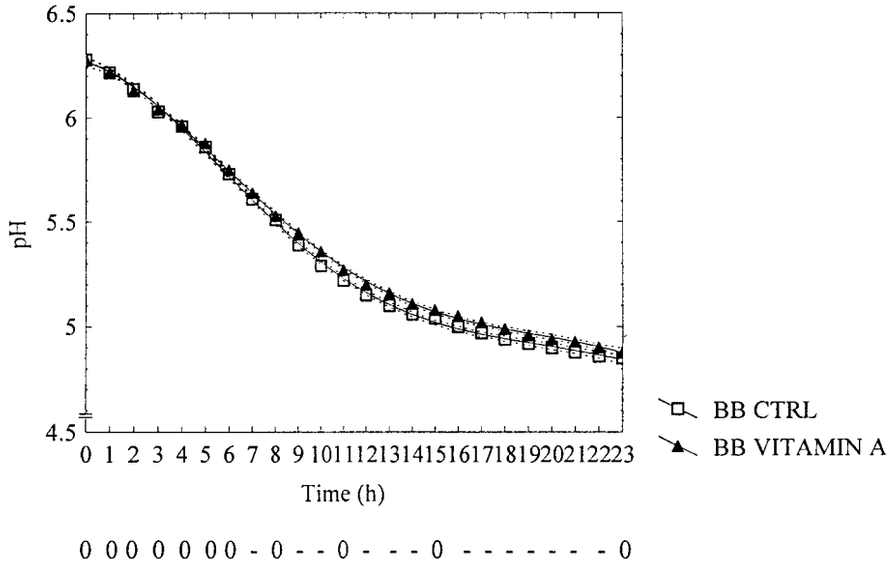


Fig. 50 Effect of vitamin A on acid development by *Bifidobacterium bifidum* Bb-12 in milk

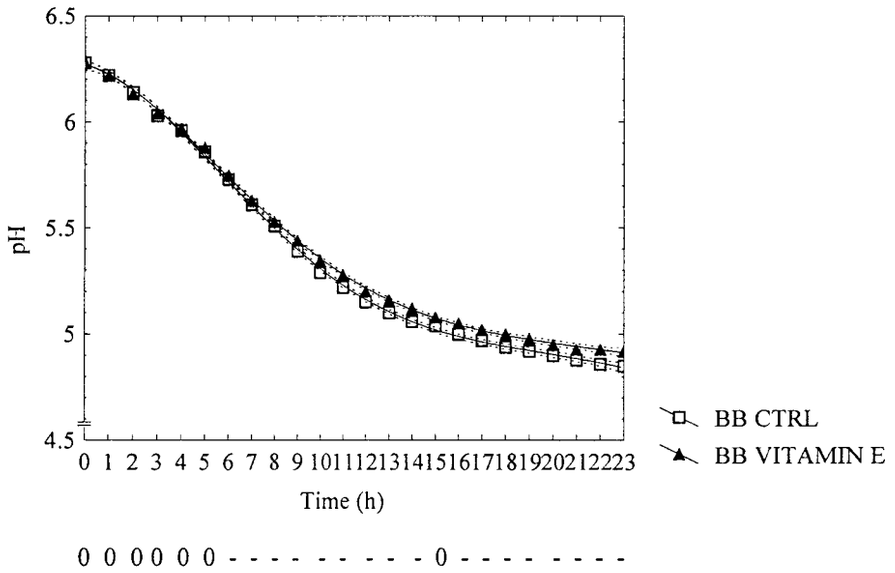


Fig. 51 Effect of vitamin E on acid development by *Bifidobacterium bifidum* Bb-12 in milk

The water-soluble vitamins (B-complex, C) did not have a significant effect on the acid production of *Bifid. bifidum*, although the addition of vitamin C caused some stimulation of the strain tested. The fat-soluble vitamins (A, E), however, retarded the acid production of *Bifid. bifidum* significantly.

Tables 10a and 10b and Figs 52-56 display the effect of addition of nitrogenous compounds on the rate of acid development by *Bifid. bifidum*.

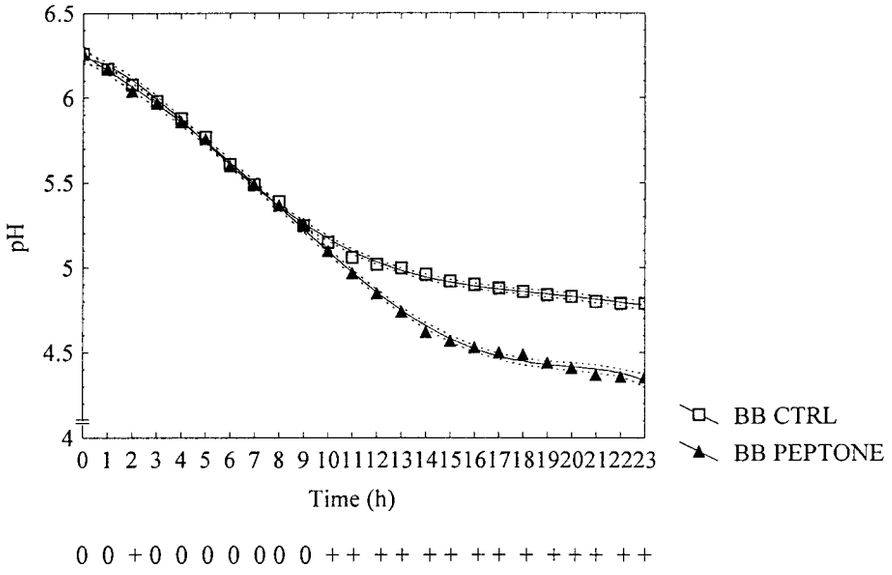


Fig. 52 Effect of peptone on acid development by *Bifidobacterium bifidum* Bb-12 in milk

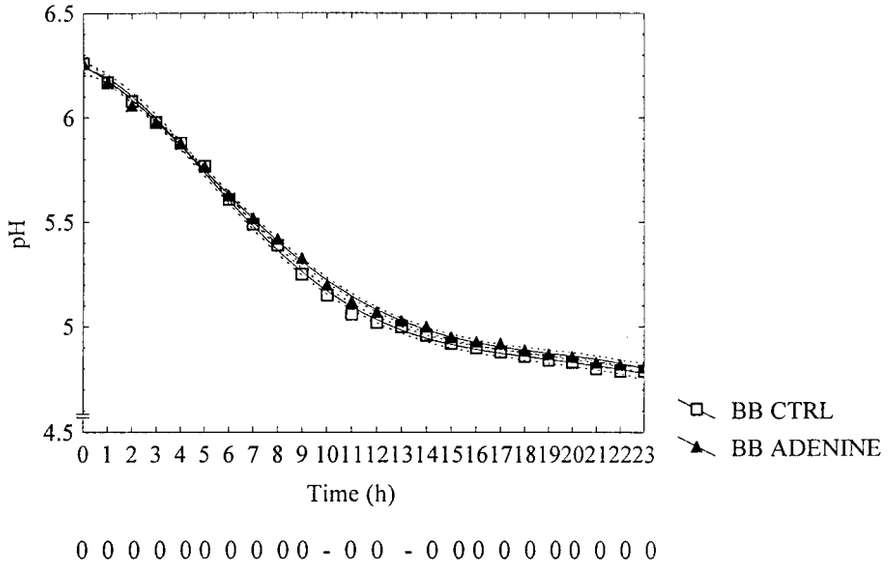


Fig. 53 Effect of adenine on acid development by *Bifidobacterium bifidum* Bb-12 in milk

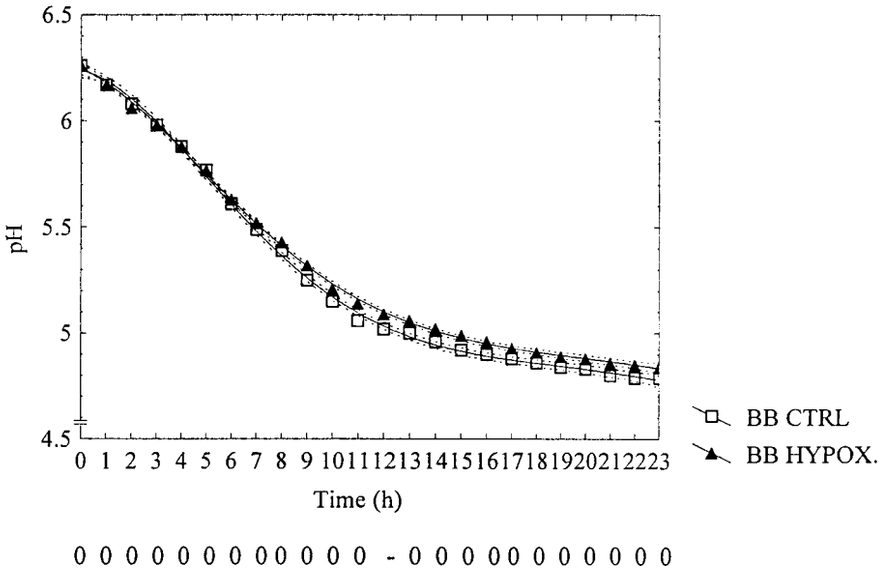


Fig. 54 Effect of hypoxanthine on acid development by *Bifidobacterium bifidum* Bb-12 in milk

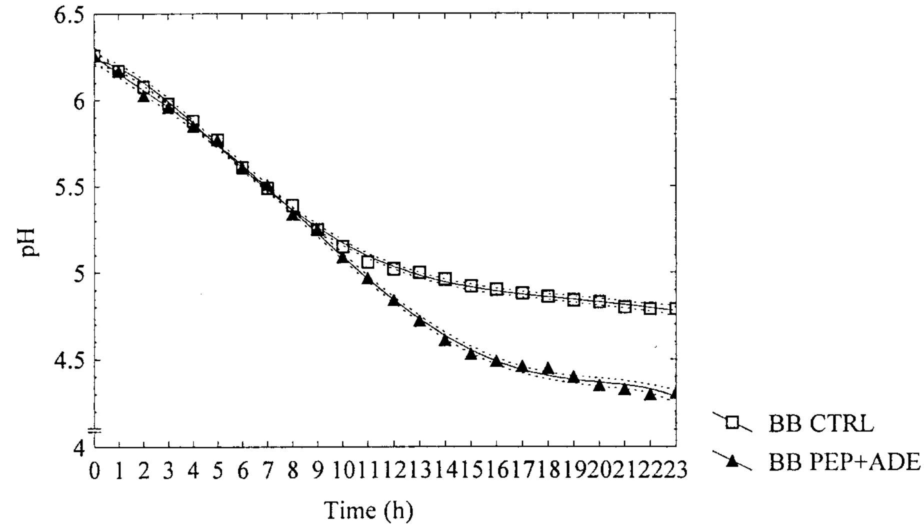


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Fig. 55 Effect of peptone and adenine on acid development by *Bifidobacterium bifidum* Bb-12 in milk

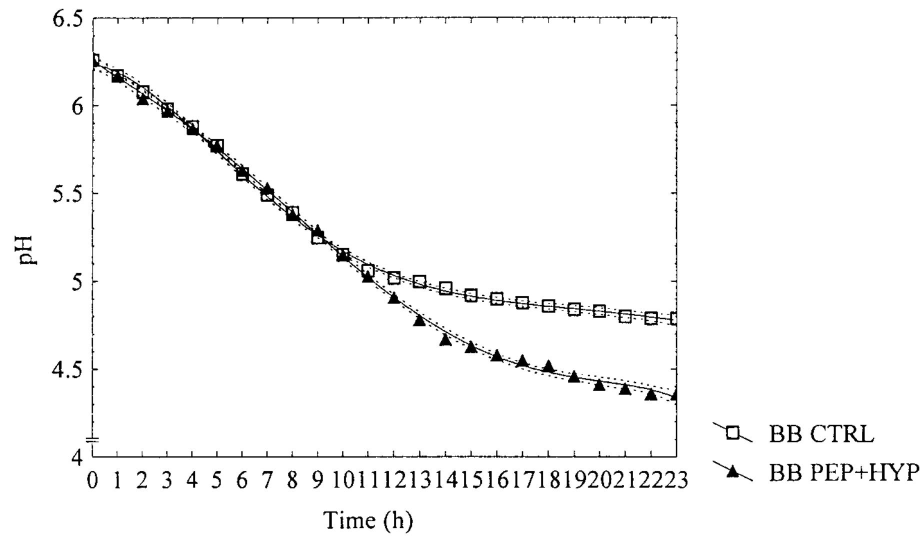

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Fig. 56 Effect of peptone and hypoxanthine on acid development by *Bifidobacterium bifidum* Bb-12 in milk

As for acid production, contrary to the three lactic acid bacterial strains tested, *Bifid. bifidum* was either adversely affected or completely unaffected by the addition of adenine and that of hypoxanthine. The influence of adenine employed in combination with peptone, however, proved to be stimulatory on acid production.

On the whole, peptone was the only substance tested to stimulate acid production of *Bifid. bifidum* significantly but this stimulation did not account for the one caused by the cyanobacterial biomass. Consequently, the *Spir. platensis* biomass must have also contained further effective components, besides peptone.

4.2 Effect of the *Spirulina platensis* biomass on acid production and growth of combinations of thermophilic dairy starter culture strains in a model milk medium

Further researches were done in order to reveal the effect of the cyanobacterial biomass on various combinations of the four single strains. Preliminary experiments had been conducted so that the levels of inoculation giving approximately the same counts of colony forming units (cfu) of starter culture organisms at the end of the fermentation process could be determined. On the basis of the results obtained, the rate of inoculation employed was between 0.1% (v/v) and 6.0% (v/v) with respect to the single strains.

4.2.1 Effect of the Spirulina platensis biomass on combination of Streptococcus thermophilus CH-1 and Lactobacillus bulgaricus CH-2 in a model milk medium

Fig. 57 shows acid development by the mixed culture of *Strep. thermophilus* CH-1 and *Lact. bulgaricus* CH-2 (0.5-0.5%, v/v) in milk and in milk supplemented with 3 g l^{-1} *Spir. platensis* biomass.

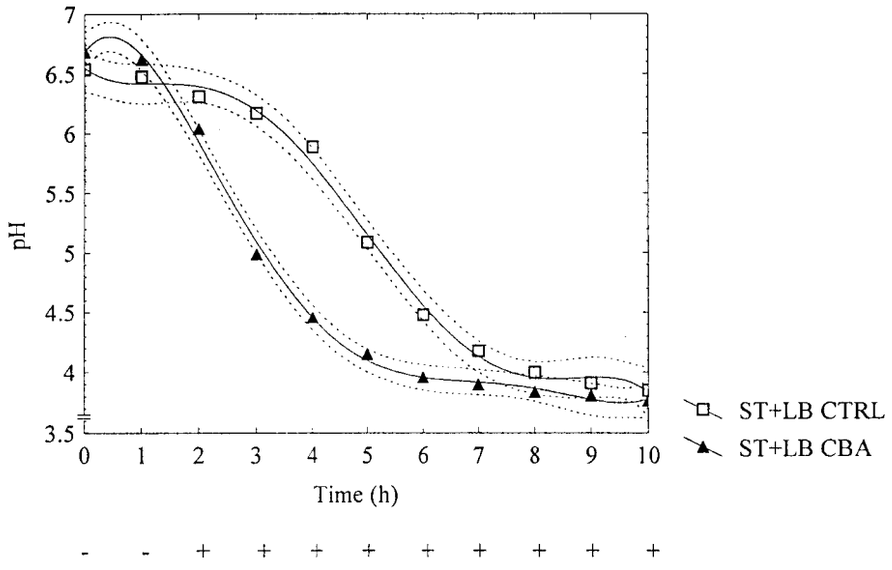


Fig. 57 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by the mixed culture of *Streptococcus thermophilus* CH-1 and *Lactobacillus bulgaricus* CH-2 in milk

The average differences in pH between cyanobacterial and control samples are shown in Tables 11a and 11b.

Table 11a Effect of CBA biomass* on pH of milk samples inoculated with various combinations of strains

Combination tested	Average decrease in pH during fermentation compared to controls						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
ST+LB 0.5-0.5%†	**-0.15**	**-0.15**	**+0.27**	**+1.18**	**+1.41**	**+0.94**	**+0.52**
ST+LA 0.1-1.0%†	**-0.13**	**-0.13**	**-0.14**	**-0.16**	**-0.18**	**-0.17**	**+0.25**
ST+BB 0.1-6.0%†	**-0.09**	**-0.08**	**-0.10**	**-0.13**	-0.02	-0.04	-0.03
LA+BB 1.0-6.0%†	**-0.06**	**-0.06**	**-0.04**	-0.01	+0.05	**+0.20**	**+0.35**
ST+LA+BB 0.1-1.0-6.0%†	**-0.14**	**-0.14**	**-0.14**	**-0.10**	0	**+0.02**	**+0.02**

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; ST, *Streptococcus thermophilus* CH-1; LB, *Lactobacillus bulgaricus* CH-2; LA, *Lactobacillus acidophilus* La-5; BB, *Bifidobacterium bifidum* Bb-12; †, Rate of inoculation (v/v) respectively; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

Table 11b Effect of CBA biomass* on pH of milk samples inoculated with various combinations of strains

Combination tested	Average decrease in pH during fermentation compared to controls						
	7 h	8 h	9 h	10 h	11 h	12 h	13 h
ST+LB 0.5-0.5%†	**+0.28**	**+0.16**	**+0.10**	**+0.08**	—	—	—
ST+LA 0.1-1.0%†	**+0.65**	**+0.77**	**+0.61**	**+0.42**	**+0.28**	**+0.18**	**+0.13**
ST+BB 0.1-6.0%†	**-0.07**	**-0.07**	**-0.07**	**-0.07**	—	—	—
LA+BB 1.0-6.0%†	**+0.49**	**+0.52**	**+0.54**	**+0.53**	**+0.47**	**+0.41**	**+0.36**
ST+LA+BB 0.1-1.0-6.0%†	-0.01	0	-0.01	-0.01	—	—	—

*, 3 g l^{-1} cyanobacterial biomass enriched with iodine, zinc and selenium; ST, *Streptococcus thermophilus* CH-1; LB, *Lactobacillus bulgaricus* CH-2; LA, *Lactobacillus acidophilus* La-5; BB, *Bifidobacterium bifidum* Bb-12; †, Rate of inoculation (v/v) respectively; -, Retardation of acid production; +, Stimulation of acid production; **Bold numbers, Significantly different at the P=0.05 level.**

It is seen in Fig. 57, Tables 11a and 11b and can be calculated on the basis of Fig. 4 that acid production of this mixed culture was over 4 times higher in the cyanobacterial samples than in controls during the first 3 h of the fermentation process.

