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Effect of a Spray-Dried *Arthrospira* (*Spirulina*) *platensis* Biomass on Acid Development by Various Strains of *Lactococcus lactis* in Milk

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Abstract. *Arthrospira* (*Spirulina*) *platensis*, which is a planktonic cyanobacterium belonging to prokaryotic algae, has been approved for human nutrition by many governments and health agencies and has recently been marketed and consumed as a safe human food worldwide. The influence of 3 g/L powdered *A. platensis* biomass on the rate of acid development by various strains of major lactic acid producers in mesophilic dairy starter cultures such as *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* was evaluated in cow's milk. Acid production of the starter culture strains screened was mostly stimulated significantly ($P < 0.05$), although to varying degrees. The *A. platensis* biomass rich in trace elements, vitamins, and other bioactive substrates also had a beneficial effect on the nutritional value of milk, thus providing a new opportunity for the manufacture of functional fermented milks, i.e., *Arthrospira*-enriched cultured cream and buttermilk.

1. Introduction

Cyanobacteria 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 [Ördög, 1998]. *Arthrospira* (*Spirulina*) *platensis* is a planktonic cyanobacterium that forms massive populations in tropical and subtropical water bodies characterized by high levels of carbonate and bicarbonate and pH values of up to 11 [Tomaselli, 1997]. The dried biomass of *A. platensis* typically contains 3 to 7% moisture, 55 to 60% protein, 6 to 8% lipids, 12 to 20% carbohydrate, 7 to 10% ash, 8 to 10% fiber, 1 to 1.5% chlorophyll *a*, and a wide range of vitamins [Belay, 1997], [Cohen, 1997], [Vonshak, 1997a]. *A. platensis* is especially rich in proteins. The proteins with the highest economic potential are the biliproteins (e.g., c-phycocyanin and allophycocyanin), which are water-soluble blue pigments. The protein fraction may have a phycocyanin content of up to 20% [Cohen, 1997]. Fatty acid composition is largely influenced by environmental conditions. *Arthrospira platensis* can be characterized by about 25 to 30% saturated and 70 to 75% unsaturated fatty acids. Up to 30% of fatty acids is γ-linolenic acid, a rare polyunsaturated fatty acid claimed to have medicinal properties. Beneficial *Arthrospira* strains and an efficient processing procedure should yield biomass with at least 1% γ-linolenic acid [Cohen, 1997], [Vonshak, 1997a]. Since the late 1970s, *A. platensis* has been marketed and consumed as a safe human food and has been approved for human nutrition by many governments, health agencies, and associations of some 80 countries. Based on 30 yr of safety and quality research, several countries and organizations have established *Arthrospira* Quality and Safety Standards. More than 70% of the current *Arthrospira* market is for human consumption, mainly as health food. The total annual production of food-grade *Arthrospira* biomass is estimated to be approximately 1000 to 1500 tonnes [Belay, 1997], [Vonshak, 1997b].

It is our belief that consumers would need to ingest considerably less medicine and artificially produced vitamin and mineral supplements if fermented milks were enriched with vitamins, proteins, essential fatty acids and trace elements of natural origin. A simple way of attaining this goal is the use of cyanobacteria in manufacture of cultured dairy foods [Varga et al., 2002]. Productivity is one of the main concerns of dairy processors. Therefore, the influence of an *A. platensis* biomass on the rate of acid development by single strains of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* was evaluated in milk. The objectives of this research were (i) to determine the optimal concentration of the cyanobacterial biomass in regards to sensory properties and cost, and (ii) to select *Lactococcus* strains whose acid production can be stimulated to a large degree by addition of the *Arthrospira* biomass.

2. Materials and Methods

2.1. Raw Material

Commercially available UHT milk containing (per liter) 36 g of fat, 34 g of protein, 47 g of lactose, and 7 g of ash was used, to which 10 g of skim milk powder per liter was added. This raw material was heated to 90°C and held for 10 min before being cooled to inoculation temperature to insure adequate whey protein denaturation [Kessler, 1988].

2.2. *Lactococcus* Strains

The *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* starter strains tested were kindly supplied in freeze-dried form by the Hungarian Dairy Research Institute, Inc. (Mosonmagyaróvár, Hungary). Before the start of the trials, the *Lactococcus* strains were subcultured twice at 30°C for 24 h.

