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ORIGINAL ARTICLE

Sodium Formate as an Alternative for the Replacement of Streptococci in the Elaboration of Quartirolo Cheese

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Influence of sodium formate (SF) on the specific proteolytic activity (SPA) of *L. delbrueckii* subsp. *bulgaricus* (LBB, LBP and LBR) and its application in the elaboration of Quartirolo cheese was studied. A comparison between Quartirolo-type cheese, prepared with selected mixed starters of lactobacilli and streptococci (LBB + CP2 and LBP + CP2) with respect to pure lactobacilli starters (LBB and LBP), developed with and without sodium formate was also performed as regards organoleptic and microbiological properties.

Different elaborations were performed with each starter and two cheese loaves of each one were analyzed at 10 and 20 days of ripening. For each sample were analyzed: pH, bacterial counts, proteolytic activity, meltability, moisture content, texture and sensorial properties. Statistical analysis was applied to experimental data.

Bacterial suspensions obtained from cultures with strain LBB grown with sodium formate (SF) showed higher proteolytic activity than those cultures grown without SF.

Texture properties, meltability and proteolytic activity in cheese prepared with lactobacilli LBB + streptococci CP2 were similar to those found in cheese prepared with LBB + SF. These results suggest that formate could replace the streptococci on cheese elaboration. The assay of sensorial acceptability indicated that the formate could be supplemented to a pure starter and be employed instead of the streptococci since organoleptic properties were similar on both kinds of cheese.

Key Words: sodium formate; quartirolo cheese; cheese ripening; streptococci.

INTRODUCTION

Lactic acid bacteria play an important role in the production and conservation of foodstuffs, especially in the dairy industry. Mixed "starters cultures" containing selected strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are generally used in the elaboration of yoghurt and soft cheese. Both species present a synergistic relationship (Beal and Corrieu, 1994; Higashio *et al*., 1977). Proteolytic enzymes produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* degrade casein releasing low molecular weight peptides and aminoacids. These molecules were

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identified as growth factors for *Streptococcus thermophilus* (Rajagopal and Sandine, 1990). The growth of lactobacilli is stimulated by carbon dioxide and formic acid produced by the streptococci. These thermophilic cultures are used not only on the elaboration of products whose processing involves high temperatures (yoghurt, hard cheese), but also in processes where the temperature never exceeds 32°C (soft cheese).

Soft cheese production in Argentina represents 55% of the total cheese production. Statistical data for the last years indicate that the consumption of this type of cheese shows a clear growing tendency (Elaboración de Leches y Productos Lácteos, 1997). Quartirolo cheese, a soft Argentinean cheese (De La Canal, 1994) is prepared with pasteurized milk and ripened at 10°C for 20 days.

Texture is one of the features that determine not only the type of cheese but also its quality. Variations in texture may reflect structural changes during ripening. Texture depends on moisture content, pH and degree of casein proteolysis (De Jong, 1978; Creamer and Olson, 1982). Considering the organoleptic properties of cheese, hardness is one of the most important traits of texture regarding consumer's preferences. Hardness could be defined as the force required to penetrate a cheese sample with the teeth and can also be quantified by an instrumental assay (uniaxial compression test) as the maximal force employed to penetrate the sample. Values obtained for this parameter both through the equipment and by sensorial analysis show good correlation if experimental conditions are appropriately chosen (Bevilacqua, 1997).