This was due to the stimulatory effect produced by the CBA biomass on the growth of *Lact. bulgaricus* (Table 12).

Table 12 Average growth rate of components of the mixed culture of *Streptococcus thermophilus* CH-1 (0.5%, v/v) and *Lactobacillus bulgaricus* CH-2 (0.5%, v/v) in milk and in milk supplemented with CBA biomass, at 42.5°C, between hours 0-3, 3-6 and 0-6

	Control	CBA
$\mu_{ST(0;3)}$	1.28	1.30
$\mu_{ST(3;6)}$	0.85	0.74
$\mu_{ST(0;6)}$	1.07	1.02
$\mu_{LB(0;3)}$	0.44	0.74
$\mu_{LB(3;6)}$	1.26	0.81
$\mu_{LB(0;6)}$	0.85	0.78

As a result of this, the increase in average viable cell count of *Lact. bulgaricus* was 0.67 log cycle in the cyanobacterial samples and only 0.40 in the controls during the period mentioned (Fig. 59). These values are significantly different.

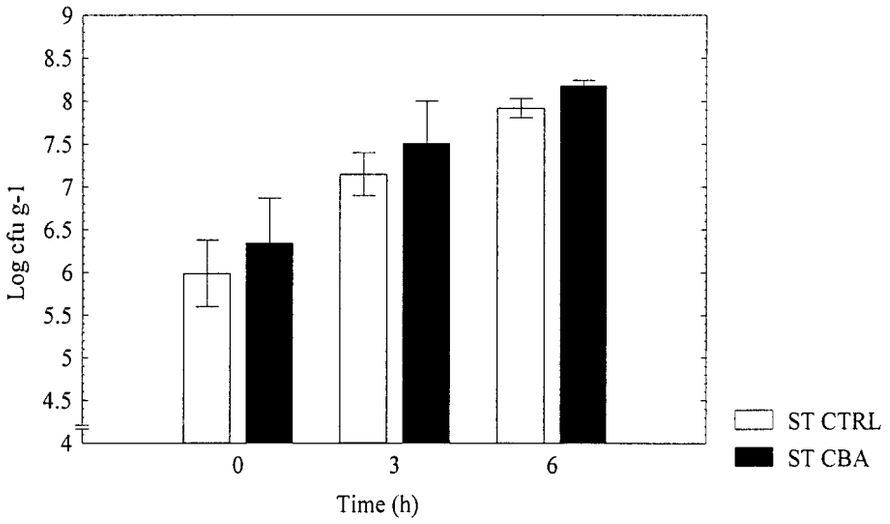


Fig. 58 Average viable cell counts of *Streptococcus thermophilus* CH-1 in milk and in milk supplemented with CBA biomass, grown in combination with *Lactobacillus bulgaricus* CH-2

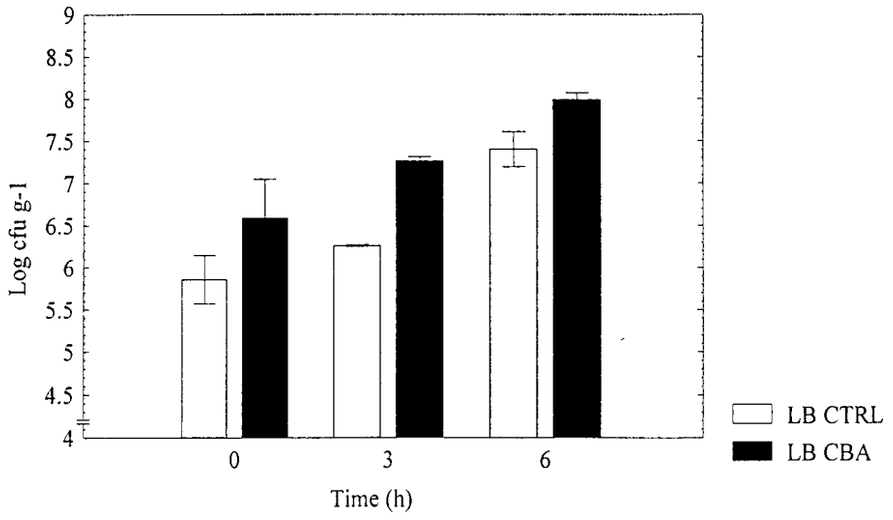


Fig. 59 Average viable cell counts of *Lactobacillus bulgaricus* CH-2 in milk and in milk supplemented with CBA biomass, grown in combination with *Streptococcus thermophilus* CH-1

The results of growth rate calculations and the average viable cell counts indicate that the *Spir. platensis* biomass had no influence on the growth of *Strep. thermophilus* in the course of this model experiment (Table 12 and Fig. 58).

4.2.2 Effect of the Spirulina platensis biomass on combination of Streptococcus thermophilus CH-1 and Lactobacillus acidophilus La-5 in a model milk medium

The drop in pH of milk and that of cyanobacterial milk inoculated with 0.1% (v/v) *Strep. thermophilus* and 1.0% (v/v) *Lact. acidophilus* showed the same tendency during the first 5 h of fermentation (Tables 11a and 11b). The significantly negative differences were largely due to the higher initial pH and greater buffering capacity of the cyanobacterial milk. The results showed an increase in the rate of acid production 6-10 h after the start of fermentation, which was 2-2.5 times greater in the cyanobacterial samples than in controls (Fig. 60).

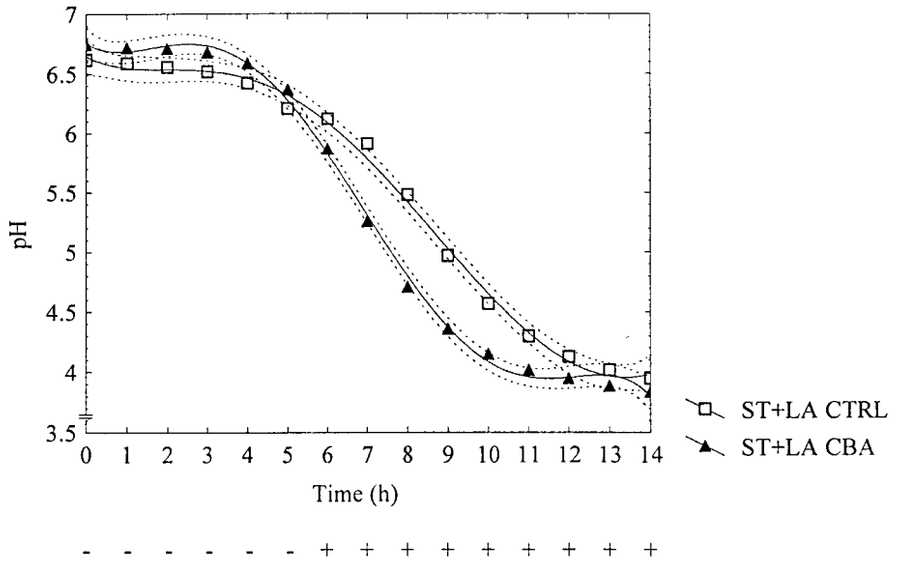


Fig. 60 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by the mixed culture of *Streptococcus thermophilus* CH-1 and *Lactobacillus acidophilus* La-5 in milk

This could be accounted for by the significantly higher average growth rate and average viable cell count values of both *Strep. thermophilus* and *Lact. acidophilus* in the CBA samples than in controls during the first 10 h of fermentation (Table 13, Figs 61 and 62).

Table 13 Average growth rate of components of the mixed culture of *Streptococcus thermophilus* CH-1 (0.1%, v/v) and *Lactobacillus acidophilus* La-5 (1.0%, v/v) in milk and in milk supplemented with CBA biomass, at 37.5°C, between hours 0-5, 5-10 and 0-10

	Control	CBA
$\mu_{ST(0;5)}$	0.57	0.52
$\mu_{ST(5;10)}$	-0.17	0.11
$\mu_{ST(0;10)}$	0.20	0.32
$\mu_{LA(0;5)}$	0.47	0.57
$\mu_{LA(5;10)}$	0	0.09
$\mu_{LA(0;10)}$	0.23	0.33

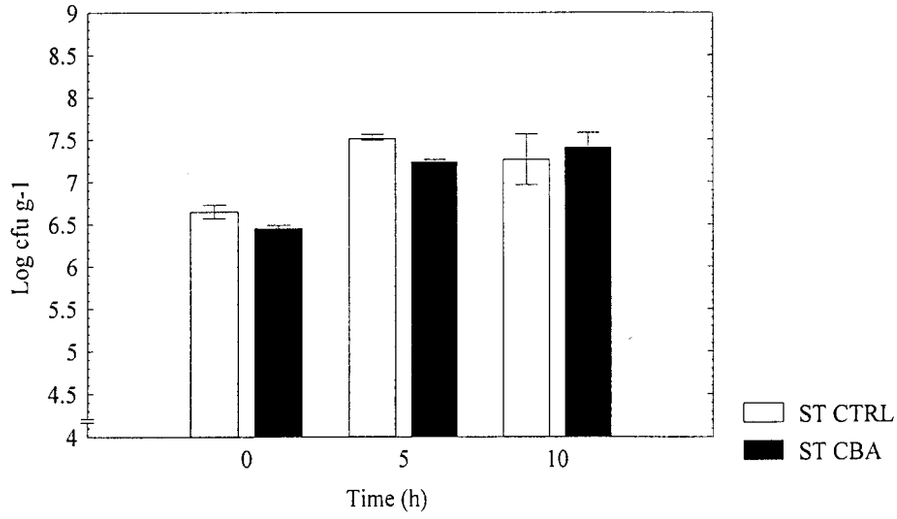


Fig. 61 Average viable cell counts of *Streptococcus thermophilus* CH-1 in milk and in milk supplemented with CBA biomass, grown in combination with *Lactobacillus acidophilus* La-5

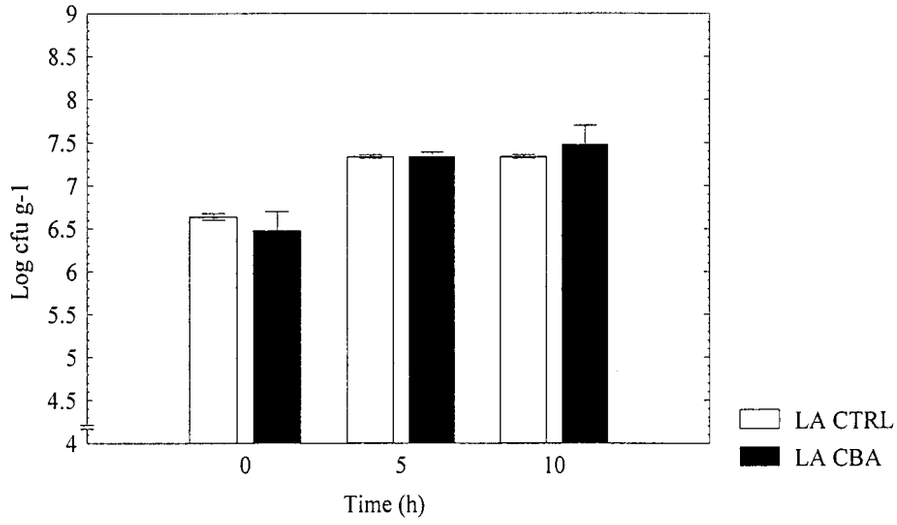
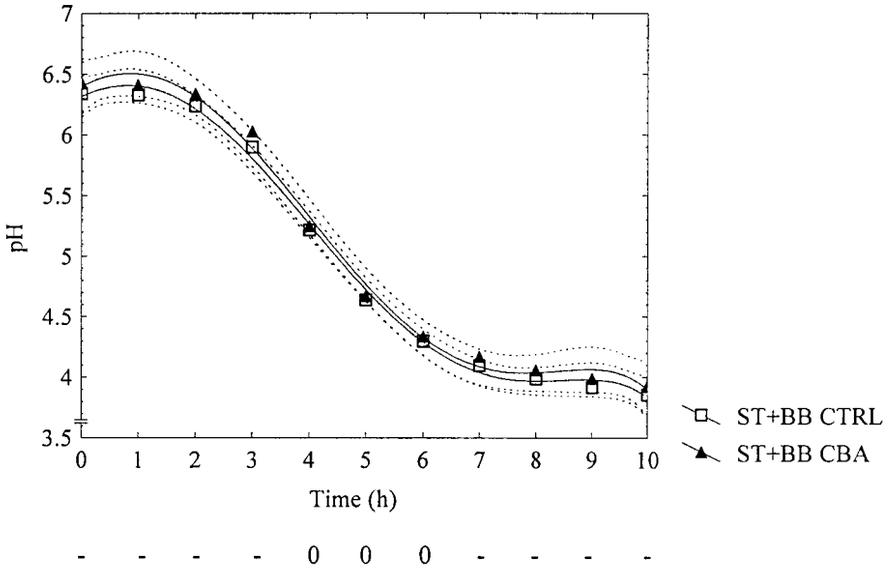


Fig. 62 Average viable cell counts of *Lactobacillus acidophilus* La-5 in milk and in milk supplemented with CBA biomass, grown in combination with *Streptococcus thermophilus* CH-1

4.2.3 *Effect of the Spirulina platensis biomass on combination of Streptococcus thermophilus CH-1 and Bifidobacterium bifidum Bb-12 in a model milk medium*

Actually, there was no difference in pH-decrease between cyanobacterial and control milks inoculated with 0.1% (v/v) *Strep. thermophilus* and 6.0% (v/v) *Bifid. bifidum*, but addition of the *Spir. platensis* biomass increased the pH of milk to a slight degree (Tables 11a and 11b). The acid production curves, the growth results and the average viable cell counts did not show marked differences, thereby indicating that the CBA biomass had no influence either on fermentation activity or on growth of the mixed culture of *Strep. thermophilus* and *Bifid. bifidum* (Figs 63, 64, 65 and Table 14).



- - - - 0 0 0 - - - -

Fig. 63 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by the mixed culture of *Streptococcus thermophilus* CH-1 and *Bifidobacterium bifidum* Bb-12 in milk

Table 14 Average growth rate of components of the mixed culture of *Streptococcus thermophilus* CH-1 (0.1%, v/v) and *Bifidobacterium bifidum* Bb-12 (6.0%, v/v) in milk and in milk supplemented with CBA biomass, at 37.5°C, between hours 0-5, 5-10 and 0-10

	Control	CBA
$\mu_{ST(0;5)}$	1.46	1.58
$\mu_{ST(5;10)}$	1.08	0.85
$\mu_{ST(0;10)}$	1.27	1.22
$\mu_{BB(0;5)}$	0.33	0.33
$\mu_{BB(5;10)}$	0.40	0.33
$\mu_{BB(0;10)}$	0.36	0.33

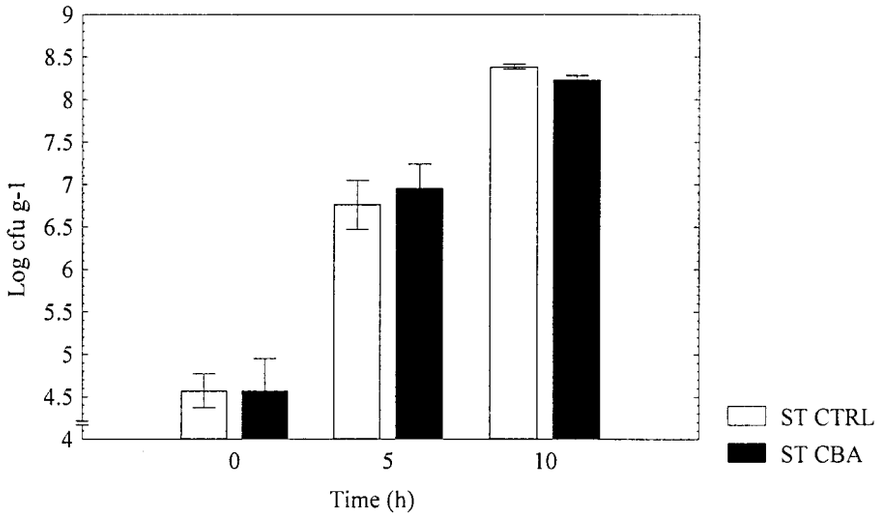


Fig. 64 Average viable cell counts of *Streptococcus thermophilus* CH-1 in milk and in milk supplemented with CBA biomass, grown in combination with *Bifidobacterium bifidum* Bb-12

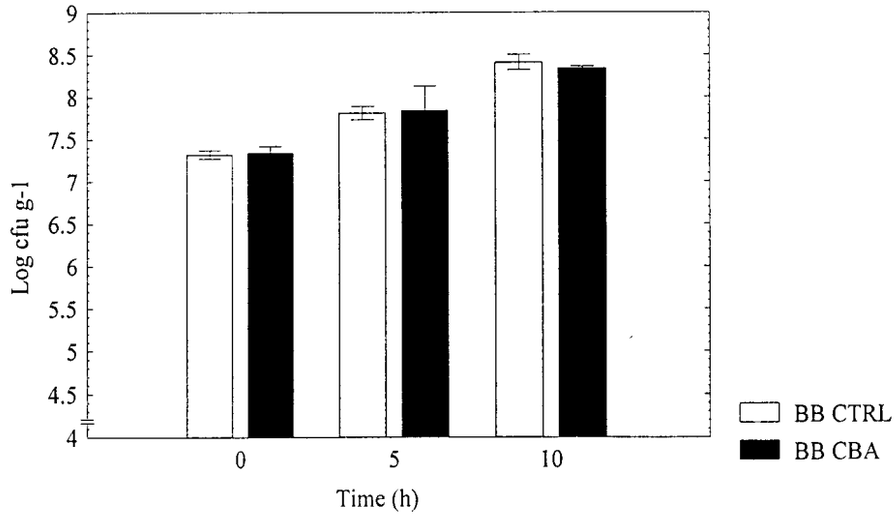


Fig. 65 Average viable cell counts of *Bifidobacterium bifidum* Bb-12 in milk and in milk supplemented with CBA biomass, grown in combination with *Streptococcus thermophilus* CH-1

4.2.4 Effect of the Spirulina platensis biomass on combination of Lactobacillus acidophilus La-5 and Bifidobacterium bifidum Bb-12 in a model milk medium

As regards acid development, the mixed culture of *Lact. acidophilus* (1.0%, v/v) and *Bifid. bifidum* (6.0%, v/v) gave results similar to those produced by the combination of *Strep. thermophilus* and *Lact. acidophilus* in the case of cyanobacterial and control samples alike (Tables 11a and 11b, Figs 60 and 66).