2.3. *Arthrospira* (*Spirulina*) *platensis* Biomass

The *A. platensis* Hau biomass was obtained from the Institute of Cereal Processing, Inc. (Bergholz-Rehbrücke, Germany). It contained (per kilogram) 941 g of DM, 576 g of protein, 111 g of total lipids, and 114 g of ash.

2.4. Inoculation, Incubation, and pH-Measurement

The heat-treated and cooled *Arthrospira*-supplemented and control milks were inoculated with the *Lactococcus* strains tested at the rate of 1% and were then incubated at 30°C. The pH value of three replicate samples from both treatments was measured at regular intervals (i.e., every 2 h) with a HANNA 8521 pH-meter and combined glass electrode. The entire experimental program was repeated twice.

2.5. Statistical Analysis

The influence of *A. platensis* on acid production of lactococci during the fermentation process was analyzed with the Student's *t*-test, by means of the STATISTICA data analysis software system, version 6 [StatSoft, 2001]. Significance of difference was set at $P < 0.05$ in all cases.

3. Results and Discussion

The optimal concentration of the cyanobacterial biomass in regards to effectiveness, sensory properties, and cost was determined by using selected strains of *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*. The results obtained are presented in Tables 1 and 2.

Table 1. Effect of *Arthrospira* (*Spirulina*) *platensis* biomass used at various concentrations on acid production[1] of *Lactococcus lactis* subsp. *lactis* Ha-2 in milk

Time (h)	Batch containing *Arthrospira* (*Spirulina*) *platensis* biomass at				
	0%	0.1%	0.3%	0.5%	0.8%
0	6.64	6.64	6.65	6.67	6.63
2	6.61	6.61	6.63	6.65	6.54
4	6.49	6.45	6.46	6.47	6.43
6	6.30	6.18*	6.19*	6.18*	6.16*
8	5.82	5.59*	5.58*	5.58*	5.68*
10	5.34	5.05*	5.01*	5.04*	5.13*
12	4.88	4.63*	4.61*	4.65*	4.76*
14	4.58	4.48	4.48	4.49	4.59
24	4.33	4.28	4.23	4.31	4.37

*Significant difference ($P < 0.05$) compared to the respective control (i.e., 0%).
[1]Figures are mean pH values, based on six observations (three samples, two replicates).

Table 2. Effect of *Arthrospira* (*Spirulina*) *platensis* biomass used at various concentrations on acid production[1] of *Lactococcus lactis* subsp. *cremoris* W-24 in milk

Time (h)	Batch containing *Arthrospira* (*Spirulina*) *platensis* biomass at				
	0%	0.1%	0.3%	0.5%	0.8%
0	6.63	6.65	6.66	6.69	6.54
2	6.61	6.62	6.63	6.68	6.54
4	6.47	6.46	6.44	6.49	6.40
6	6.22	6.16	6.10*	6.13*	6.08*
8	5.70	5.56*	5.48*	5.56*	5.58*
10	5.09	4.88*	4.83*	4.91*	4.94*
12	4.61	4.50*	4.48*	4.55*	4.59*
14	4.44	4.41	4.41	4.44	4.49
24	4.25	4.22	4.22	4.26	4.29

*Significant difference ($P < 0.05$) compared to the respective control (i.e., 0%).
[1]Figures are mean pH values, based on six observations (three samples, two replicates).

It is clearly visible that, between h 6 and h 12 of the fermentation process, *A. platensis* significantly stimulated ($P < 0.05$) acid production of both strains. The use of 0.8% cyanobacterial biomass had no advantage over the concentration of 0.1%. For this and a variety of other reasons, our subsequent trials were carried out with 0.3% (i.e., 3 g/L) *Arthrospira* biomass (Tables 3 and 4).