The purpose of this work was to study the influence of sodium formate (SF) on the specific proteolytic activity (SPA) of *L. delbrueckii* subsp. *bulgaricus* and its application in the elaboration of Quartirolo cheese. Besides, a comparison between Quartirolo-type cheese prepared with selected mixed starters with respect to pure starters was performed as regards organoleptic and microbiological properties.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Lactobacillus delbrueckii subsp. *bulgaricus* CIDCA 331 (LBB), CIDCA 332 (LBP), CIDCA 333 (LBR), and *S. thermophilus* CIDCA 321 (CP2) were isolated and identified in our laboratory (Abraham *et al*., 1992; De Antoni *et al*., 1991; Gómez Zavaglia *et al*., 1997; De Urraza, 1997). Strains were maintained at −80°C in milk. Stock cultures were propagated in UHT (ultra high temperature) skim milk, 12% milk solids, 0% fat and 3.3% protein, (Parmalat S.A, Buenos Aires (1649), Argentina) at 37°C for 18 h and then subcultured in milk at 37°C until pH 5 (6–8 h to obtain an active inoculum).

Harvest of Bacteria and Preparation of Bacterial Suspensions

For all experiments, 50 mL of milk with 40 ppm of sodium formate or without sodium formate were inoculated with 5% of active inoculum and incubated at 30°C, for 18 and 24 h, respectively. Bacteria were harvested from those milk cultures at pH 5 after treatment with 2% (w/v) Na_2EDTA, pH 12 (Abraham *et al*., 1992) and centrifuged at $5000 \times g$ for 15 min at 10°C. The bacterial pellet was washed twice and resuspended in 0.5 M K_2HPO_4 buffer pH 7 to obtain a bacterial suspension 10-fold concentrated ($1–4 \times 10^9$ cfu/ml).

Determination of Proteolytic Activity of the Bacterial Suspension

Diluted milk was used as substrate to measure proteolytic activity. The reaction mixture consisted of 0.5 mL of bacterial suspension ($1–4 \times 10^9$ cfu/mL) and 2 mL of

diluted milk (protein content: 2.5 mg/mL). The reaction mixture was incubated at 30°C during 8 and 24 h. Optical Density determined according to Kanasaki (Kanasaki *et al*., 1975) did not increase or decrease during the enzymatic assay. The reaction was stopped by the addition of 5 mL of TCA (12% w/v). After centrifuging at $7000 \times g$ for 15 min TCA soluble compounds on the supernatant were evaluated according to the method of Citti and expressed as mg Tyr/100 g (Citti *et al*., 1963).

Determination of Specific Caseinolytic Activity of Bacterial Suspensions

Hydrolysis of protein was determined using a reaction mixture containing 0.5 mL of bacterial suspension ($OD_{600} = 1.0$ equivalent to $1–4 \times 10^9$ cfu/mL) and 2 mL of diluted milk (1/10). Samples (0.5 mL) were incubated at 30°C, during 24 and 48 h, and the reaction was stopped by freezing at −80°C. To perform electrophoretic runs, samples were treated with 9 M urea and 2% 2-mercaptoethanol. Samples were diluted 1:1 in buffer (0.14 M Tris, 0.001 M Na_2EDTA, 1% SDS, 10% glycerol and 0.01% bromophenol blue, pH 7). Each sample was analyzed by SDS-PAGE by the method of Laemmli (1970) on gels prepared with 12.8% acrylamide in a vertical system Bio-Rad Mini Protean II (Bio Rad Lab, Richmond, CA 94804, U.S.A.). Gel slabs were stained with 0.1% Coomasie Brillant Blue R250 in water:methanol:acetic acid (5:5:2).

Cheese Elaboration

Starter. Milk with 40 ppm sodium formate or without sodium formate was inoculated with an active culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and incubated at 30°C for 18 and 24 h, respectively, to approximately reach pH 5. In another set of experiments, mixed starters prepared in milk with an active culture of lactobacilli and streptococci in a 1:1 ratio were incubated at 30°C during 18 h, to reach pH 5.

Quartirolo cheese (Marsili *et al*., 1981; AOAC, 1984) was prepared with 500 L of pasteurized milk (17°D). At 30–32°C, 150 g of $CaCl_2$ and 5 L of starter were added to the milk. The mixture was incubated approximately for 40–60 min in order to increase the acidity in 5°D (AOAC, 1984). Then, 300 mL of rennet were added and temperature was raised to 32–34°C for 30–40 min until milk coagulation. Whey was removed, curd was pressed, salted and finally packed in low gas permeability films. Cheeses were ripened at 10°C for 20 days. Experiments were performed using five different starters for cheese elaborations. From each batch, 16–20 cheese loaves were obtained and two loaves of each one were analyzed to study the ripening. In the case of starters LBB, LBB plus SF and LBB plus CP2 two different batches were elaborated.