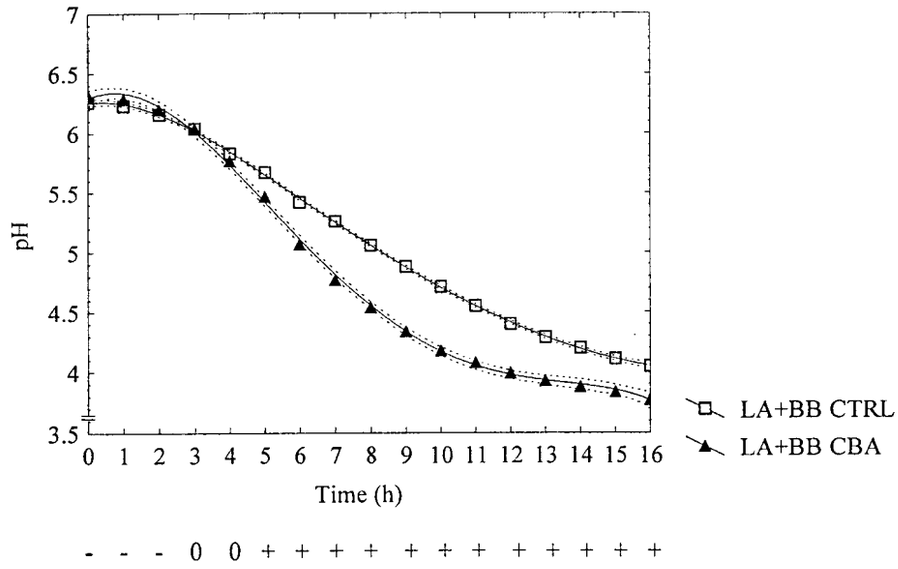


Fig. 66 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by the mixed culture of *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 in milk

The CBA biomass had a stimulatory effect on the growth of *Lact. acidophilus* in both mixed cultures (Tables 13 and 15, Figs 62 and 67). As shown in Table 15 and Figs 67 and 68, the CBA biomass stimulated the acid production of this combination of strains by increasing the average growth rate and average viable cell count values of both *Lact. acidophilus* and *Bifid. bifidum* during the first 10 h of fermentation.

Table 15 Average growth rate of components of the mixed culture of *Lactobacillus acidophilus* La-5 (1.0%, v/v) and *Bifidobacterium bifidum* Bb-12 (6.0%, v/v) in milk and in milk supplemented with CBA biomass, at 37.5°C, between hours 0-5, 5-10, 0-10

	Control	CBA
$\mu_{LA(0;5)}$	0.63	0.74
$\mu_{LA(5;10)}$	0.55	0.62
$\mu_{LA(0;10)}$	0.59	0.68
$\mu_{BB(0;5)}$	0.19	0.18
$\mu_{BB(5;10)}$	0.36	0.48
$\mu_{BB(0;10)}$	0.28	0.33

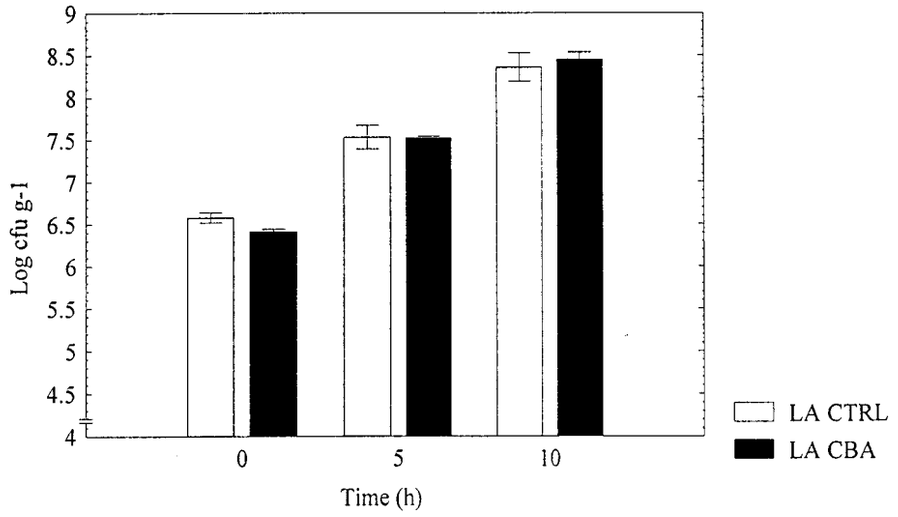


Fig. 67 Average viable cell counts of *Lactobacillus acidophilus* La-5 in milk and in milk supplemented with CBA biomass, grown in combination with *Bifidobacterium bifidum* Bb-12

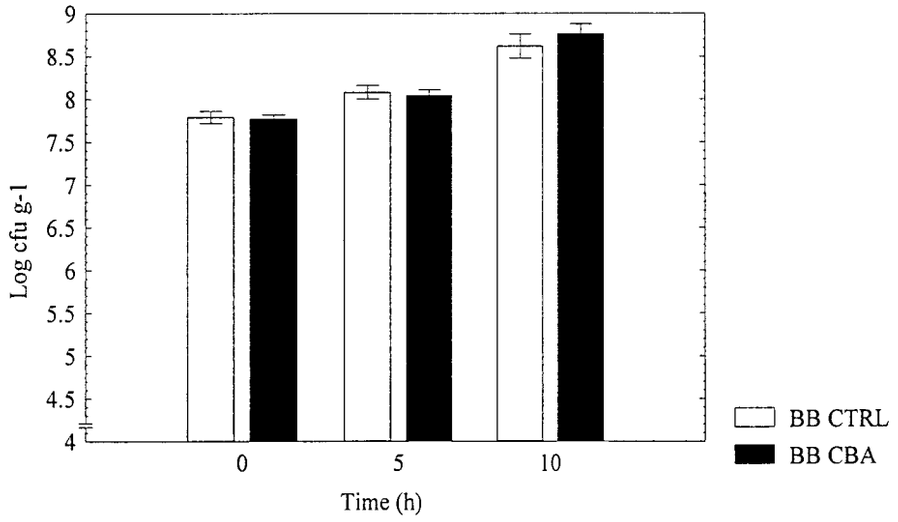


Fig. 68 Average viable cell counts of *Bifidobacterium bifidum* Bb-12 in milk and in milk supplemented with CBA biomass, grown in combination with *Lactobacillus acidophilus* La-5

4.2.5 Effect of the Spirulina platensis biomass on combination of Streptococcus thermophilus CH-1, Lactobacillus acidophilus La-5 and Bifidobacterium bifidum Bb-12 in a model milk medium

Fig. 69 illustrates the drop in pH of cyanobacterial and control milks caused by the mixed culture of *Strep. thermophilus* (0.1%, v/v), *Lact. acidophilus* (1.0%, v/v) and *Bifid. bifidum* (6.0%, v/v).

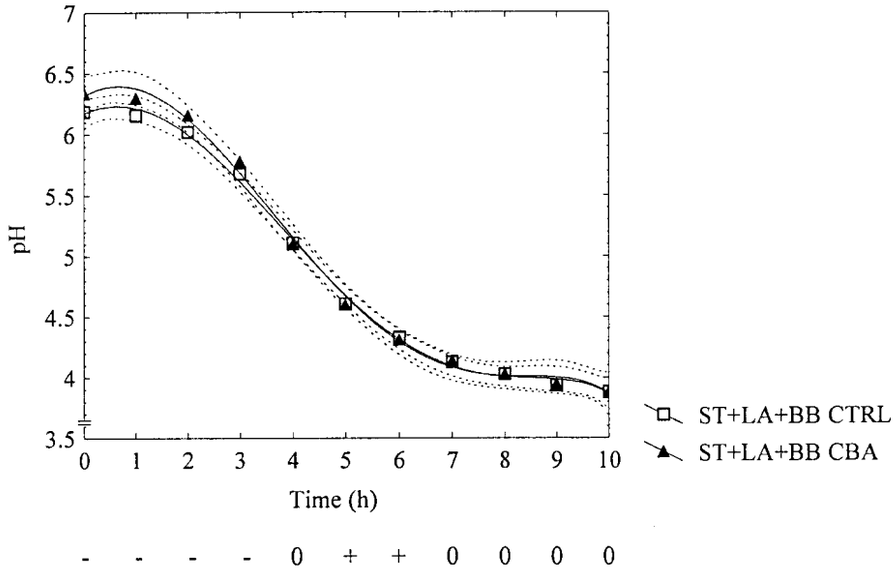


Fig. 69 Effect of 3 g l^{-1} cyanobacterial biomass on acid development by the mixed culture of *Streptococcus thermophilus* CH-1, *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 in milk

It is seen in Fig. 69 and Tables 11a and 11b that the average differences in pH between cyanobacterial and control samples were minimal.

The growth of *Lact. acidophilus* was stimulated whereas that of *Strep. thermophilus* was not affected at all by the *Spir. platensis* biomass. Although the acid-producing activity of *Bifid. bifidum* was not so pronounced as that of the other two

starter culture organisms, its growth results were significantly better in the CBA samples than in controls (Table 16, Figs 70, 71 and 72).

Table 16 Average growth rate of components of the mixed culture of *Streptococcus thermophilus* CH-1 (0.1%, v/v), *Lactobacillus acidophilus* La-5 (1.0%, v/v) and *Bifidobacterium bifidum* Bb-12 (6.0%, v/v) in milk and in milk supplemented with CBA biomass, at 37.5°C, between hours 0-5, 5-10 and 0-10

	Control	CBA
$\mu_{ST(0;5)}$	1.11	1.09
$\mu_{ST(5;10)}$	0	0.01
$\mu_{ST(0;10)}$	0.55	0.55
$\mu_{LA(0;5)}$	0.66	0.70
$\mu_{LA(5;10)}$	-0.37	-0.20
$\mu_{LA(0;10)}$	0.14	0.25
$\mu_{BB(0;5)}$	0.07	0.25
$\mu_{BB(5;10)}$	0.05	0.17
$\mu_{BB(0;10)}$	0.06	0.21

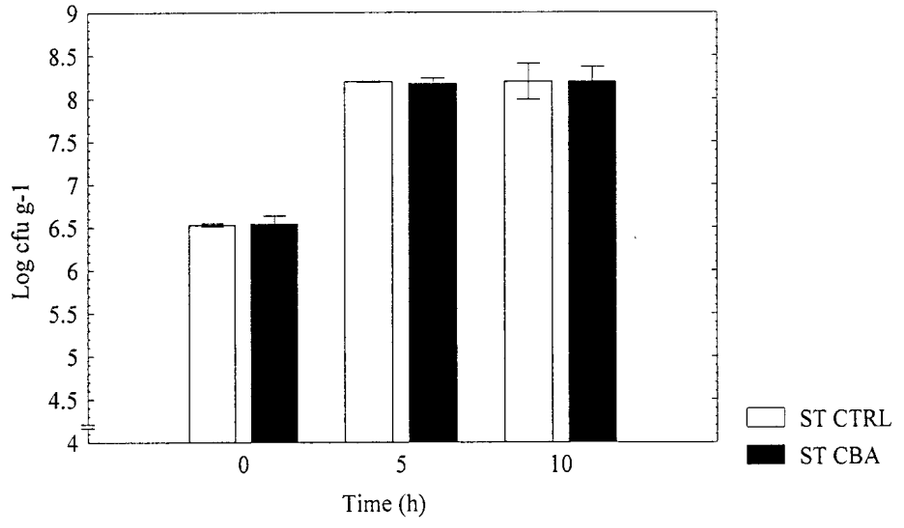


Fig. 70 Average viable cell counts of *Streptococcus thermophilus* CH-1 in milk and in milk supplemented with CBA biomass, grown in combination with *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12

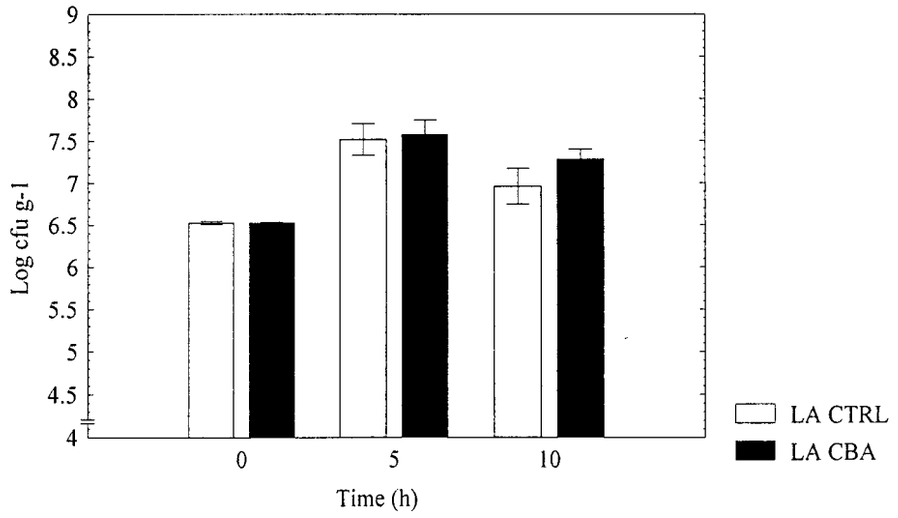


Fig. 71 Average viable cell counts of *Lactobacillus acidophilus* La-5 in milk and in milk supplemented with CBA biomass, grown in combination with *Streptococcus thermophilus* CH-1 and *Bifidobacterium bifidum* Bb-12

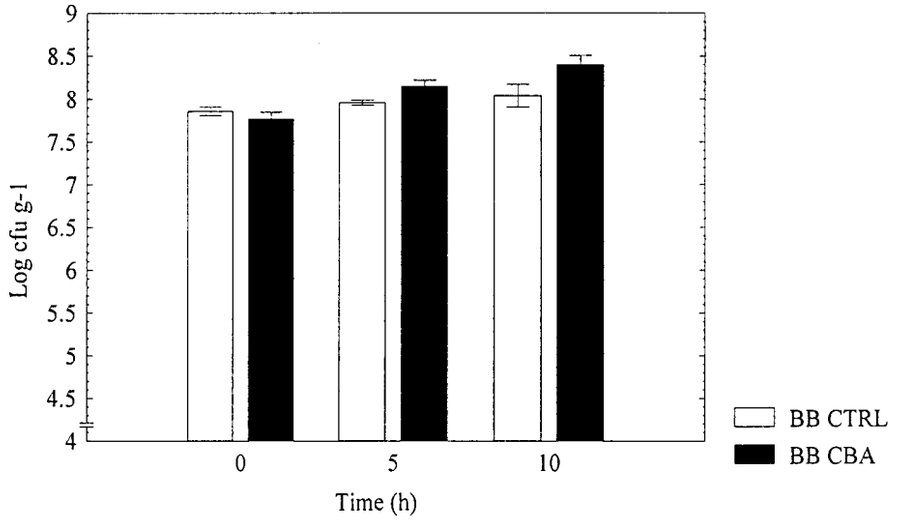


Fig. 72 Average viable cell counts of *Bifidobacterium bifidum* Bb-12 in milk and in milk supplemented with CBA biomass, grown in combination with *Streptococcus thermophilus* CH-1 and *Lactobacillus acidophilus* La-5

The results of experiments carried out in order to reveal the effect of the *Spir. platensis* biomass on combinations of thermophilic dairy starter culture strains clearly indicate that the cyanobacterial biomass did not always have the same effect on the same strain in various combinations. This is well illustrated by the example of *Strep. thermophilus*. As regards the mixed culture of *Strep. thermophilus* and *Lact. bulgaricus*, the *Spir. platensis* biomass had a significant stimulatory effect on acid development but it did not influence the growth of *Strep. thermohilus*. In the case of the combination of *Strep. thermophilus* and *Lact. acidophilus*, the cyanobacterial biomass stimulated both acid production and the growth rate of *Strep. thermophilus*. As for the mixed culture of *Strep. thermophilus* and *Bifid. bifidum*, neither acid production nor the growth of *Strep. thermophilus* was affected by the *Spir. platensis* biomass.

As can be seen from the results, it was the mixed cultures containing *Lact. bulgaricus* or *Lact. acidophilus* that the cyanobacterial biomass had the most stimulatory effect on. The growth rate of these two strains was accelerated in each case and *Bifid. bifidum* was also stimulated whenever it was combined with a rod-shaped starter organism (i.e. *Lact. acidophilus*). As to the mixed culture of *Strep. thermophilus*, *Lact. acidophilus* and *Bifid. bifidum*, the growth of *Bifid. bifidum* was found to be significantly faster in the cyanobacterial samples than in controls. Since such an effect was not observed in the case of the combination of *Strep. thermophilus* and *Bifid. bifidum*, it is supposed that the growth rate of *Bifid. bifidum* was accelerated by certain metabolic products of *Lact. acidophilus* and/or by some specific nutrients found in the *Spir. platensis* biomass.

On the whole, the cyanobacterial biomass stimulated the rod-shaped starter bacteria to a greater extent than the coccus-shaped ones, but its effect on cocci was largely dependent on what kind of rod the cocci were combined with. In other words, the effect of the *Spir. platensis* biomass on mixed cultures also depended on the interaction between/among strains composing the mixed cultures.

4.3 Microbiological and physical-chemical changes in cyanobacterial and control yogurts during storage

Experiments were carried out to reveal the changes in the microflora of yogurt samples containing 3 g l^{-1} *Spir. platensis* biomass and in those of control (i.e. regular natural yogurt) samples stored for a period of 35 days at 4°C and 15 days at 15°C. Along with the microbiological investigations, the pH of samples was measured so that data concerning the degree of post-acidification would also be obtained.