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Table 3. Effect of 3 g/L *Arthrospira* (*Spirulina*) *platensis* biomass on acid production of *Lactococcus lactis* subsp. *lactis* strains in milk

Time (h)	Decrease in pH compared to control[1] of strain no.					
	89.07	89.04	Ha-2	88.05	CH-1	Ha-1
0	–0.07	0.00	–0.01	–0.04	–0.02	–0.04
2	–0.04	0.00	–0.02	–0.07	–0.03	–0.04
4	+0.02	+0.01	+0.03	–0.03	–0.06	0.00
6	+0.32*	+0.33*	+0.11*	+0.18*	+0.09*	+0.13*
8	+0.65*	+0.44*	+0.24*	+0.28*	+0.50*	+0.32*
10	+0.53*	+0.45*	+0.33*	+0.21*	+0.55*	+0.31*
12	+0.14*	+0.18*	+0.27*	+0.09*	+0.15*	+0.14*
24	+0.10	+0.15	+0.10	+0.06	+0.04	+0.06

*Significant difference ($P < 0.05$); +: stimulation of acid production, –: inhibition of acid production.
[1]Values are means based on six observations (three samples, two replicates).

Table 4. Effect of 3 g/L *Arthrospira* (*Spirulina*) *platensis* biomass on acid production of *Lactococcus lactis* subsp. *cremoris* strains in milk

Time (h)	Decrease in pH compared to control[1] of strain no.			
	W-24	86.04	89.07	CH-1
0	–0.03	–0.02	–0.03	–0.04
2	–0.02	–0.02	0.00	–0.04
4	+0.03	+0.02	+0.05	+0.01
6	+0.12*	+0.39*	+0.45*	+0.24*
8	+0.22*	+0.62*	+0.92*	+0.61*
10	+0.26*	+0.55*	+0.86*	+0.73*
12	+0.13*	+0.16*	+0.24*	+0.21*
24	+0.03	0.00	+0.01	+0.04

*Significant difference ($P < 0.05$); +: stimulation of acid production, –: inhibition of acid production.
[1]Values are means based on six observations (three samples, two replicates).

It should be noted that acid production of all the *Lactococcus* strains screened was stimulated significantly ($P < 0.05$), although to varying degrees. In the case of both species, the strain designated 89.07 gave the best result.
Our findings are in accordance with previous reports by Varga et al. [1999], who stated that acid production and growth rate of thermophilic dairy starter cultures could be stimulated significantly ($P < 0.05$) by an *A. platensis* biomass. The substances responsible for the stimulatory properties of this cyanobacterial biomass were identified as adenine, hypoxanthine and free amino acids.

4. Conclusions

Arthrospira (*Spirulina*) *platensis* had beneficial effects on mesophilic lactic acid bacteria commonly used for manufacture of fermented milks by stimulating their acid production, thereby potentially increasing the profitability of cultured dairy foods manufacture. In addition, the *A. platensis* biomass rich in trace elements, vitamins, and other bioactive substrates also beneficially influenced the nutritional value of milk, thus providing a new opportunity for the manufacture of functional fermented milks, i.e., *Arthrospira*-enriched cultured cream and buttermilk.

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References

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

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

Kessler, H. G. 1988. Erhitzen und Auswirkungen. Pages 132–195 in *Lebensmittel- und Bioverfahrenstechnik—Molkereitechnologie*. H. G. Kessler, ed. Verlag A. Kessler, Freising, Germany.

Ördög, V. 1998. Prokariota algák (Prokaryotic algae). Pages 189–190 in *Mezőgazdasági Növénytan* (*Agricultural Botany*). G. Turcsányi, ed. Mezőgazdasági Szaktudás Kiadó, Budapest, Hungary.

StatSoft. 2001. *STATISTICA Data Analysis Software System*. *Version 6*. StatSoft Inc., Tulsa, OK.

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

Varga, L., J. Szigeti, R. Kovács, T. Földes, and S. Buti. 2002. Influence of a *Spirulina platensis* biomass on the microflora of fermented ABT milks during storage. *Journal of Dairy Science* 85:1031–1038.

Varga, L., J. Szigeti, and V. Ördög. 1999. Effect of a *Spirulina platensis* biomass and that of its active components on single strains of dairy starter cultures. *Milchwissenschaft* 54:187–190.

Vonshak, A. 1997a. Appendices. Pages 213–226 in Spirulina platensis *(*Arthrospira*): Physiology, Cell-biology and Biotechnology*. A. Vonshak, ed. Taylor and Francis Ltd., London, UK.

Vonshak, A. 1997b. Use of *Spirulina* biomass. Pages 205–212 in Spirulina platensis *(*Arthrospira*): Physiology, Cell-biology and Biotechnology*. A. Vonshak, ed. Taylor and Francis Ltd., London, UK.