In those cheese elaborated with sodium formate, this was added to the milk at a final concentration of 40 ppm and immediately after the addition of the starter culture. Cheese without sodium formate and cheese elaborated with mixed starters were prepared with the starters developed in milk.

Control of Ripening Parameters (10 *and* 20 *days*)

*pH was determined at 25°C using a Cole-Parmer (Chicago, IL) combined glass-calomel microelectrode.

*Bacterial counts. Aerobic mesophilic bacteria counts were performed on plate count agar medium (PCA), lactic acid bacteria were counted on LEE differential medium (Lee *et al*., 1974), molds and yeasts were counted on YGC medium (agar, yeast extract, glucose and cloranfenicol).

*Proteolytic activity. Cheese samples were diluted 1/10 (w/v) in tryptone 0.1% (w/v), proteins were precipitated with TCA 8% (w/v) (Kuchroo and Fox, 1982). After

centrifuging, at $7000 \times g$ during 15 min, the TCA-soluble concentration was determined in the supernatant with Folin's reagent and expressed as mg Tyr/100 g of cheese. Absorbance at 650 nm was determined in a Shimadzu (Kyoto, Japan) double-beam spectrophotometer (Citti *et al*., 1963).

*Meltability. Meltability is defined as the ability of cheese particles to flow together forming a continuous and uniform mass (Kindstedt, 1993). To determine this parameter, six portions 5 mm thick and 18 mm in diameter were cut from the inside of a cheese sample using a large cork borer-type cutter. The test portions were placed in glass Petri dishes and were arranged on the shelf in a pre-selected order. The oven was pre-heated to 100°C. Cold test portions (4°C) were placed in Petri dishes, covered, tempered at room temperature (20 ± 2°C) for 30 min, and placed in the oven at 100°C for 1 h. Petri dishes were removed from the oven and cooled at room temperature for 1 h. The diameter of each melted test portion was measured to the nearest 0.01 cm at four different angles, and the means were calculated. The mean diameter was compared to the initial diameter and results were expressed as % of meltability (Kosikowski, 1982; Bertola *et al*., 1996).

*Moisture content. This parameter was determined as the water loss of a sample placed in an oven at 100°C until constant weight. Results were expressed as g water/100 g cheese.

*Hardness analysis. Cylinders of cheese samples (1.5 cm in diameter and 2.5 cm high) were obtained from the inside of each cheese using a cork borer-type cutter. Cheese cylinders were compressed to 80% of their original height using a 3.5 cm diameter plate at a crosshead speed of 10 cm/min in an Instron Universal Testing Machine (1132 model, Instron Corporation, Canton, MA, U.S.A.), with a compression cell of 50 kg. Tests were run at 20°C. The uniaxial compression test was performed and the peak height was considered as the hardness value and expressed in Newton, N (Bevilacqua, 1997). Each test was repeated at least six times for each one of the two cheese loaves, belonging to the same production, and mean values were reported.

*Sensorial analysis. A sensorial acceptability assay was designed as follows: after 20 days of storage, the cheeses were evaluated by a consumer panel of 36 (18 males and 18 females) volunteers. Each sample of cheese was cut into several dices of 1.5 cm and was placed in plates labeled with three numbers and held at room temperature (22°C) for 1 h before presentation to participants. Each participant received four cheese samples and additional sample belonging to a well-known trademark as a good control of organoleptic properties. Sensory attributes (color, taste, global preference and texture) of the cheese were scored on a nine-point hedonic scale (9 = like extremely to 1 = dislike extremely).