4.3.1 Storage at 15°C

Figs 73-75 illustrate the survival of the characteristic microbial flora of yogurts stored at 15°C.

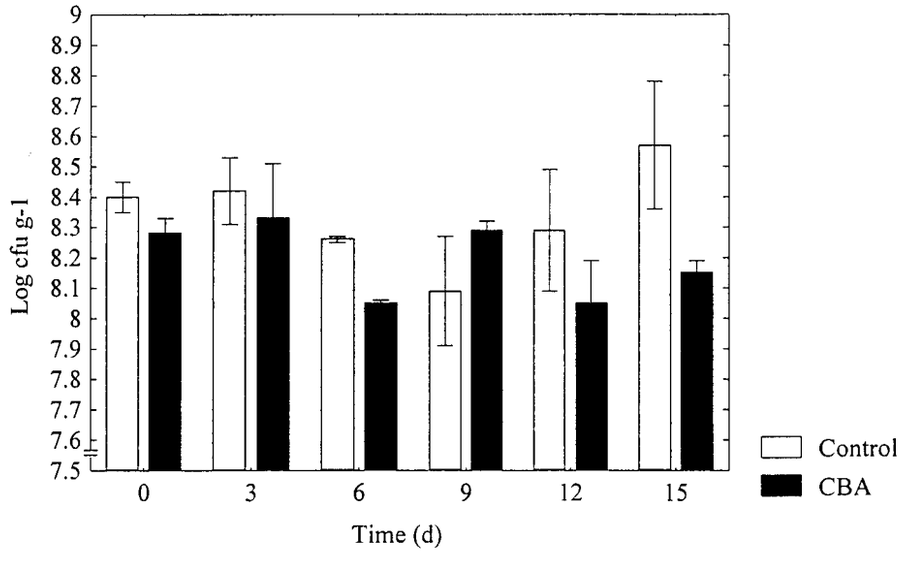


Fig. 73 Changes in the viable cell counts of *Strep. thermophilus* during storage at 15°C

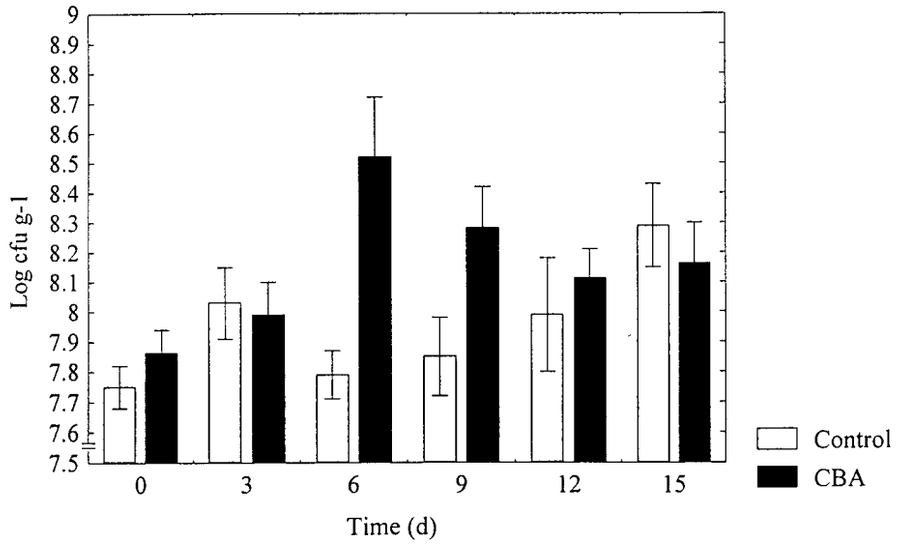


Fig. 74 Changes in the viable cell counts of *Lact. bulgaricus* during storage at 15°C

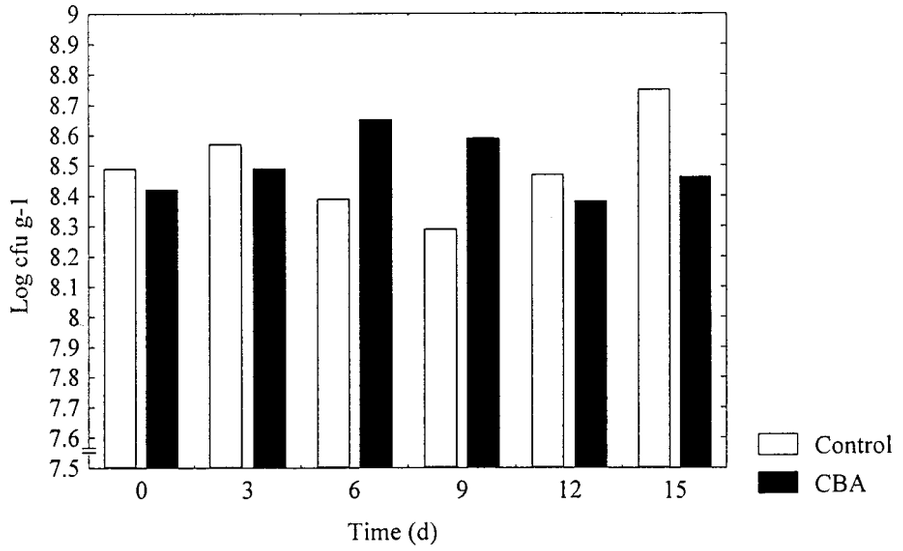


Fig. 75 Changes in the characteristic viable cell counts of yogurts during storage at 15°C (calculated values: *Strep. thermophilus* count + *Lact. bulgaricus* count)

As shown in Figs 73 and 74, the viable cell counts of both yogurt starter organisms exceeded the value of 10^8 cfu g^{-1} in the samples containing cyanobacterial biomass. The stimulating effect of the CBA biomass on *Lact. bulgaricus* was noticeable throughout the storage period, the most pronounced stimulation being observed on day 6.

As for the characteristic viable cell counts, both the control and the cyanobacterial yogurts satisfied even the most stringent requirements detailed in subchapter 2.7 (Fig. 75).

Enterococci or coliform organisms were detected neither in control nor in cyanobacterial yogurts during the entire storage period.

Fig. 76 shows that pronounced post-acidification occurred in the control and cyanobacterial samples alike, which was due to the relatively high storage temperature.

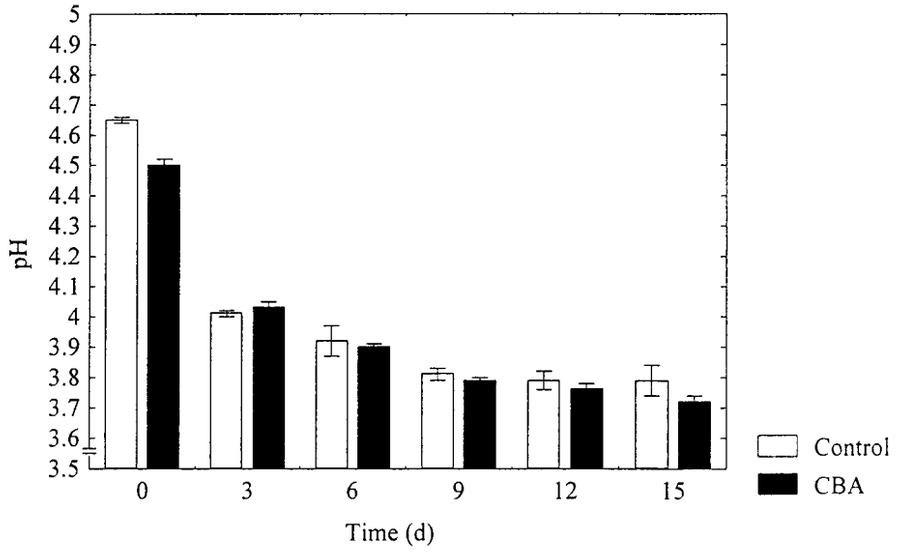


Fig. 76 Post-acidification of control and cyanobacterial yogurts stored at 15°C

Owing to the non-sterile manufacturing conditions and the high storage temperature, the count of yeasts and molds rose to a level of 10^1 cfu g^{-1} by the sixth day and to a level of 10^5 cfu g^{-1} by the 15th day of the storage period. No significant difference was found between controls and cyanobacterial samples in this regard.

4.3.2 Storage at 4°C

Figs 77-79 illustrate the survival of the characteristic microbial flora of yogurts stored at 4°C.

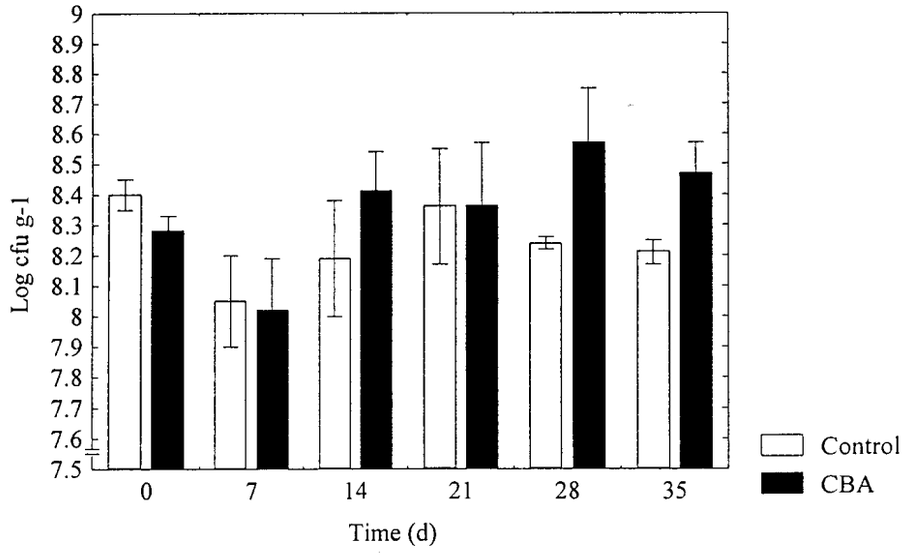


Fig. 77 Changes in the viable cell counts of *Strep. thermophilus* during storage at 4°C

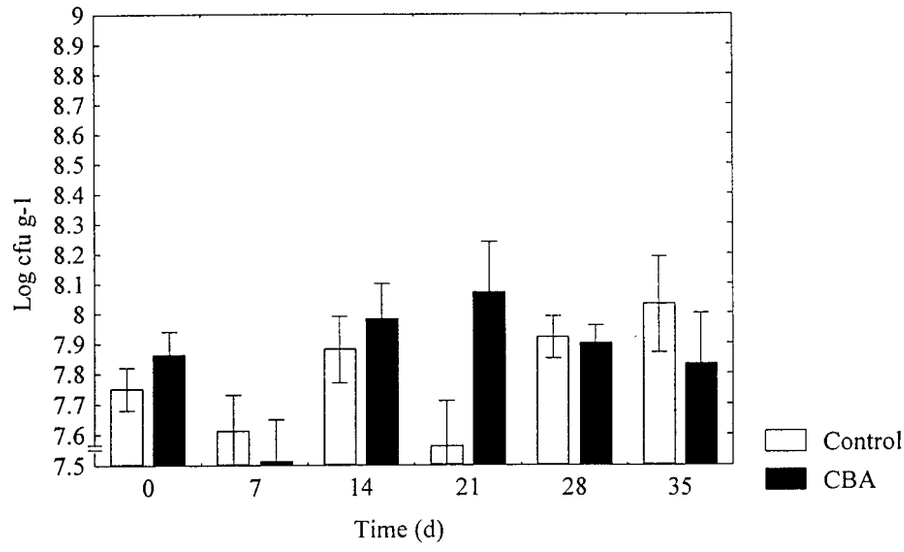


Fig. 78 Changes in the viable cell counts of *Lact. bulgaricus* during storage at 4°C

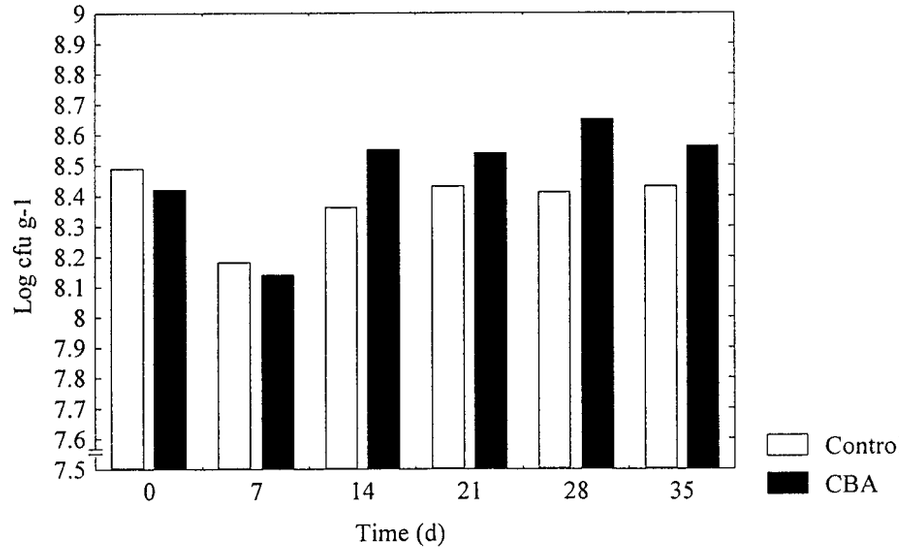


Fig. 79 Changes in the characteristic viable cell counts of yogurts during storage at 4°C (calculated values: *Strep. thermophilus* count + *Lact. bulgaricus* count)

Figs 77 and 78 show that the viable cell counts of *Strep. thermophilus* and *Lact. bulgaricus* did not change basically during the storage period, either in the case of control yogurt samples or in that of cyanobacterial ones.

As for the characteristic viable cell counts, both the control and the cyanobacterial yogurts fulfilled even the most stringent requirements of the European Union throughout the storage period (Gläser 1992). Furthermore, the viable cell counts were found to be noticeably higher in the cyanobacterial samples than in controls (Fig. 79).

None of the samples contained enterococci or coliform organisms.

No excessive post-acidification occurred at this temperature since, during a storage period of 35 days, neither the pH of control yogurt samples nor that of cyanobacterial ones dropped below a value characteristic of such products. There was not much difference between control and cyanobacterial yogurts in this regard (Fig. 80).

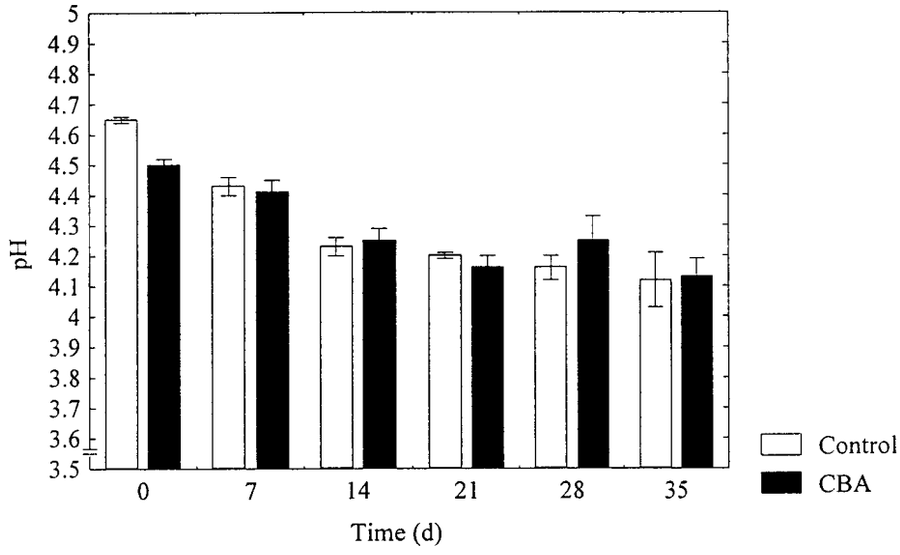


Fig. 80 Post-acidification of control and cyanobacterial yogurts stored at 4°C

The influence of the *Spir. platensis* biomass on growth of yeasts and molds in the product during storage at 4°C is illustrated in Fig. 81.

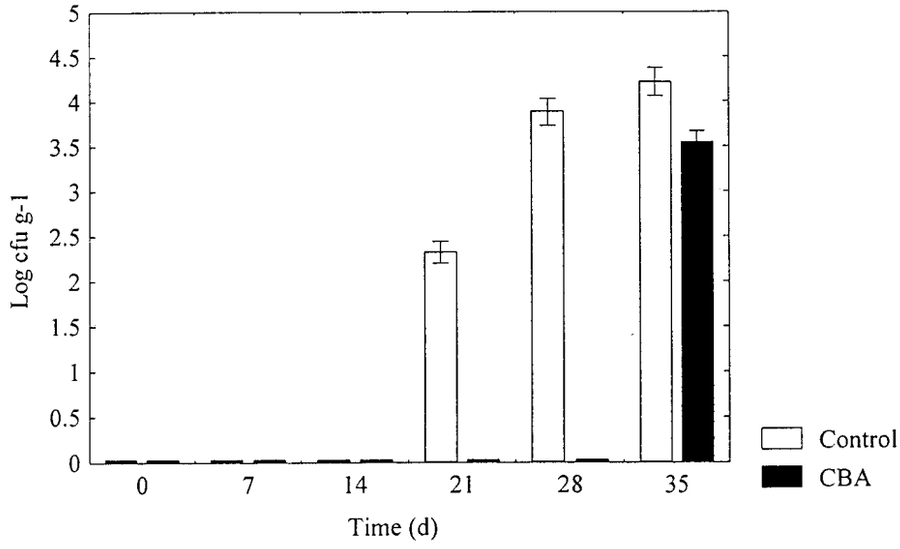


Fig. 81 Changes in the counts of yeasts and molds found in control and cyanobacterial yogurts stored at 4°C

On day 21 of the storage period, the count of yeasts and molds found in control yogurt samples slightly exceeded the value of 10^2 cfu g^{-1} whereas the cyanobacterial yogurt proved to have a shelf-life of 1 month.