Statistical Analysis

Statistical analysis was applied to experimental data using Systat-Software (Systat Version 5-0, Systat, Inc. U.S.A.). Analysis of Least-Significant-Difference Test (LSD) was performed at $\alpha < 0.05$.

RESULTS AND DISCUSSION

Influence of Sodium Formate on the Specific Proteolytic Activity (SPA) of Lactobacillus delbrueckii subsp. bulgaricus

Table 1 shows the effect of sodium formate on the specific proteolytic activity of the bacterial suspension grown in milk with and without sodium formate (SF) and

TABLE 1

Proteolytic activity of bacterial suspension in diluted milk at 30°C

Bacterial suspension[1]		Casein hydrolysis after 48 h of incubation		TCA soluble-N (mg tyr/100 mL)	
		α_{s-1}	β	8 h	24 h
LBB	SF +	+	+	3.05 ± 0.05	5.50 ± 0.50
	SF −	−	+	1.55 ± 0.05	2.55 ± 0.05
LBP	SF +	−	+	1.90 ± 0.50	3.10 ± 0.10
	SF −	−	+	1.50 ± 0.01	2.75 ± 0.05
LBR	SF +	−	+	2.65 ± 0.05	3.15 ± 0.05
	SF −	−	+	2.55 ± 0.05	2.80 ± 0.01

Note: (+): positive (−): negative. Data is the average of at least three independent experiments. No significant differences ($\alpha > 0.05$) between the specific proteolytic activity of bacteria grown with and without SF were observed in the case of LBP and LBR after 24 and 48 h of incubation at 30°C.

[1]Bacterial suspensions of lactobacilli (LBB, LBP and LBR) were obtained from milk cultures with sodium formate (SF +) and without sodium formate (SF −) at 30°C as indicated in the Materials and Methods section.

assayed in diluted milk at 30°C. Bacterial suspensions obtained from LBB cultures grown with SF showed higher proteolytic activity than those cultures grown without SF ($\alpha < 0.05$). The same bacterial concentration was used in both enzymatic assays. These results indicate that bacteria grown in milk with SF had a higher specific proteolytic activity. No significant differences were observed in the specific proteolytic activity of LBP and LBR strains grown with and without SF after 24 and 48 h of incubation at 30°C. Table 1 shows, also, the effect of SF on the specific caseinolytic activity. Bacterial suspension of LBB grown in milk with SF produce the hydrolysis of α_{s-1} and β casein's after 48 h of incubation. In contrast, cells grown without SF did not show significant hydrolysis of α_{s-1} casein during the same incubation period. No significant differences between the specific caseinolytic activity of bacteria grown with and without SF were observed in the case of LBP and LBR strains after 48 h of incubation at 30°C.

Effect of Sodium Formate on the Organoleptic and Proteolytic Properties of Quartirolo Cheese

Quartirolo cheese was elaborated with pure and mixed starters as described in Materials and Methods. The strains for mixed cultures were selected on the basis of the results obtained on screening of strains in milk (Moreira *et al*., 2000a), in which an important synergistic effect between strains was observed regarding the rheological properties of the coagulum, good syneresis properties and a growth stimulus produced by lactobacilli on streptococci (Moreira *et al*., 2000b). Considering the aforementioned reasons, it seemed interesting to evaluate the selected strains as cheese starters and to compare the characteristics of the resulting cheese prepared with mixed and pure cultures.

As shown in Table 2, the pH of the cheese elaborated with mixed starters and LBB plus 40 ppm sodium formate reached the desired value (5.0 ± 0.50) at the end of the ripening stage, while those prepared with LBB and LBP strains as pure starter, reached a higher pH after ripening (6.0 ± 0.2). The meltability improved at 10 and 20 days in cheese prepared with mixed starters (LBB + CP2 and LBP + CP2) and in the cheese prepared with LBB supplemented with 40 ppm of sodium formate, in comparison with control cheese ($\alpha < 0.05$). There were no significant differences in moisture

TABLE 2

Parameters employed to evaluate the ripening of Quartirolo cheese

Parameters	Ripening time (days)	LBB	LBB + formate	LBB + CP2 1:1	LBP	LBP + CP2 1:1
pH	10	5.90 ± 0.070	5.69 ± 0.14	5.57 ± 0.014	6.33 ± 0.091	5.51 ± 0.18
	20	5.88 ± 0.014	5.35 ± 0.084	5.50 ± 0.035	6.19 ± 0.12	5.50 ± 0.035
Meltability	10	25.0 ± 1.41	44.5 ± 2.12	48.5 ± 2.12	13.95 ± 3.80	18.30 ± 2.40
(%)	20	26.5 ± 9.19	46.5 ± 9.19	60.0 ± 14.14	13.00 ± 2.82	50.0 ± 14.14
Moisture	10	46.95 ± 1.06	50.35 ± 1.06	52.60 ± 0.42	52.30 ± 0.42	56.25 ± 0.77
content	20	51.10 ± 0.28	52.0 ± 0.84	53.40 ± 0.56	51.30 ± 1.27	53.70 ± 0.70
(g water/100 g cheese)						
Hardness	10	19.4 ± 3.9	14.7 ± 0.98	10.4 ± 2.2	29.1 ± 0.63	10.2 ± 3.2
(N)	20	17.4 ± 3.6	8.9 ± 1.9	6.5 ± 0.4	27.9 ± 0.77	5.8 ± 0.14
TCA-soluble	10	87.0 ± 6.3	77.4 ± 3.6	83.1 ± 8.4	85.8 ± 7.5	119.1 ± 9.9
Nitrogen	20	206.7 ± 18.3	327.0 ± 10.8	296.1 ± 7.2	110.1 ± 20.1	252.0 ± 35.4
(mg Tyr/100 g)						
Total	10	0.45 ± 0.070	1.27 ± 1.10	22.0 ± 11.31	40.0 ± 2.82	1.95 ± 1.48
mesophiles	20	0.90 ± 0.14	0.65 ± 0.49	12.0 ± 1.41	65.0 ± 6.36	8.0 ± 4.75
(10^6 cfu/g)						
Lactic acid	10	7.25 ± 0.35	5.50 ± 0.35	3.0b[2]	ND[1]	1.5b
bacteria				30.0c		20.0c
(10^6 cfu/g)	20	3.75 ± 2.47	18.0 ± 8.48	0.2b	ND	0.3b
				12.0c		8.0c
Molds and	10	20.50 ± 15.50	9.89 ± 8.0	6.25 ± 5.30	240.0 ± 226.2	16.50 ± 4.95
yeast	20	4.24 ± 4.04	1.50 ± 0.70	13.50 ± 9.19	123.0 ± 112.0	2.00 ± 0.05
(10^3 cfu/g)						

Note: pH values as well as viable counts correspond to an average of duplicates obtained from two cheese loaves belonging to the same batch production. Values of TCA-soluble nitrogen correspond to an average of four replicates from two cheese loaves belonging to the same batch production. Hardness, meltability and moisture content values correspond to an average of six replicates from two cheese loaves belonging to the same batch production. Values of all parameters of cheeses prepared with LBB and LBB + sodium formate correspond to an average of six replicates from four cheese loaves belonging to two different batch productions.

[1] ND: not determined.