The literature provides a satisfactory explanation of these findings. De Cano *et al.* (1990) tested phenolic compounds extracted from *Nostoc muscorum* cyanobacteria for antifungal properties. The cyanobacterial phenolic compounds were found to inhibit the growth of *Candida albicans* significantly (89.1%). Extracellular products were extracted from a wide range of cyanobacteria by De Caire *et al.* (1993). One third of the 36 strains tested proved to produce compounds which inhibited the growth of *C. albicans*. In another experiment by Miura *et al.* (1993), three out of ten *Chlorella* strains had an inhibitory effect on the growth of *Saccharomyces cerevisiae* under dark conditions. A light-activated compound responsible for the antimicrobial effect was isolated. Two polyhalogenated aromatic compounds extracted from the blue-green alga *Fischerella ambigua* were found to exhibit antibacterial activity against *Escherichia coli* and antifungal activity against *Penicillium oxalicum* (Falch *et al.* 1992). Falch *et al.* (1995)

tested 54 cyanobacterial extracts for antibacterial and antifungal properties, 46 of which were active against *Bacillus subtilis*, *E. coli* and/or *Micrococcus luteus* and 13 against *P. oxalicum*.

On the whole, the findings of these storage experiments are in line with those of Rohm *et al.* (1990), which were detailed in subchapter 2.7.

The properly stored (cooled) cyanobacterial yogurt was found to have a shelf-life of one month, despite the fact that it had been produced according to regular technology of manufacture.

5 CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

- The *Spir. platensis* biomass, which is rich in essential amino acids, trace elements, unsaturated fatty acids and vitamins, has a beneficial effect on the nutritional value of cow's milk.

- The cyanobacterial biomass significantly increases the rate of acid development by and growth rate of certain thermophilic dairy starter cultures (i.e. *Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus*, *Bifid. bifidum*).

- In the case of combinations of strains, the *Spir. platensis* biomass stimulates the rod-shaped starter bacteria to a greater extent than the coccus-shaped ones, but its effect on cocci is dependent on what kind of rod the cocci are combined with. In other words, the effect of the cyanobacterial biomass on mixed cultures also depends on the interaction between/among strains composing the mixed cultures.

- The stimulating effect of the *Spir. platensis* biomass on thermophilic dairy starter organisms is largely due to nitrogenous substances (i.e. free amino acids, hypoxanthine, adenine).

- In certain instances, the vitamin (B-complex, C, A, E) and trace element (iodine, zinc, selenium) contents of the cyanobacterial biomass may have a stimulatory or retardative effect on the above-mentioned starter bacteria. However, this is of slight importance from a practical point of view.

- The *Spir. platensis* biomass inhibits the growth of yeasts and molds contaminating the fermented dairy products and it maintains the count of characteristic micro-organisms at a high level provided that the product is stored at a low temperature.

5.2 Suggestions

The use of *Spir. platensis* biomass for the manufacture of fermented milk products can be recommended for various reasons.

Owing to its composition, the cyanobacterial biomass increases the trace element and vitamin contents and improves the fatty acid composition of cow's milk. However, these beneficial effects are largely dependent on the employed concentration of the biomass. The effective and economic concentration resulting in good sensory properties has been found to be 3 g l^{-1}.

The abundance of bioactive components in the *Spir. platensis* biomass is of paramount importance from a nutritional point of view because the cyanobacterial biomass thus creates a new opportunity for the manufacture of functional dairy products.

The vitamins and trace elements are also beneficial components of the *Spir. platensis* biomass in that they have either slight or no effect on acid development by the starter cultures, that is to say the presence of these bioactive substances does not affect adversely the cyanobacterial biomass.

The significant stimulatory effect of the *Spir. platensis* biomass on acid production (and growth) of thermophilic dairy starter cultures is of practical importance because thus shorter time is needed for the manufacture of the same amount of fermented milk and consequently productivity will improve. Besides, a rapid rate of acid production prevents the growth of undesirable micro-organisms and is also essential for texture and flavor. Stimulation of growth and acid production is extremely important in the case of *Bifid. bifidum* because this species grows, and produces acetic and lactic acids, very slowly in milk. Considering this, the fact that *Bifid. bifidum* Bb-12 is stimulated by the *Spir. platensis* biomass to a very high degree can be regarded as one of the major findings of this work.

The discovery that, in mixed cultures, the cyanobacterial biomass stimulates the rod-shaped starter organisms to a greater extent than the coccus-shaped ones offers a new way of ensuring the optimal ratio of cocci to rods in the finished product.

By inhibiting the growth of yeasts and molds which contaminate the fermented dairy products and maintaining the count of characteristic micro-organisms at a high level during storage, the *Spir. platensis* biomass extends the shelf-life of products stored at a low temperature (i.e. 4°C).

When the question of using the cyanobacterial biomass for the manufacture of fermented milk products is considered, the economic aspect of the issue must also be looked at. The current market price of the *Spir. platensis* biomass is 38-40 DM/kg (Vonshak 1997b and 1997c). If roughly 3 g of biomass was employed for the making of 1 kg of finished product, the cost of production would increase by 0.12 DM/kg. In Germany, the sales value of functional (probiotic) fermented milks was 5.25 DM/kg in 1995 and 5.40 DM/kg in 1997 (Heasman and Mellentin 1998) and it is estimated to be about 5.55 DM/kg in 1999. This means that the cost of the cyanobacterial biomass amounts to approximately 2% of the average selling price of probiotic fermented milks.

In several countries, consumers are wise enough to give credit to scientifically established and documented health claims. If dairy companies invest heavily in marketing communications, the market of functional products will grow dynamically year by year. A functional fermented milk, launched in the first half of the 1990s in Denmark, owing to its well-advertised health claims, became the biggest and fastest success ever of Denmark's top dairy producer, despite a 70% price premium over regular yogurt (Heasman and Mellentin 1998). Under such conditions, an increase of a couple of per cents in the cost of production cannot be regarded as significant and the functional product is supposed to gain a high market share provided that the company has a proper marketing strategy. If the *Spir. platensis* biomass is used for the manufacture of regular yogurt, instead of a probiotic fermented milk product, the increase in the cost of production is relatively higher but the beneficial effect of the cyanobacterial biomass can be better highlighted in this case and thus the cyanobacterial yogurt can also be made marketable. Despite all the foregoing, the most important point is that consumers should be well-informed about nutritional issues, they should also be eager for new products and be able to afford to buy goods at prices higher than usual.

The situation is somewhat different in Hungary because the market of functional dairy products is not so lively as in the European Union or in North America. People do not receive enough information about nutritional issues, that is the reason why some experts have their doubts as to whether the majority of consumers would be ready to buy functional fermented milks possessing specific health benefits. The addition of *Spir. platensis* biomass would increase the cost of production to a greater extent in Hungary than in Germany since the current selling price of yogurt in Hungary is about 2-2.5 DM/kg on average. However, certain fancy yogurts might as well cost 3.5-4 DM/kg. Calculations show that, in Hungary, the cost of the cyanobacterial biomass, employed at a concentration of 3 g l^{-1}, amounts to approximately 5-6% of the average market price of flavored yogurts. Therefore, the *Spir. platensis* biomass should be used for the manufacture of dairy products which cost more than yogurts because people who spend on fancy foods are likely to be able to afford cyanobacterial products. Moreover, it is essential that consumers should be kept informed about nutritional issues through advertisements and newspaper/magazine articles so that functional dairy products will sell well when the purchasing power of people starts to increase.

6 SUMMARY

The consumption of fermented dairy products shows an upward tendency world-wide. The importance of probiotic micro-organisms has recently come into prominence in several countries which have highly developed dairy production.

A simple way of further improving the high nutritional value of fermented dairy products is the use of *Spirulina platensis* cyanobacterial biomass enriched with trace elements for the manufacture of fermented milks.

Productivity is one of the main concerns of dairy technologists and scientists. According to German and Japanese authors, acid development by and growth rate of certain lactic acid bacteria can be stimulated by the addition of green algal extracts (Shirota *et al.* 1964; Stengel 1970; Zielke *et al.* 1978; Kurita *et al.* 1979; Webb 1982).

This work aimed to find out whether stimulation of thermophilic dairy starter cultures could be brought about by *Spir. platensis* biomass and identify the substances responsible for the effects observed. The effect of 3 g l^{-1} *Spir. platensis* biomass enriched with trace elements (iodine, zinc, selenium) on the rate of acid development by and growth rate of pure and synchronized mixed cultures of *Streptococcus salivarius* subsp. *thermophilus* CH-1, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2, *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 was evaluated in a model milk medium. The components of the cyanobacterial biomass responsible for the stimulation caused were also identified in laboratory simulations wherein trace elements (iodine, zinc, selenium), vitamins (B-complex, C, A, E) and nitrogenous compounds (peptone, adenine, hypoxanthine) were tested.

As for the experiments done with single strains, the rate of inoculation was 1% (v/v) except for *Bifid. bifidum* (6%, v/v). *Strep. thermophilus* and *Lact. bulgaricus* were incubated at 42.5°C whereas *Lact. acidophilus* and *Bifid. bifidum* at 37.5°C. Acid development was checked by hourly pH measurements. All the experimental results shown in this Dissertation are means of 3 trials (n=3).

It could be concluded that addition of the cyanobacterial biomass resulted in increasing the rate of acid development by all four starter culture strains significantly, although to varying degrees.

The *Spir. platensis* biomass enhanced acid development by *Strep. thermophilus* significantly during hours 2-5 of the fermentation process. This increase in acid production was partly due to the presence of trace elements and to a greater extent to that of nitrogenous compounds (peptone, adenine, hypoxanthine). The addition of vitamins C, A and E, in which cyanobacteria are abundant, also resulted in a considerable drop in pH.

The cyanobacterial biomass stimulated acid production of *Lact. bulgaricus* to a greater extent than that of *Strep. thermophilus*. The increase in the rate of acid development during the main phase of fermentation could be attributed to the additive effect of nitrogenous substances (peptone, adenine, hypoxanthine). The acid tolerance of this moderate acid-producing strain was substantially improved by the cyanobacterial biomass, which could be partly accounted for by the stimulating effect of vitamin C on fermentation activity during the stationary phase of fermentation. The addition of inorganic selenium resulted in a small, although significant decrease in the rate of acid development.

Acid production of *Lact. acidophilus* was also stimulated significantly by the *Spir. platensis* biomass. Nitrogenous compounds (mainly peptone) and vitamin C were found to be the most stimulatory of all the individual substrates tested whereas the inhibitory effect of selenium and that of vitamin E were clearly visible too; i.e. among the antioxidants preventing membrane lipid peroxidation by free radicals, only vitamin C proved to stimulate acid production of *Lact. acidophilus* while the rest of them (vitamins A, E and selenium) retarded it to some extent. The B-complex vitamins reduced the rate of acid production as well.

Most of the substrates tested had similar effects on *Bifid. bifidum* and on *Lact. acidophilus*, although the two species differ widely in some of their major characteristics, including their metabolic system. However, adenine and hypoxanthine had an adverse effect or no effect at all on acid production of *Bifid. bifidum*. The

influence of adenine employed in combination with peptone proved to be stimulatory on acid production, which was in contrast to what had been experienced when adenine being tested alone. Peptone was the only substance to stimulate acid development by *Bifid. bifidum* significantly but this stimulation did not account for the one caused by the cyanobacterial biomass. The *Spir. platensis* biomass must have also contained other effective components than peptone.

Further experiments were carried out in order to reveal the effect of the cyanobacterial biomass on combinations of the above-mentioned single strains. Preliminary experiments had been conducted so that the levels of inoculation giving approximately the same counts of colony forming units of starter culture organisms at the end of the fermentation process could be determined. The rate of inoculation was thus between 0.1% (v/v) and 6.0% (v/v) with respect to the single strains. The mixed culture of *Strep. thermophilus* and *Lact. bulgaricus* was incubated at 42.5°C whereas the combinations of strains containing *Lact. acidophilus* and/or *Bifid. bifidum* at 37.5°C. Acid production was determined by hourly pH measurements and growth was checked by enumeration of micro-organisms.

The effect of the cyanobacterial biomass on the combinations of strains could only partly be accounted for by what had been experienced with the single strains because the combinations of strains formed more complex systems than the individual strains.

The cyanobacterial biomass did not always have the same effect on the same strain in various mixed cultures. This is well illustrated by the example of *Strep. thermophilus*. As regards the mixed culture of *Strep. thermophilus* and *Lact. bulgaricus*, the *Spir. platensis* biomass had a significant stimulatory effect on acid development but it did not influence the growth of *Strep. thermohilus*. In the case of the combination of *Strep. thermophilus* and *Lact. acidophilus*, the cyanobacterial biomass stimulated both acid production and the growth rate of *Strep. thermophilus*. As for the mixed culture of *Strep. thermophilus* and *Bifid. bifidum*, neither acid production nor the growth of *Strep. thermophilus* was affected by the *Spir. platensis* biomass.

It was the mixed cultures containing *Lact. bulgaricus* or *Lact. acidophilus* that the cyanobacterial biomass had the most stimulatory effect on. The growth rate of these two

strains was accelerated in each case and *Bifid. bifidum* was also stimulated whenever it was combined with a rod-shaped starter organism (i.e. *Lact. acidophilus*). As to the mixed culture of *Strep. thermophilus*, *Lact. acidophilus* and *Bifid. bifidum*, the growth of *Bifid. bifidum* was found to be significantly faster in the cyanobacterial samples than in controls. Since such an effect was not observed in the case of the combination of *Strep. thermophilus* and *Bifid. bifidum*, it is supposed that the growth rate of *Bifid. bifidum* was accelerated by certain metabolic products of *Lact. acidophilus* and/or by some specific nutrients found in the *Spir. platensis* biomass.

On the whole, the cyanobacterial biomass stimulated the rod-shaped starter bacteria to a greater extent than the coccus-shaped ones, but its effect on cocci was largely dependent on what kind of rod the cocci were combined with. In other words, the effect of the *Spir. platensis* biomass on mixed cultures also depended on the interaction between/among strains composing the mixed cultures.

Thereafter storage experiments were performed to reveal the changes in the characteristic and undesirable microbial flora of cyanobacterial and control yogurts produced according to regular technology of manufacture. The samples were stored for 35 days at 4°C and 15 days at 15°C. Along with the microbiological investigations, the pH of samples was measured so that data concerning the degree of post-acidification would also be gained.

Characteristic viable cell counts of over 10^8 cfu g^{-1} were found both in control and cyanobacterial yogurts, regardless of storage temperature. However, the viable cell counts were significantly higher in the cyanobacterial samples than in controls at 4°C.

The storage temperature of 15°C resulted in considerable post-acidification in control and cyanobacterial yogurts alike while the pH of all the samples stored at 4°C remained above 4.0 during the entire storage period.

The count of yeasts and molds, both in the cyanobacterial and control samples, rose to a level of 10^1 cfu g^{-1} by the 6^{th} day and to a level of 10^5 cfu g^{-1} by the 15^{th} day of the storage period at 15°C; whereas the cyanobacterial yogurt, after one month of storage at 4°C, had a significantly lower count of yeasts and molds than the control

yogurt. These results suggest that substance(s) possessing fungistatic properties can be found in the *Spir. platensis* biomass.

None of the samples contained enterococci or coliform organisms at either temperature during the storage period.

All things considered, the properly stored (cooled) cyanobacterial yogurt was found to have a shelf-life of one month, despite the fact that it had been produced according to regular technology of manufacture.

On the basis of the results obtained, the following conclusions can be drawn.

The *Spir. platensis* biomass, which is rich in essential amino acids, trace elements, unsaturated fatty acids and vitamins, has a beneficial effect on the nutritional value of cow's milk.

The cyanobacterial biomass significantly increases the rate of acid development by and growth rate of certain thermophilic dairy starter cultures (i.e. *Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus*, *Bifid. bifidum*).

In the case of combinations of strains, the *Spir. platensis* biomass stimulates the rod-shaped starter bacteria to a greater extent than the coccus-shaped ones, but its effect on cocci is dependent on what kind of rod the cocci are combined with. In other words, the effect of the cyanobacterial biomass on mixed cultures also depends on the interaction between/among strains composing the mixed cultures.

The stimulating effect of the *Spir. platensis* biomass on thermophilic dairy starter organisms is largely due to nitrogenous substances (i.e. free amino acids, hypoxanthine, adenine).

In certain instances, the vitamin (B-complex, C, A, E) and trace element (iodine, zinc, selenium) contents of the cyanobacterial biomass may have a stimulatory or retardative effect on the above-mentioned starter bacteria. However, this is of slight importance from a practical point of view.

The *Spir. platensis* biomass inhibits the growth of yeasts and molds contaminating the fermented dairy products and it maintains the count of characteristic micro-organisms at a high level provided that the product is stored at a low temperature.

The use of *Spir. platensis* biomass for the manufacture of fermented milk products can be recommended for various reasons.

Owing to its composition, the cyanobacterial biomass increases the trace element and vitamin contents and improves the fatty acid composition of cow's milk. However, these beneficial effects are largely dependent on the employed concentration of the biomass. The effective and economic concentration resulting in good sensory properties has been found to be 3 g l^{-1}.

The abundance of bioactive components in the *Spir. platensis* biomass is of great importance from a nutritional point of view because the cyanobacterial biomass thus creates a new opportunity for the manufacture of functional dairy products.

The vitamins and trace elements are also beneficial components of the *Spir. platensis* biomass in that they have either slight or no effect on acid development by the starter cultures, that is to say the presence of these bioactive substances does not affect adversely the cyanobacterial biomass.

The significant stimulatory effect of the *Spir. platensis* biomass on acid production (and growth) of thermophilic dairy starter cultures is of practical importance because thus shorter time is needed for the manufacture of the same amount of fermented milk and consequently productivity will improve. Besides, a rapid rate of acid production prevents the growth of undesirable micro-organisms and is also essential for texture and flavor. Stimulation of growth and acid production is extremely important in the case of *Bifid. bifidum* because this species grows, and produces acetic and lactic acids, very slowly in milk. Considering this, the fact that *Bifid. bifidum* Bb-12 is stimulated by the *Spir. platensis* biomass to a very high degree can be regarded as one of the major findings of this work.

The discovery that, in mixed cultures, the cyanobacterial biomass stimulates the rod-shaped starter organisms to a greater extent than the coccus-shaped ones offers a new way of ensuring the optimal ratio of cocci to rods in the finished product.