[2] "b and c" represent differential counts of lactobacilli (LBB and LBP) and streptococci (CP2), respectively in mixed cultures.

content among all cheese. As regards proteolytic activity, TCA-soluble nitrogen was significantly different ($\alpha < 0.05$) in cheese prepared with LBB + CP2, LBP + CP2 and with LBB + formate in comparison to their corresponding controls, after 20 days of ripening. The cheese prepared with LBB + formate reached similar soluble nitrogen levels to those of cheese prepared with mixed starters. Texture properties were significantly different between cheese prepared with mixed starters or LBB + formate and controls. Texture properties, meltability and proteolytic activity in cheese prepared with LBB + CP2 were similar to those found in cheese prepared with LBB + formate. These results suggest that formate could replace the streptococci in cheese elaboration. The increment in TCA-soluble nitrogen content during the ripening had a good correlation with the decrease in hardness (Table 2). Since the water content did not change either at 10 or 20 days of ripening, it was possible to detect an increment in soluble nitrogen content and the corresponding drop in the hardness values. As regards total bacteria counts, cheese prepared with LBB + CP2 and LBP showed the highest values. Lactic acid bacteria counts, after 20 days were 10^6 cfu/g for the cheese prepared with LBB and 10^7 cfu/g for cheese prepared with LBB + formate. In cheese prepared with mixed starters, streptococci were one logarithm unit higher

TABLE 3

Sensorial acceptability of Quartirolo cheese after 20 days of ripening

Attribute	Cheese sample				
	LBB	LBB + formate	LBB + CP2 (1 : 1)	LBP + CP2 (1 : 1)	Commercial cheese
Global preference	4.71a	4.82ab	6.65c	5.65b	6.56c
Color	5.91a	5.91a	6.39b	6.39b	6.76b
Taste	4.88a	6.74b	6.91b	6.29b	7.06b
Texture	4.68a	4.92ab	6.68b	5.50ab	7.15b

Note: Values correspond to an average of 72 data for each elaboration (non-trained jury composed of 36 people) obtained from two cheese loaves.

a, b and c: Numbers with the same letters showed no significant differences ($\alpha < 0.05$).

Sensory attributes of the cheese were scored on a nine-point hedonic scale.

than lactobacilli, both at 10 and 20 days of ripening. Fungi and yeast counts did not differ amongst cheese, the only exception being the cheese prepared with LBP. Due to the high content of fungi and yeast in cheese prepared with LBP, this cheese was not included in the sensorial analysis.

As shown in Table 3, the results indicate that as a global preference, consumers did not find differences between reference cheese (well-known trademark) and cheese prepared with LBB + CP2. Differences were found with regard to color between cheese prepared with pure cultures and cheese prepared with mixed starters as well as with the commercial cheese. As regards taste, consumers did not find differences between reference cheese and cheese number 2, 3 and 4, suggesting that the characteristics obtained in the cheese prepared with LBB + formate were similar to that obtained in cheese prepared with mixed starters, but different to that in cheese with LBB. As regards texture, cheese 3 prepared with the mixed starter LBB + CP2 seemed to be similar to the commercial cheese, but different to the cheese prepared with LBB. On the other hand, cheese prepared with LBB + formate showed texture properties in between those for the aforementioned.

The assay of sensorial acceptability indicated that the formate could be supplemented to a pure starter and be employed instead of the streptococci since organoleptic properties were similar in both kinds of cheese. Besides, the cheese prepared with LBB + CP2 showed similar properties to the commercial cheese with regard to texture, taste and global preference, but was different to the cheese prepared with a pure culture.

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REFERENCES

Abraham, A., De Antoni, G., and Añon, C. (1992). Proteolytic activity of *L. bulgaricus* grown in milk. *J. Dairy Sci.* **76,** 1498–1505.

AOAC (Association of Official Analytical Chemists) (1984). Official Methods of Analysis. AOAC 16.023.

Beal, C. and Corrieu, G. (1994). Viability and acidification activity of pure and mixed starters of *S. thermophilus* 404 and *L. bulgaricus* 398 at the different steps of their production. *Lebensm. Wiss. Technol.* **27,** 86–92.

Bertola, N., Califano, A., Bevilacqua, A., and Zaritzky, N. (1996). Effect of freezing conditions on functional properties of low moisture mozzarella cheese. *J. Dairy Sci.* **79,** 185–190.