By inhibiting the growth of yeasts and molds which contaminate the fermented dairy products and maintaining the count of characteristic micro-organisms at a high

level during storage, the *Spir. platensis* biomass extends the shelf-life of products stored at a low temperature (i.e. 4°C).

As to the economic aspect of the issue, the current market price of the *Spir. platensis* biomass is 38-40 DM/kg (Vonshak 1997b and 1997c). If roughly 3 g of biomass was employed for the making of 1 kg of finished product, the cost of production would increase by 0.12 DM/kg. The market of functional dairy products is not so lively in Hungary as in the European Union or in North America. People do not receive enough information about nutritional issues, that is the reason why some experts have their doubts as to whether the majority of Hungarian consumers would be ready to buy functional fermented milks possessing specific health benefits. The use of *Spir. platensis* biomass would increase the cost of yogurt production to a greater extent in Hungary than in Germany, for instance, since the selling price of yogurt is considerably lower in Hungary than in Germany. Calculations show that, in Hungary, the cost of the cyanobacterial biomass, employed at a concentration of 3 g l^{-1}, amounts to approximately 5-6% of the average market price of flavored yogurts. Therefore, the *Spir. platensis* biomass should be used for the manufacture of dairy products which cost more than yogurts because people who spend on fancy foods are likely to be able to afford cyanobacterial products. Moreover, it is essential that consumers should be kept informed about nutritional issues through advertisements and newspaper/magazine articles so that functional dairy products will sell well when the purchasing power of people starts to increase.

7 ACKNOWLEDGMENTS

I wish to thank Dr. Dr. h. c. János Iváncsics for providing me with the opportunity to join in the Ph.D. Main Program supervised by him.

I am extremely grateful to Dr. habil. Jenő Szigeti for his guidance, suggestions, invaluable comments and for critically reviewing the manuscript of this Dissertation.

Thanks are due to the staff at the Institute of Food Science (Pannon University of Agricultural Sciences, Faculty of Agricultural Sciences at Mosonmagyaróvár) for their excellent technical assistance in performing all the experiments. Special thanks are extended to Mr. Ferenc Németh and Mrs. Árpád Novák for laboratory assistance and to Dr. Zsolt Ajtony for advice on statistics.

The constructive criticism of certain parts of the manuscript by Dr. Vince Ördög is gratefully acknowledged.

I also thank the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár and the Institute of Cereal Processing Inc. (Institut für Getreideverarbeitung GmbH) at Bergholz-Rehbrücke (Germany) for the generous provision of bacterial cultures and cyanobacterial biomass respectively.

Additional thanks are due to all the professors, particularly Dr. habil. Sándor Szakály, Dr. habil. József Fenyvessy, Dr. Ádám Krász and Dr. György Babella, who taught me subjects related to Dairy Science.

Appreciation is extended to my family (wife, parents and brother) for supporting and encouraging me throughout my studies.

And last but not least I am very grateful to Ms. Anikó Szegedi for showing me the beauty of English during my high school years.

8 REFERENCES

Agerbaek, M., Gerdes, L.V. and Richelsen, B. (1995) Hypocholesterolaemic effect of a new fermented milk product in healthy middle-aged men. *European Journal of Clinical Nutrition* **49**, 346-352.

Aggett, P.J. (1989) Severe zinc deficiency. In *Zinc in Human Biology* ed. Mills, C.F. pp. 259-280. London: Springer-Verlag.

Aggett, P.J. and Comerford, J.G. (1995) Zinc and human health. *Nutrition Reviews* **53** (9), (II)S16-S22.

Agrawal, V., Usha, M.S. and Mital, B.K. (1986) Preparation and evaluation of acidophilus milk. *Asian Journal of Dairy Research* **5**, 33-38.

Ahmed, B., Mital, B.K. and Garg, S.K. (1990) Effect of magnesium and manganese ions on the growth of *Lactobacillus acidophilus*. *Journal of Food Science and Technology* **27**, 228-229.

Alm, L. (1982) Effect of fermentation on B-vitamin content of milk in Sweden. *Journal of Dairy Science* **65**, 353-359.

Alm, L. (1991) The therapeutic effects of various cultures — an overview. In *Therapeutic Properties of Fermented Milks* ed. Robinson, R.K. pp. 45-64. London: Elsevier Applied Science.

Alm, L., Ryd-Kjellen, E., Setterberg, G. and Blomquist, L. (1993) Effect of a new fermented milk product "Cultura" on constipation in geriatric patients. *1st Lactic Acid Bacteria Computer Conference*, Stockholm, May 1 - June 30, 4 pp.

Anderson, A.W. and Elliker, P.R. (1953) The nutritional requirements of lactic streptococci isolated from starter cultures. II. A stimulatory factor required for rapid growth of some strains in reconstituted nonfat milk solids. *Journal of Dairy Science* **36**, 608-613.

Anke, M., Groppel, E. and Scholz, E. (1993) Iodine in the food chain. In *Trace Elements in Man and Animals* (TEMA 8, Proceedings of the 8th International Symposium on Trace Elements in Man and Animals) ed. Anke, M., Meissner, D. and Mills, C.F. pp. 1049-1053. Gersdorf: Verlag Media Touristik.

Anon. (1995) *Chr. Hansen's Nu-trish™ Cultures*. 34 pp. Hørsholm: DotZero.

Ayebo, A.D. and Shahani, K.M. (1980) Role of cultured dairy products in the diet. *Cultured Dairy Products Journal* **15** (4), 21-29.

Badran, I.I. and Reichart, O. (1995) Studies on the growth and acid production of pure and mixed cultures of *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. *Acta Alimentaria* **24**, 277-287.

Ballongue, J. (1993) Bifidobacteria and probiotic action. In *Lactic Acid Bacteria* ed. Salminen, S. and von Wright, A. pp. 357-428. New York: Marcel Dekker Inc.

Balloni, W., Tomaselli, L., Giovannetti, L. and Margheri, M.C. (1980) Biologia fondamentale del genere *Spirulina*. In *Prospettive della Coltura di Spirulina in Italia* ed. Materassi, R. p. 49. Roma: CNR.

Baril, T.S. and Cardwell, J.T. (1984) The effect of UHT milk on viable cell count and organoleptic qualities of unflavored yoghurt. *Journal of Dairy Science* **67**, 227-228.

Bateup, J.M., McConnell, M.A., Jenkinson, H.F. and Tannock, G.W. (1995) Comparison of *Lactobacillus* strains with respect to bile salt hydrolase activity, colonisation of the gastrointestinal tract and growth rate of the murine host. *Applied and Environmental Microbiology* **61**, 1147-1149.

Belay, A. (1997) Mass culture of *Spirulina* outdoors – the Earthrise Farms experience. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 131-158. London: Taylor & Francis Ltd.

Black, F.T., Andersen, P.L., Ørskov, J., Ørskov, F., Gaarslev, K. and Laulund, S. (1989) Prophylactic efficacy of lactobacilli on traveler's diarrhea. *Travel Medicine* 333-335.

Black, F.T., Einarsson, K., Lidbeck, A., Orrhage, K. and Nord, C.E. (1991) Effect of lactic acid producing bacteria on the human intestinal microflora during ampicillin treatment. *Scandinavian Journal of Infectious Diseases* **23**, 247-254.

Black, F.T., Gaarslev, K., Ørskov, F., Ørskov, J., Stenderup, A., Stenderup, J. and Christensen, J. (1983) Mecillinam, a new prophylactic for travellers' diarrhoea. *Scandinavian Journal of Infectious Diseases* **15**, 189-193.

Blanc, B. (1974) Der Wert der Sauermilchprodukte in der modernen Ernährung. *Deutsche Milchwirtschaft* **25**, 1030-1032, 1057-1062.

Bouhnik, Y. (1993) Survie et effets chez l'homme des bactéries ingérées dans les laits fermentés. *Le Lait* **73**, 241-247.

Bourlioux, P. and Pochart, P. (1988) Nutritional and health properties of yogurt. *World Review of Nutrition and Dietetics* **56**, 217-258.

Bundeskanzleramt (1990) Kapitel B 12 "Milch und Milchprodukte". Teilkapitel "Milchmischerzeugnisse". *Österreichisches Lebensmittelbuch* (III. Auflage). 16 pp.

Buttriss, J. (1997) Nutritional properties of fermented milk products. *International Journal of Dairy Technology* **50** (1), 21-27.

Bylund, G. (1995) Recent developments in cultured milk products. In *Dairy Processing Handbook* ed. Teknotext AB pp. 261-262. Lund: Tetra Pak Processing Systems AB.

Campbell, D.M. (1988) Trace element needs in human pregnancy. *Proceedings of the Nutrition Society* **47**, 45-53.

Carminati, D., Brizzi, A., Giraffa, G. and Neviani, E. (1994) Effects of amino acids on *Streptococcus salivarius* subsp. *thermophilus* growth in modified milk deprived of non-protein nitrogen fraction. *Milchwissenschaft* **49**, 486-489.

Carminati, D., Giraffa, G., Gatti, M. and Neviani, E. (1993) Characterization of *Streptococcus thermophilus* to be used in dairy technology. In *Proceedings of the Symposium on Biotechnology and Molecular Biology of Lactic Acid Bacteria for the Improvement of Foods and Feeds Quality* ed. Zamorani, A., Manachini, P.L., Bottazzi, V. and Coppola, S. pp. 116-126. Roma: Istituto Poligrafico e Zecca dello Stato.

Casey, C.E. (1988) Selenophilia. *Proceedings of the Nutrition Society* **47**, 55-62.

Černá, J., Picková, J. and Blattná, J. (1972) Effect of dairy cultures on the contents of vitamins in milk. *Dairy Science Abstracts* **35**, 413.

Chandra, R.K., Hambræus, L., Puri, S., Au, B. and Kutty, K.M. (1993) Immune responses of healthy volunteers given supplements of zinc or selenium. *FASEB Journal* 7, A723.

Citti, J.E., Sandine, W.E. and Elliker, P.R. (1965) Comparison of slow and fast acid-producing *Streptococcus lactis*. *Journal of Dairy Science* **48**, 14-18.

Cohen, Z. (1997) The chemicals of *Spirulina*. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 175-204. London: Taylor & Francis Ltd.

Coolbear, T., Pillidge, C.J. and Crow, V.L. (1994) The diversity of potential cheese ripening characteristics of lactic acid starter bacteria: 1. Resistance to cell lysis and levels and cellular distribution of proteinase activities. *International Dairy Journal* **4**, 697-721.

Crow, V.L., Holland, R., Pritchard, G.G. and Coolbear, T. (1994) The diversity of potential cheese ripening characteristics of lactic acid starter bacteria: 2. The levels and subcellular distributions of peptidase and esterase activities. *International Dairy Journal* **4**, 723-742.

Davis, J.G. (1970) Dietary or health yoghurt. *Dairy Industries* **35**, 827-830.

De Caire, G.Z., De Cano, M.M.S., De Mule, M.C.Z. and De Halperin, D.R. (1993) Screening of cyanobacterial bioactive compounds against human pathogens. *Phyton Buenos Aires* **54** (1), 59-65.

De Cano, M.M.S., De Mule, M.C.Z., De Caire, G.Z. and De Halperin, D.R. (1990) Inhibition of *Candida albicans* and *Staphylococcus aureus* by phenolic compounds from the terrestrial cyanobacterium *Nostoc muscorum*. *Journal of Applied Phycology* **2** (1), 79-81.

Deeth, H.C. and Tamime, A.Y. (1981) Yoghurt: nutritive and therapeutic aspects. *Journal of Food Protection* **44**, 78-86.

Dellaglio, F., Torriani, S., Vlaeminck, G. and Cornet, R. (1992) Specific characteristics of micro-organisms used for new fermented milks. In *Bulletin of the International Dairy Federation* No. 277, 4-16.

Desmazeaud, M. (1988) Metabolismo dei batteri lattici termofili. In *Yogurt e i Latti Fermentati.* Atti del Convegno Internazionale in collaborazione con INTERLAB, Thiene 25 maggio, pp. 6-37.

Desmazeaud, M.J. (1994) Le lait milieu de culture. In *Bactérie Lactique* (vol. 2) ed. De Roissart, H. and Luquet, F.M. pp. 25-36. Uriage: Lorica.

Desmazeaud, M.J. and Juge, M. (1976) Caractérisation de l'activité protéolytique et fractionnement des dipeptidases et des aminopeptidases de *Streptococcus thermophilus. Le Lait* **56**, 241-260.

De Smet, I., Van Horde, L., De Saeyer, N., Van de Woestyne, M. and Verstraete, M. (1994) In vitro study of bile salt hydrolase (BSH) activity of BSH isogenic *Lactobacillus plantarum* 80 strains and estimation of cholesterol lowering through enhanced BSH activity. *Microbial Ecology in Health and Disease* 7, 315-329.

Driessen, F.M. and DeBoer, R. (1989) Fermented milks with selected intestinal bacteria: a healthy trend in new products. *Netherlands Milk and Dairy Journal* **43**, 367-382.

Driessen, F.M., Kingma, F. and Stadhouders, J. (1982) Evidence that *Lactobacillus bulgaricus* in yogurt is stimulated by carbon dioxide produced by *Streptococcus thermophilus. Netherlands Milk and Dairy Journal* **36**, 135-144.

DuPont, H.L., Ericsson, C.D., Johnson, P.C. and Cabada, F.J. (1986) Antimicrobial agents in the prevention of travelers' diarrhea. *Review of Infectious Diseases* (Suppl. 2) **8**, 167-171.

Egli, F. (1980) Production of sterile yoghurt of long storage life — containing modifying additive composing of agar, starch, gelatin and sugar. *Patent* No. US 4235934 A.

Falch, B.S., König, G.M. and Wright, A.D. (1992) Antibacterial and cytotoxic compounds from the blue-green alga *Fischerella ambigua. Planta Medica* **58**, A654-A655.

Falch, B.S., König, G.M., Wright, A.D., Sticher, O., Angerhofer, C.K. and Pezzuto, J.M. (1995) Biological activities of cyanobacteria: evaluation of extracts and pure compounds. *Planta Medica* **61**, 321-328.

Foissy, H. (1983) Ernährungsphysiologische Aspekte von Joghurt. In *Milchwirtschaftliche Berichte aus den Bundesanstalten Wolfpassing und Rotholz* No. 75, pp. 137-142.

Friend, B.A., Fiedler, J.M. and Shahani, K.M. (1983) Influence of culture selection on the flavor, antimicrobial activity, β-galactosidase and B-vitamins of yogurt. *Milchwissenschaft* **38**, 133-136.

Gerome, J., Pirlet, J., Steils, A. and Wats, F. (1994) Drink containing semi-skimmed milk and concentrate including vitamin(s) etc. — is suitable for the elderly and provides at least 50 per cent of essential nutrients whilst avoiding excessive intake. *Derwent Information Ltd.* WO 9405159 A2, 25 pp.

Gilliland, S.E. (1975) In *Bacterial Starter Cultures for Foods* p. 177. Boca Raton: CRC Press Inc.

Gilliland, S.E. (1989) Acidophilus milk products: a review of potential benefits to consumers. *Journal of Dairy Science* **72**, 2483-2494.

Gilliland, S.E. and Speck, M.L. (1969) Biological response of lactic streptococci and lactobacilli to catalase. *Applied Microbiology* **17**, 797-800.

Gilliland, S.E. and Walker, D.K. (1990) Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. *Journal of Dairy Science* **73**, 905-911.

Gläser, H. (1992) Living bacteria in yoghurt and other fermented milk products. *European Dairy Magazine* **4** (1), 6-15.

Glass, L. and Hedrick, T.I. (1976) Bacterial growth and vitamin content of milk. *Journal of Milk and Food Technology* **39**, 325-327.

Goh, J.S., Kwon, I.K., Ahn, J.K. and Yoon, Y.H. (1987) Fermentation of milk by bifidobacteria, and improvement of usability of *Bifidobacterium bifidum* ATCC 11863 by lactic acid bacteria for the fermentation of milk. *Korean Journal of Dairy Science* **9**, 211-219.

Golden, M.H. (1989) The diagnosis of zinc deficiency. In *Zinc in Human Biology* ed. Mills, C.F. pp. 323-333. London: Springer-Verlag.

Gorbach, S.L. (1990) Lactic acid bacteria and human health. *Annals of Medicine* **22**, 37-41.

Gurr, M.I. (1987) Nutritional aspects of fermented milk products. *FEMS Microbiology Reviews* **46**, 337-342.

Gutknecht, K.W. (1992) Microbes confer healthful benefits on ice cream & yogurt. *Utah Science* **52** (4), 134-135.

György, P., Kuhn, R., Rose, C.S. and Zilliken, F. (1954a) Bifidus factor. II. Its occurrence in milk from different species and in other natural products. *Archives of Biochemistry and Biophysics* **48**, 202-208.

György, P., Norris, R.F. and Rose, C.S. (1954b) Bifidus factor. I. A variant of *Lactobacillus bifidus* requiring a special growth factor. *Archives of Biochemistry and Biophysics* **48**, 193-201.

Halfhide, B. (1994) Yogurt: single strains combine. *Dairy Industries International* **59** (3), 35-37.

Hambidge, K.M. (1988) Assessing the trace element status of man. *Proceedings of the Nutrition Society* **47**, 37-44.

Hambidge, K.M. (1989) Mild zinc deficiency in human subjects. In *Zinc in Human Biology* ed. Mills, C.F. pp. 281-296. London: Springer-Verlag.

Hamilton-Miller, J.M.T. (1996) Probiotics — panacea or nostrum? In *BNF Nutrition Bulletin* No. 79, vol. 21, pp. 199-203. London: British Nutrition Foundation.

Harriman, L.A. and Hammer, B.W. (1931) Variations in the coagulation and proteolysis of milk by *Streptococcus lactis*. *Journal of Dairy Science* **14**, 40-49.

Hartman, A.M. and Dryden, L.P. (1974) The vitamins in milk and milk products. In *Fundamentals of Dairy Chemistry* (2nd edition) ed. Webb, B.H., Johnson, A.H. and Alford, J.A. pp. 325-401. Westport: AVI Publishing Co. Inc.

Hatcher, G.E. and Lambrecht, R.S. (1993) Augmentation of macrophage phagocytic activity by cell-free extracts of selected lactic acid-producing bacteria. *Journal of Dairy Science* **76**, 2485-2492.