Bevilacqua, A. (1997). Propiedades fisico-químicas y comportamiento reológico en la maduración de quesos, Ph.D. Thesis. Facultad de Ingeniería, Universidad Nacional de La Plata, Bs.As., Argentina.

Citti, J., Sandine, W., and Elliker, P. (1963). Some observations on the Hull method for measurement of proteolysis in milk. *J. Dairy Sci.* **46,** 337–339.

Creamer, L. K. and Olson, N. F. (1982). Rheological evaluation of maturing Cheddar Cheese. *J. Food Sci.* **47**(2), 631–636.

De Antoni, G., Perez, P., Abraham, A., and Añon, C. (1991). Trehalose, a cryoprotectant for *L. bulgaricus*. *Cryobiology* **26,** 149–153.

De Jong, L. (1978). The influence of the moisture content on the consistency of protein breakdown of cheese. *Neth. Milk Dairy J.* **32,** 1–14.

De La Canal ASOC. (1994). Código Alimentario Argentino Actualizado; art. 621, p. 209. Buenos Aires, Argentina.

De Urraza, P. (1997). Obtención de bacterias lacticas para cultivos starters, Ph.D. Thesis. Facultad de Ciencias Exactas, Universidad Nacional de La Plata.

Elaboracion de Leches y Productos Lacteos: Lechería (1997). Dirección Nacional de Alimentación, Subsecretaría de Alimentación y Mercados. Secretaría de Agricultura, Ganadería, Pesca y Alimentación.

Gomez Zavaglia, A., Abraham, A., Giorgieri, S., and De Antoni, G. (1997). Application of polyacrilamide gel electrophoresis and capillary gel electrophoresis to the analysis of *Lactobacillus delbrueckii* whole-cell-proteins. *J. Dairy Sci.* **82**(5), 870–877.

Higashio, K., Yoshioka, Y., and Kikuchi, T. (1977). Isolation and identification of a growth factor of *S. thermophilus* produced by *L. bulgaricus*. *J. Agric. Chem. Soc. Jpn* **51,** 209–215.

Kanasaki, M., Breheny, S., Hillier, A., and Jago, G. (1975). Effects of temperature on the growth and acid production of lactic acid bacteria. A rapid method for the estimation of bacterial populations in milk. *Aust. J. Dairy Technol.* **30,** 142–145.

Kindstedt, P. S. (1993). Effect of manufacturing factors, composition and proteolysis on the functional characteristics of Mozzarella cheese. *Crit. Rev. Food Sci. Nutr.* **33**(2), 167–187.

Kosikowski, F. V. (1982). Cheese and Fermented Milk Foods, 2nd edn. Edwards Brothers Inc., Arbor, Michigan, U.S.A.

Kuchroo, C. N. and Fox, P. F. (1982). Fractionation of the water soluble nitrogen from Cheddar cheese: chemical methods. *Milchwissenschaft* **37,** 651–653.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London) **227,** 680–685.

Lee, S., Vedamuthu, E., Washam, C., and Reinhold, G. (1974). An agar medium for the differential enumeration of yoghurt starter bacteria. *J. Milk Food Technol.* **37,** 272–276.

Marsili, R., Ostapenko, H., Simons, R., and Green D. (1981). A controlled approach to cheese technology. *J. Food Sci.* **46,** 52–57.

Moreira, M., Abraham, A., and De Antoni, G. (2000a). Protocooperative Interaction between different strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* y *Streptococcus thermophilus* at suboptimal temperature. *Rev. Argent. Lactol.* **18,** 42–54.

Moreira, M., Abraham, A., and De Antoni, G. (2000b). Technological properties of milks fermented with thermophilic LAB at suboptimal temperature. *J. Dairy Sci.* **83,** 395–400.

Rajagopal, S. N. and Sandine, W. E. (1990). Associative growth and proteolysis of *S. thermophilus* and *L. bulgaricus* in skim milk. *J. Dairy Sci.* **73,** 894–899.