Heasman, M. and Mellentin, J. (1998) Battling for Europe's gut. In *International Food Ingredients* No. 4, pp. 40-41.

Hegóczki, J., Janzsó, B., Suhajda, Á. and Vereczkey, G. (1997) Krómos, szilíciumos és titános élesztők — Új típusú mikroelemforrások. *Élelmezési Ipar* **51**, 50-55.

Helmeczi, B. (1994) Az algatermesztés és -felhasználás kérdései. In *Mezőgazdasági Mikrobiológia* ed. Helmeczi, B. pp. 342-350. Budapest: Mezőgazda Kiadó.

Hewitt, D. and Bancroft, H.J. (1985) Nutritional value of yogurt. *Journal of Dairy Research* **52**, 197-207.

Higashio, K., Yoshioka, Y. and Kikuchi, T. (1977) Symbiosis in yogurt culture. 1. Isolation and identification of a growth factor for *Streptococcus thermophilus* produced by *Lactobacillus bulgaricus*. *Journal of the Agricultural Chemical Society of Japan* **51**, 203-208.

Hood, S.K. and Zottola, E.A. (1989) An electron microscopic study of the adherence of *Lactobacillus acidophilus* to human intestinal cells in vitro. *Food Microstructure* **8**, 91-97.

Hooper, P.L., Visconti, L., Garry, P.J. and Johnson, G.E. (1980) Zinc lowers high-density lipoprotein-cholesterol levels. *JAMA* **244**, 1960-1961.

Huhtanen, C.N. and Williams, W.L. (1963) Factors which increase acid production in milk by lactobacilli. *Applied Microbiology* **11**, 20-22.

Hunger, W. (1989) Bifidobakterien und *Lactobacillus acidophilus* für Sauermilchprodukte. *Deutsche Molkerei Zeitung* **10**, 1178-1185.

Iizuka, T., Takeuchi, D., Uehara, K., Kenichi, U., Taizo, T. and Tokio, I. (1994) Food materials, used as additive — are marine microalgae containing docosahexaenoic acid. *Derwent Information Ltd.* EP 622027 A2, 12 pp.

International Dairy Federation (1983) Cultured dairy foods in human nutrition. In *Bulletin of the International Dairy Federation* No. 159, pp. 1-28.

International Dairy Federation (1985) Milk and milk products. Enumeration of coliforms. Colony count technique and most probable number technique at 30°C. *International IDF Standard* No. 73A, 8 pp.

International Dairy Federation (1988) Yogurt. Enumeration of characteristic micro-organisms. Colony count technique at 37°C. *International IDF Standard* No. 117A, 4 pp.

International Dairy Federation (1990) Enumeration of yeasts and moulds. Colony count technique at 25°C. *International IDF Standard* No. 94B, 2 pp.

International Dairy Federation (1991a) Butter, fermented milks and fresh cheese. Enumeration of contaminating micro-organisms. Colony count technique at 30°C. *International IDF Standard* No. 153, 3 pp.

International Dairy Federation (1991b) Cultured dairy products in human nutrition. In *Bulletin of the International Dairy Federation* No. 255, pp. 1-23.

Ip, C. (1985) Selenium inhibitions of chemical carcinogenesis. *Federation Proceedings* **44**, 2573-2578.

Ip, C. and Sinha, D.K. (1981) Enhancement of mammary tumorigenesis by dietary selenium deficiency in rats with high polyunsaturated fat intake. *Cancer Research* **41**, 31-34.

Ito, M., Deguchi, Y., Miyamori, A., Matsumoto, K., Kikuchi, H., Kobayashi, Y., Yajima, T. and Kan, T. (1990) Effects of administration of galacto-oligosaccharides on the human faecal microflora, stool weight and abdominal sensation. *Microbial Ecology in Health and Disease* **3**, 285-292.

Janzsó, B., Suhajda, Á., Hegóczki, J. and Pais, I. (1995) Mikroelemekkel dúsított élesztők előállítása és alkalmazása. *Élelmezési Ipar* **49**, 201-204.

Johnson, M.C., Ray, B. and Bhowmik, T. (1987) Selection of *Lactobacillus acidophilus* strains for use in "acidophilus products". *Antonie van Leeuwenhoek* **53**, 215-231.

Kawaguchi, J., Nakamura, S. and Nagahara, H. (1989) Emulsion stabilizer for milk drink. *Patent Abstracts of Japan* JP 01157365 A. Unexamined Applications, Section C, Sect. No. 637, vol. 13, No. 419, p. 51.

Keeling, P.W.N., O'Day, J.O., Ruse, W. and Thompson, R.P.H. (1982) Zinc deficiency and photoreceptor dysfunction in chronic liver disease. *Clinical Science* **62**, 109-111.

Kennedy, H.E. and Speck, M.L. (1954) Studies on corn steep liquor in the nutrition of certain lactic acid bacteria. *Journal of Dairy Science* **37**, 635.

Kennedy, H.E. and Speck, M.L. (1955) Studies on corn steep liquor in the nutrition of certain lactic acid bacteria. *Journal of Dairy Science* **38**, 208-216.

Kennedy, H.E., Speck, M.L. and Aurand, L.W. (1955) Studies on a growth stimulant from corn steep using *Lactobacillus casei*. *Journal of Bacteriology* **70**, 70-77.

Keshan Disease Research Group (1979) Epidemiological studies on the etiologic relationship of selenium and Keshan disease. *Chinese Medical Journal* **92**, 477-482.

Kessler, H.G. (1988a) Erhitzen und Auswirkungen. In *Lebensmittel- und Bioverfahrenstechnik – Molkereitechnologie* ed. Kessler, H.G. pp. 132-195. Freising: Verlag A. Kessler.

Kessler, H.G. (1988b) Technologie der Sauermilchprodukte – Milcherzeugnisse und Hydrokolloidanwendung. In *Lebensmittel- und Bioverfahrenstechnik – Molkereitechnologie* ed. Kessler, H.G. pp. 404-426. Freising: Verlag A. Kessler.

Kieffer, F. (1991) Metals as essential trace elements for plants, animals, and humans. In *Metals and Their Compounds in the Environment — Occurrence, Analysis, and Biological Relevance* ed. Merian, E. pp. 481-489. Weinheim, New York, Basel, Cambridge: VCH.

Kim, H.S. (1990) Fermentierte Milch und Gesundheit. Primäre und Sekundäre Wirkungen. *DMZ-Lebensmittelindustrie und Milchwirtschaft* **111**, 208-217.

Kim, H.Y., Bae, H.S. and Baek, Y.J. (1991) In vivo antitumor effects of lactic acid bacteria on sarcoma 180 and mouse Lewis lung carcinoma. *Journal of Korean Cancer Association* **23**, 188-195.

Kirchgessner, M. and Roth, H.P. (1983) Nutritional influence of zinc on the activity of enzymes and hormones. In *Metal Ions in Biological Systems* ed. Sigel, H. pp. 363-413. New York: Marcel Dekker Inc.

Klaenhammer, T.R. (1982) Microbiological considerations in selection and preparation of *Lactobacillus* strains for use as dietary adjuncts. *Journal of Dairy Science* **65**, 1339-1349.

Klaver, F.A.M., Kingma, F. and Weerkamp, A.H. (1993) Growth and survival of bifidobacteria in milk. *Netherlands Milk and Dairy Journal* **47**, 151-164.

Klaver, F.A.M. and Van der Meer, R. (1993) The assumed assimilation of cholesterol by lactobacilli and *Bifidobacterium bifidum* is due to their bile salt deconjugating activity. *Applied and Environmental Microbiology* **59**, 1120-1124.

Kneifel, W. (1989) Abbau und Synthese von Vitaminen während der Fermentation von Sauermilchprodukten. In *Milchwirtschaftliche Berichte aus den Bundesanstalten Wolfpassing und Rotholz* No. 99, pp. 110-116.

Kneifel, W., Holub, S. and Wirthmann, M. (1989) Monitoring of B-complex vitamins in yogurt during fermentation. *Journal of Dairy Research* **56**, 651-656.

Koburger, J.A., Speck, M.L. and Aurand, L.W. (1963) Identification of growth stimulants for *Streptococcus lactis*. *Journal of Bacteriology* **85**, 1051-1055.

Kon, S.K. (1962) In *XVI. International Dairy Congress* vol. D, p. 613. — Ref.: Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations* p. 118. Vanløse, Copenhagen: Technical Dairy Publishing House.

Kon, S.K. (1964) *Some Aspects of the Nutritive Value of Milk and the Effect of Processing*. Melbourne: Dairy Produce Board. — Ref.: Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations* p. 118. Vanløse, Copenhagen: Technical Dairy Publishing House.

Kovács, Á. (1997) Tejsavas fermentáció. In *Az Élelmiszertudomány Alapjai III. — Az Élelmiszerek Mikrobiológiája és Mikroökológiája* ed. Kovács, Á. pp. 97-99. Pécs: Pécsi Orvostudományi Egyetem, Egészségügyi Főiskolai Kar.

Kurita, H., Tajima, O. and Fukimbara, T. (1979) Isolation and identification of nucleosides in *Chlorella* extract. *Nippon Nôgeikagaku Kaishi* **53** (4), 131-133.

Kurmann, J.A. (1980) Újabb adatok a bifidobaktérium kultúrákról. In *XVIII. Óvári Tudományos Napok.* A plenáris előadások összefoglalói. A tejgazdaság biológiai, műszaki és ökonómiai problémái pp. 94-98. Mosonmagyaróvár: Agrártudományi Egyetem, Keszthely, Mezőgazdaságtudományi Kar.

Kurmann, J.A. and Rašić, J.L. (1988) Technology of fermented special products. In *Bulletin of the International Dairy Federation* No. 227, pp. 101-109.

Lampert, L.M. (1970) *Modern Dairy Products*. New York: Chemical Publishing Company Inc. — Ref.: Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations* p. 114. Vanløse, Copenhagen: Technical Dairy Publishing House.

Lankaputhra, W.E.V. and Shah, N.P. (1995) Survival of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in the presence of acids and bile salts. *Cultured Dairy Products Journal* **30** (3), 2-7.

Lee, S.Y., Vedamuthu, E.R., Washam, C.J. and Reinbold, G.W. (1974) An agar medium for the differential enumeration of yogurt starter bacteria. *Journal of Milk and Food Technology* **37**, 272-276.

Levander, O.A. (1985) In *Trace Elements in Human and Animal Nutrition* (5th edition, vol. 2) pp. 209-279. New York: Academic Press.

Lidbeck, A., Orrhage, K. and Nord, C.E. (1995) Effect of oral supplementation with lactic acid bacteria during intake of an antimicrobial agent. *International Dairy Lactic Acid Bacteria Conference*, Palmerston North, February 19-23, Poster presentation.

Liggett, R.W. and Koffler, H. (1948) Corn steep liquor in microbiology. *Bacteriology Reviews* **12**, 297-311.

Lin, M.Y., Savaiano, D. and Harlander, S. (1991) Influence of nonfermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. *Journal of Dairy Science* **74**, 87-95.

Link-Amster, H., Rochat, F., Saudan, K.Y., Mignot, O. and Aeschlimann, J.M. (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immunology and Medical Microbiology* **10**, 55-63.

Magyar Élelmiszerkönyv Bizottság (1996) Tej és tejtermékek. 3. Savanyú tej- és tejszínkészítmények. *Magyar Élelmiszerkönyv / Codex Alimentarius Hungaricus* No. MÉ 2 – 51/03 (Bizottsági anyagok), 5 pp.

Marshall, V.M.E. (1984) Fermented milks: new developments in the biochemistry of starter cultures. *Biochemical Society Transactions* **12**, 1150-1152.

Marshall, V.M., Cole, W.M. and Vega, J.R. (1982) A yoghurt-like product made by fermenting ultrafiltered milk containing elevated whey proteins with *Lactobacillus acidophilus*. *Journal of Dairy Research* **49**, 665-670.

Marshall, V.M. and Tamime, A.Y. (1997) Starter cultures employed in the manufacture of biofermented milks. *International Journal of Dairy Technology* **50** (1), 35-41.

Marshall, V.M. and Taylor, E. (1995) Ability of neonatal human *Lactobacillus* isolates to remove cholesterol from liquid medium. *International Journal of Food Science and Technology* **30**, 577-584.

Mayer, H. (1990) Ernährungsphysiologische Aspekte von Sauermilchprodukten. In *Milchwirtschaftliche Berichte aus den Bundesanstalten Wolfpassing und Rotholz* No. 102, pp. 23-28.

McDowell, L.R. (1992) Iodine; Zinc; Selenium. In *Minerals in Animal and Human Nutrition* pp. 224-245 and 265-332. San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: Academic Press Inc.

McKellar, R.C. and Modler, H.W. (1989) Metabolism of fructo-oligosaccharides by *Bifidobacterium* spp. *Applied Microbiology and Biotechnology* **31**, 537-541.

Mertz, W. (1987) The practical importance of interactions of trace elements. *Trace Subst. Environ. Health* **21**, 526-532.

Mertz, W. (1993) Essential trace metals: new definitions based on new paradigms. *Nutrition Reviews* **51** (10), 287-295.

Mertz, W. (1995) Risk assessment of essential trace elements: new approaches to setting recommended dietary allowances and safety limits. *Nutrition Reviews* **53** (7), 179-185.

Midwinter, R.G. and Pritchard, G.G. (1994) Aminopeptidase N from *Streptococcus salivarius* subsp. *thermophilus* NCDO 573: purification and properties. *Journal of Applied Bacteriology* **77**, 288-295.

Miller, B. and Puhan, Z. (1980) Möglichkeiten zur Verkurzung der Fermentation bei Acidophilus Milch. *Schweizerische Milchwirtschaftliche Forschung* **9**, 49-56.

Mitić, S., Otenhajmer, I. and Obradović, D. (1973) A study of bacterial antagonism of strains of *Lactobacillus acidophilus* towards some test-organisms. *Acta Veterinaria* **23**, 141-147.

Miura, Y., Sode, K., Narasaki, Y. and Matsunaga, T. (1993) Light-induced antimicrobial activity of extracts from marine *Chlorella*. *Journal of Marine Biotechnology* **1** (3), 143-146.

Modler, H.W., McKellar, R.C. and Yaguchi, M. (1990) Bifidobacteria and bifidogenic factors. *Canadian Institute of Food Science and Technology Journal* **23** (1), 29-41.

Monnet, V. and Grippon, J.C. (1994) Métabolisme azoté des bactéries lactiques. In *Bactérie Lactique* (vol. 1) ed. Roissart, H. and Luquet, F.M. pp. 331-347. Uriage: Lorica.

Nath, K.R. and Ledford, R.A. (1971) Stimulation of the rate of acid production by lactobacilli in media containing a capsular substance from micrococci. *Journal of Dairy Science* **54**, 1784-1789.

Nath, K.R. and Wagner, S.J. (1973) Stimulation of lactic acid bacteria by a *Micrococcus* isolate: evidence for multiple effects. *Applied Microbiology* **26**, 49-55.

National Dairy Council (1984) Cultured and culture-containing dairy foods. *Dairy Council Digest* **55** (3), 15.

National Research Council (1983) *Selenium in Nutrition*. Washington, DC: National Academy of Sciences.

National Research Council, Food and Nutrition Board (1989) *Recommended Dietary Allowances* (10th edition) ed. Havel, R.J. 258 pp. Washington, DC: National Academy of Sciences, National Academy Press.

Navert, B., Sandstrom, B. and Cederblad, A. (1985) Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. *British Journal of Nutrition* **53**, 47-53.

Neviani, E., Giraffa, G., Brizzi, A. and Carminati, D. (1995) Amino acid requirements and peptidase activities of *Streptococcus salivarius* subsp. *thermophilus*. *Journal of Applied Bacteriology* **79**, 302-307.

Oberg, C.J. and Broadbent, J.R. (1993) Thermophilic starter cultures: another set of problems. *Journal of Dairy Science* **76**, 2392-2406.

Ördög, V. (1998) Prokariota algák. In *Mezőgazdasági Növénytan* ed. Turcsányi, G. pp. 189-190. Budapest: Mezőgazdasági Szaktudás Kiadó.

Özbaş, Z.Y. (1993) Bifidobakter'ler ve *Lactobacillus acidophilus*: özellikleri, diyetetik amaçlar için kullanimlari, yararli etkileri ve ürün uygulamalari (Bifidobacteria and *Lactobacillus acidophilus*: behavior, using for dietary purposes, beneficial effects and applications of products). *Gida* **18**, 247-251.

Pais, I. (1992) Újabb kutatási eredmények az élelmiszertermékekben található mikroelemek táplálkozásélettani hatásáról. *Élelmezési Ipar* **46**, 161-165.

Pais, I. (1993) Mikroelemek – táplálkozás – egészség. *Élelmezési Ipar* **47**, 357-361.

Pette, J.W. and Lolkema, H. (1950) Yoghurt I. Symbiosis and antibiosis in mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Netherlands Milk and Dairy Journal* **4**, 197.

Philippe, M. (1994) Boisson aux vertus apaisantes et nutritionelles. *Patent* No. FR 2699371 A1, 6 pp.

Poirer, K.A. and Milner, J.A. (1983) Factors influencing the antitumorigenic properties of selenium in mice. *Journal of Nutrition* **113**, 2147-2154.

Pritchard, G.G. and Coolbear, T. (1993) The physiology and biochemistry of the proteolytic system in lactic acid bacteria. *FEMS Microbiology Reviews* **12**, 179-206.

Proulx, M., Ward, P., Gauthier, S.F. and Roy, D. (1994) Comparison of bifidobacterial growth-promoting activity of ultrafiltered casein hydrolyzate fractions. *Le Lait* **74**, 139-159.

Rao, D.R., Reddy, A.V., Pulusani, S.R. and Cornwell, P.E. (1984) Biosynthesis and utilization of folic acid and vitamin B_{12} by lactic cultures in skim milk. *Journal of Dairy Science* **67**, 1169-1174.

Rao, D.R. and Shahani, K.M. (1987) Vitamin content of cultured milk products. *Cultured Dairy Products Journal* **22** (2), 6-10.

Rašić, J.L. (1972) In *Zbornik Ref. Konf. Novoj Techn. a Technike Vyr. Kisloml. Vyr. a Ost. Mlieč. Spec.*, Vratna Dol., 24.-26. 10., p. 94. — Ref.: Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations* p. 118. Vanløse, Copenhagen: Technical Dairy Publishing House.

Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations*. 466 pp. Vanløse, Copenhagen: Technical Dairy Publishing House.

Rauch, P. and Králová, B. (1986) Substances with biological activity of vitamin B_{12} formed during cultivation of *Propionibacterium freudenreichii* in the presence of precursors. *Nahrung* **30**, 563-564.

Reddy, K.P., Shahani, K.M. and Kulkarni, S.M. (1976) B-complex vitamins in cultured and acidified yogurt. *Journal of Dairy Science* **59**, 191-195.

Reinhold, J.G., Faradji, B., Abadi, P. and Beigi, F.I. (1976) Binding of zinc to fiber and other solids in wholemeal bread. In *Trace Elements in Human Health and Disease* ed. Prasad, A.S. and Oberleas, D. pp. 163-180. New York: Academic Press.

Renner, E. (1983) *Milk and Dairy Products in Human Nutrition.* 450 pp. München: VV-GmbH Volkswirtschaftlicher Verlag.

Rigó, J. (1992) A mikroelemek jelentősége a kiegyensúlyozott táplálkozásban és a cardio-vascularis betegségek dietoterápiájában. *MTA Előadás Kézirat.* 16 pp. — Ref.: Pais, I. (1992) Újabb kutatási eredmények az élelmiszertermékekben található mikroelemek táplálkozásélettani hatásáról. *Élelmezési Ipar* **46**, 161-165.

Rohm, H. and Lechner, F. (1988) Zur Lebendkeimzahl von Joghurt / Viable bacteria count in yogurt. *Ernährung / Nutrition* **12**, 536-540.

Rohm, H. and Lechner, F. (1989) Aspekte zur Lebendkeimzahl von Joghurt. In *Milchwirtschaftliche Berichte aus den Bundesanstalten Wolfpassing und Rotholz* No. 99, pp. 116-124.

Rohm, H., Lechner, F. and Lehner, M. (1990) Microflora of Austrian natural-set yogurt. *Journal of Food Protection* **53**, 478-480.

Roy, D., Dussault, F. and Ward, P. (1990) Growth requirements of *Bifidobacterium* strains in milk. *Milchwissenschaft* **45**, 500-502.

Saavedra, J.M., Bauman, N.A., Oung, I., Perman, J.A. and Yolken, R.H. (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *The Lancet* **344**, 1046-1049.

Sack, R.B. (1986) Antimicrobial prophylaxis of travelers' diarrhea: a selected summary. *Review of Infectious Diseases* (Suppl. 2) **8**, 160-166.

Salminen, S. and Tanaka, R. (1996) Role of cultured and culture-containing dairy products and probiotic bacteria in health and disease. In *Newsletter of the International Dairy Federation* No. 145, pp. 32-34.

Samona, A. and Robinson, R.K. (1994) Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. *Journal of the Society of Dairy Technology* **47** (2), 58-60.

Sandine, W.E., Speck, M.L. and Aurand, L.W. (1956) Identification of constituent amino acids in a peptide stimulatory for lactic acid bacteria. *Journal of Dairy Science* **39**, 1532-1541.

Sarudi, I., Lassu, I., Nagy, I. and Szakály, S. (1992a) A hazai tejek, tejporok és tejfehérje-koncentrátumok szeléntartalma. In *Az Integrált Élelmiszergazdaság Biológiai és Gazdasági Alapjainak Feltárása a Dél-Dunántúlon* ed. Széles, Gy., Csató, L., Szakály, S. and Mózsik, Gy. pp. 61-71. Kaposvár: Pannon Agrártudományi Egyetem, Állattenyésztési Kar.

Sarudi, I., Lassu, I., Rumi, Gy., Kovách, G. and Szakály, S. (1992b) Élesztőben dúsított "bioszelén" hasznosulása a sertés és az ember szervezetében. In *Az Integrált Élelmiszergazdaság Biológiai és Gazdasági Alapjainak Feltárása a Dél-Dunántúlon* ed. Széles, Gy., Csató, L., Szakály, S. and Mózsik, Gy. pp. 72-77. Kaposvár: Pannon Agrártudományi Egyetem, Állattenyésztési Kar.

Scardovi, V. (1986) Genus *Bifidobacterium*. In *Bergey's Manual of Systematic Bacteriology* (vol. 2) ed. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. pp. 1418-1434. Baltimore: Williams & Wilkins.

Schaafsma, G. (1996) State of the art concerning probiotic strains in milk products. In *Newsletter of the International Dairy Federation* No. 145, pp. 23-24.

Scherer, S. (1995) Biologische Bekämpfung von Krankheitserregern in Lebensmitteln: Option oder Fiktion? *DMZ-Lebensmittelindustrie und Milchwirtschaft* **116**, 432-442.

Schiffrin, E.J., Rochat, F., Link-Amster, H., Aeschlimann, J.M. and Donnet-Hughes, A. (1995) Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *Journal of Dairy Science* **78**, 491-497.

Schirmann, F. (1985) Produit laitier, notamment yaourt aux algues et aux plantes et procédé de préparation d'un tel produit laitier, notamment yaourt. *Patent* No. FR 2563414 A1, 7 pp.

Schwarz, K. (1954) Factors protecting against dietary necrotic liver degeneration. Nutritional factors and liver diseases. *Annals of the New York Academy of Sciences* **57**, 878-888.

Selby Smith, J., Hillier, A.J., Lees, G.J. and Jago, G.R. (1975) The nature of the stimulation of the growth of *Streptococcus lactis* by yeast extract. *Journal of Dairy Research* **42**, 123-138.

Shah, N.P. (1997a) Bifidobacteria: characteristics and potential for application in fermented milk products. *Milchwissenschaft* **52**, 16-21.

Shah, N.P. (1997b) Isolation and enumeration of bifidobacteria in fermented milk products: a review. *Milchwissenschaft* **52**, 72-76.

Shahani, K.M. (1983) Nutritional impact of lactobacillic fermented foods. In *XV. Symposia of the Swedish Nutrition Foundation* (1981, publ. 1983) pp. 103-111.

Shahani, K.M. and Chandan, R.C. (1979) Nutritional and healthful aspects of cultured and culture-containing dairy foods. *Journal of Dairy Science* **62**, 1685-1694.

Shankar, P.A. and Davies, F.L. (1977) Recent developments in yoghurt starters. III. Associative bacterial growth in yoghurt starters; initial observations on stimulatory factors. *Journal of the Society of Dairy Technology* **30** (1), 31-32.

Shino, K. and Tanaka, Y. (1981) Production of butter. *Patent Abstracts of Japan* JP 56092736 A. Unexamined Applications, Section C, Sect. No. 76, vol. 5. No. 1641, p. 17.

Shirota, M. (1971) Report on yakult-strain. In *The Summary of Reports on Yakult* No. 1. Tokyo: Yakult Honska Co. Ltd.

Shirota, M., Nagamatsu, N. and Takechi, Y. (1964) Method for cultivating lactobacilli. *Patent* No. US 3123538, 3 pp.

Souci, S.W., Fachmann, W. and Kraut, H. (1973) *Die Zusammensetzung der Lebensmittel. Nährwerttabellen Band I. und II.* Stuttgart: Wissenschaftliche Verlagsgesellschaft GmbH. — Ref.: Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations* p. 114. Vanløse, Copenhagen: Technical Dairy Publishing House.

Speck, M.L. (1980) Preparation of lactobacilli for dietary uses. *Journal of Food Protection* **43**, 65-67.

Speck, M.L. and Katz, R.S. (1980) Nutritive and health values of cultured dairy foods. *Cultured Dairy Products Journal* **15** (4), 10-12.

Spolaor, D., Zilio, F., Dal Santo, M.L., Cavallari, A., Restello, I., Zanatta, P., Loddo, A., Giacon, D. and Broggiato, F. (1988) Caratterizzazione microbiologica dello yogurt. In *Yogurt e i Latti Fermentati.* Atti del Convegno Internazionale in collaborazione con INTERLAB, Thiene 25 maggio, pp. 142-175.

Sprince, H. and Woolley, D.W. (1945) The occurrence of the growth factor strepogenin in purified proteins. *Journal of the American Chemists' Society* **67**, 1734-1736.

Springer, M., Pulz, O., Szigeti, J., Ördög, V. and Varga, L. (1998) Verfahren zur Herstellung von biologisch hochwertigen Sauermilcherzeugnissen. *Patent* No. DE 19654614 A1, 7 pp.

Stengel, E. (1970) Anlagentypen und Verfahren der technischen Algenmassenproduktion. *Berichte der Deutschen Botanischen Gesellschaft* **83**, 589-606.

Sugihara, T.F. and Kline, L. (1975) Further studies on a growth medium for *Lactobacillus sanfrancisco. Journal of Milk and Food Technology* **38**, 667-672.

Szakály, S. (1981) A pasztőrözött tej- és tejszínkészítmények gyártása. In *Tejipari Kézikönyv* ed. Balatoni, M. and Ketting, F. pp. 303-346. Budapest: Mezőgazdasági Kiadó.

Szakály, S. (1989) *Táplálkozástudományi Kutatások a Korszerű Tejipari Gyártmányfejlesztés Szolgálatában.* 14 pp. Pécs: Magyar Tejgazdasági Kísérleti Intézet.

Szakály, S. (1991) A tejtermékek táplálkozási jelentősége. In *Tejgazdaságtan* ed. Szakály, S. pp. 115-120. Kaposvár: Pannon Agrártudományi Egyetem, Állattenyésztési Kar.

Szakály, S. (1992) *Tejipari Gyártmánytan (Fontosabb Táblázatok és Ábrák).* 128 pp. Mosonmagyaróvár: Pannon Agrártudományi Egyetem, Mezőgazdaságtudományi Kar.

Szakály, S. (1994) A tej és tejtermékek táplálkozásbiológiai szerepe. *Tejipari Hírlap* **21** (4), 6.

Szakály, S. (1996) *Tejipari Gyártmánytan (Fontosabb Táblázatok és Ábrák).* 145 pp. Mosonmagyaróvár: Pannon Agrártudományi Egyetem, Mezőgazdaságtudományi Kar.

Szakály, S., Schäffer, B. and Krász, Á. (1998) A savanyított tejkészítmények szerepe az emberi egészség megóvásában (Fermented milks in maintaining human health). In *XXVII. Óvári Tudományos Napok.* Új kihívások a mezőgazdaság számára az EU-csatlakozás tükrében. Az előadások teljes terjedelemben megjelent anyagai, IV. kötet, Minőségi Élelmiszer-előállítás Szekció pp. 874-882. Mosonmagyaróvár: Pannon Agrártudományi Egyetem, Mezőgazdaságtudományi Kar.

Szakály, S. and Zsinkó, M. (1997) Pro- és prebiotikumot tartalmazó új magyar tejtermékek funkcionális minősége (Functional quality of new Hungarian dairy products containing pro- and prebiotics). *Tejgazdaság* (Különszám) **57**, 149-155.

Szigeti, E. (1995) Mikroelemekkel dúsított új típusú fermentált tejtermékek előállítása. *Diplomamunka.* 43 pp. Budapest: Kertészeti és Élelmiszeripari Egyetem, Élelmiszeripari Kar.

Tamime, A.Y. and Robinson, R.K. (1988) Fermented milks and their future trends. Part II. Technological aspects. *Journal of Dairy Research* **55**, 281-307.

Tamime, A.Y., Marshall, V.M.E. and Robinson, R.K. (1995) Microbiological and technological aspects of milks fermented by bifidobacteria. *Journal of Dairy Research* **62**, 151-187.

Taylor, E. (1995) Investigations into cholesterol-lowering ability of selected strains of lactobacilli. *Ph.D. Thesis*. University of Huddersfield.

Teodoru, V., Cucu, D. and Bologa, A. (1985) Îmbogățirea produselor lactate în iod prin administrarea algelor marine în hrana vacilor (Increasing iodine in dairy products by giving marine algae to cows). *Revista de Creşterea Animalelor* **35** (5), 60-61.

Teodoru, V., Nicolau, G., Budurus, D. and Stadniciuc, M. (1976) Valorificarea algelor marine în alimentația taurinelor în zonele deficitare în iod (Use of marine algae for feeding cows in iodine-deficient areas). *Revista de Creşterea Animalelor* **26** (7), 40-43.

Thomas, T.D. and Mills, O.E. (1981) Proteolytic enzymes of starter bacteria. *Netherlands Milk and Dairy Journal* **35**, 255-273.

Tomaselli, L. (1997) Morphology, ultrastructure and taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira* (*Spirulina*) *platensis*. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 1-15. London: Taylor & Francis Ltd.

Tredici, M.R. and Zittelli, G.C. (1997) Cultivation of *Spirulina* (*Arthrospira*) *platensis* in flat plate reactors. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 117-130. London: Taylor & Francis Ltd.

Tsakalidou, E. and Kalantzopoulos, G. (1992) Purification and partial characterization of an intracellular aminopeptidase from *Streptococcus salivarius* subsp. *thermophilus* strain ACA-DC 114. *Journal of Applied Bacteriology* **72**, 227-232.

Tucker, K. (1995) Micronutrient status and aging. *Nutrition Reviews* **53** (9), (II)S9-S15.

Turnbull, A.J. and Thompson, R.P.H. (1989) Zinc — a precious metal. In *BNF Nutrition Bulletin* No. 55, vol. 14, pp. 23-35. London: British Nutrition Foundation.

Underwood, E.J. and Mertz, W. (1987) Introduction. In *Trace Elements in Human and Animal Nutrition* (5th edition, vol. 1) ed. Mertz, W. pp. 1-19. Orlando: Academic Press.

USSI Ingeniérie (1990) La voie microalgues en biosynthèse. In *Process (Rennes)* No. 1049, pp. 54-55.

Vallee, B.L. (1982) Zinc in biology and biochemistry. In *Zinc Enzymes* ed. Spiro, T.G. pp. 3-24. New York: John Wiley and Son.

Vandierendounck, E. (1971) *Milchkommission, „Spezialkommission für Ernährungsfragen* 12. Sitzung, Bern. — Ref.: Rašić, J.L. and Kurmann, J.A. (1978) *Yoghurt — Scientific Grounds, Technology, Manufacture and Preparations* p. 114. Vanløse, Copenhagen: Technical Dairy Publishing House.

Varga, L. (1994) Joghurtféleségek mikrobaszáma és változása a tárolás során. *Diplomadolgozat.* 41 pp. Mosonmagyaróvár: Pannon Agrártudományi Egyetem, Mezőgazdaságtudományi Kar.

Vedamuthu, E.R. (1974) Cultures for buttermilk, sour cream and yoghurt with special comments on acidophilus yoghurt. *Cultured Dairy Products Journal* 9 (1), 16-21.

Vonshak, A. (1997a) Appendices. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 213-226. London: Taylor & Francis Ltd.

Vonshak, A. (1997b) Outdoor mass production of *Spirulina*: the basic concept. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 79-99. London: Taylor & Francis Ltd.

Vonshak, A. (1997c) Preface. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. ix-x. London: Taylor & Francis Ltd.

Vonshak, A. (1997d) Use of *Spirulina* biomass. In *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology* ed. Vonshak, A. pp. 205-212. London: Taylor & Francis Ltd.

Webb, L.E. (1982) Detection by Warburg manometry of compounds stimulatory to lactic acid bacteria. *Journal of Dairy Research* 49, 479-486.

Weseloh, J.H. OHG (1972) Verfahren zur Herstellung von Frucht-Joghurt. *Patent* No. DE 2129853 A1.

Whanger, P.D. (1981) Selenium and heavy metal toxicity. In *Selenium in Biology and Medicine* ed. Spallholz, J.E., Martin, J.L. and Ganther, H.E. pp. 230-255. Westport: AVI Publishing Co.

Wiesenberg, A., Kolb, E. and Engelhardt, I. (1984) Protein containing conditioning drink. *Patent* No. US 4486413.

Wilkinson, J.F. and Rose, A.H. (1963) Fermentation process. In *Biochemistry of Industrial Micro-organisms* ed. Rainbow, C. and Rose, A.H. pp. 379-414. London: Academic Press.

Wynckel, A., Jaisser, F., Wong, T., Drüeke, T. and Chanard, J. (1991) Intestinal absorption of calcium from yogurt in lactase-deficient subjects. *Reproduction, Nutrition, Development* **31**, 411-418.

Yaeshima, T. (1996) Benefits of bifidobacteria to human health. In *Bulletin of the International Dairy Federation* No. 313, pp. 36-42.

Yamazaki, H. and Matsumoto, K. (1994) Purification of Jerusalem artichoke fructans and their utilisation by bifidobacteria. *Journal of the Science of Food and Agriculture* **64**, 461-465.

Yang, G.Q. (1985) Keshan disease: an endemic selenium-related deficiency disease. In *Trace Elements in Nutrition of Children* ed. Chandra, R.K. pp. 273-290. New York: Raven Press.

Yang, G.Q., Wang, S., Zhou, R. and Sun, S. (1993) Endemic selenium intoxication of humans in China. *American Journal of Clinical Nutrition* **37**, 872-881.

Zhao, F. (1994) Healthy whole milk powder containing organic Ge and its manufacture process. *Derwent Information Ltd.* CN 1082821 A.

Zielke, H., Kneifel, H., Webb, L.E. and Soeder, C.J. (1978) Stimulation of lactobacilli by an aqueous extract of the green alga *Scenedesmus acutus* 276-3a. *European Journal of Applied Microbiology and Biotechnology* **6**, 79-86.

Zsinkó, M., Vass, A. and Szakály, S. (1997) A koleszterin-szint csökkentésének lehetőségei speciális savanyú tejtermékekkel és mérési módszerei (Possibilities of decrease of cholesterol content by special fermented dairy products and methods for its measurement). *Tejgazdaság* (Különszám) **57**, 142-148.

Zuraw, E.A., Speck, M.L., Aurand, L.W. and Tove, S.B. (1960) Purification of stimulants from condensed corn-fermentation solubles active for *Lactobacillus casei* in milk. *Journal of Bacteriology* **80**, 457-463.