Manufacture of Quartirolo cheese using exopolysaccharide-producing starter cultures

By M. MOREIRA[1, 2], A. BEVILACQUA[1, 3] and G. DE ANTONI[1, 4]

[1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos, CONICET-Facultad de Ciencias Exactas, Universidad Nacional de La Plata. 47 y 116, (1900) La Plata, Buenos Aires, Argentina. E-mail: moreira_maria@fi.mdp.edu.ar
[2]Grupo de Investigación en Ingeniería en Alimentos, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional de Mar del Plata. Argentina
[3]Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional de La Plata. Argentina
[4]Comisión de Investigaciones Científicas, Buenos Aires, Argentina

The capacity of some strains of lactic acid bacteria (LAB) to produce exopolysaccharides (EPS) has been used in dairy industry to improve the rheological characteristics of fermented milks and soft cheese. In a previous study we showed that sodium formate (SF) can stimulate the growth and proteolytic activity of *Lactobacillus bulgaricus* (LBB). In this study, the effects of SF on EPS production are evaluated for the elaboration of soft cheese, Quartirolo type. The results showed significant differences in moisture content, meltability, proteolytic activity and texture between cheeses made using EPS$^+$ strains (mixed-starter cultures prepared with *L. bulgaricus* + *S. thermophilus*, LBB+CP2 and single-strain culture plus SF, LBB+SF) and cheeses made with a control culture (LBB). On the other hand, hardness, meltability and proteolytic activity were similar in cheeses made with mixed starters cultures (LBB+CP2) and single strain plus SF (LBB+SF). As expected, cheese moisture levels and meltability were significantly higher in cheese made with mixed-starter culture (LBB+CP2) and single-strain plus SF (LBB+ SF) compared to the control, LBB. These results suggest that EPS$^+$ starter cultures could be used to increase cheese moisture content and to improve meltability, textural and proteolytic properties, compared to the cheeses made with single strain starter (LBB).

Herstellung von Quartirolo-Käse mit Exopolysaccharid-produzierenden Milchsäureweckern

Die Fähigkeit einiger Milchsäurebakterien (LAB) zur Bildung von Exopolysacchariden (EPS) wird in der Milchindustrie zur Verbesserung der rheologischen Eigenschaften fermentierter Milch und von Weichkäse eingesetzt. In einer früheren Studie wurde gezeigt, dass Natriumformiat (SF) Wachstum und proteolytische Aktivität von *Lactobacillus bulgaricus* (LBB) stimulieren kann. In dieser Studie wird die Wirkung von SF auf die EPS-Bildung bei der Herstellung von Weichkäse des Quartirolo-Typs bewertet. Die Ergebnisse zeigten signifikante Unterschiede bei Wassergehalt, Schmelzverhalten, proteolytischer Aktivität und Textur zwischen Käsen, die mit EPS$^+$ Stämmen (gemischte Starterkulturen aus *L. bulgaricus* + *S. thermophilus*, LBB+CP2 und Einzelstammkulturen + SF, LBB + SF) und mit einer Kontrollkultur (LBB) hergestellten Käsen. Andererseits waren Härte, Schmelzverhalten und proteolytische Aktivität in Käsen, hergestellt mit Mischstarterkulturen (LBB + CP2) und Einzelstamm plus SF (LBB + SF) ähnlich. Wie erwartet waren Wassergehalt und Schmelzverhalten der Käse signifikant höher in Käse mit Mischstarterkultur (LBB + CP2) und Einzelstamm plus SF (LBB + SF) im Vergleich zu der Kontrolle LBB. Diese Ergebnisse lassen vermuten, dass EPS$^+$-Starterkulturen zur Erhöhung von Wassergehalt und zur Verbesserung von Schmelzverhalten, Textur- und proteolytischen Eigenschaften des Käses beitragen könnten im Vergleich zu den mit Einzelstammsäureweckern (LBB) hergestellten Käsen.

56 **Quartirolo cheese** (exopolysaccharide production of starter cultures)

56 **Quartirolo-Käse** (Exopolysaccharidbildung von Säureweckern)

1. Introduction

Yoghurt and soft cheese are fermented dairy products, resulting from the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in milk. The two organisms have complementary metabolisms: *L bulgaricus* provides peptides for *S. thermophilus, which* in return produces formate stimulating growth and metabolism of *L bulgaricus* (1, 2).

European yogurt manufacturers have used texture-promoting or ropy cultures for many years, particularly where addition of stabilizers is prohibited. Cultures described as *ropy* and used for their texture-enhancing properties are strains capable of secreting exopolysaccharides (EPS). The capacity of some strains of LAB to produce EPS has been used in the dairy industry to improve the rheological characteristics of fermented milks and soft cheese (3, 4). Microbial polysaccharides can be used as thickeners, viscosifying, emulsifying, or gelling agents in foodstuffs (5).

Ropy strains are also used in the manufacture of fermented milk and cheese. The quantities of EPS produced by LAB vary considerably as a function of species and strain and culture conditions. *S. thermophilus* can produce from as little as 30mg/l to 890 mg/l, while *L. bulgaricus* can produce 60 mg/l to 150 mg/l. Amounts and yields of EPS are also affected by associative growth. (4, 6).

In this study, the strains for mixed cultures (LBB+CP2) were selected on the basis of the results obtained from a screening of strains in milk (7). Selected cultures presented an important synergistic effect on the rheological properties of the coagulum, good syneresis properties and a growth stimulus produced by lactobacilli on streptococci (8). SF stimulates growth and proteolytic activity of *L. bulgaricus* (LBB) (2).

The purpose of this work was to analyze the exopolysaccharide production capacity of single-strains cultures of lactobacilli (LBB) and streptococci (CP2) and

that of the corresponding mixed-starter cultures. The effect of SF on the production of EPS by single strain (LBB) and mixed starter cultures (LBB+CP2) was also analyzed. The application of the results herewith obtained to improve the organoleptic characteristics of a soft cheese, Quartirolo type, was also investigated.

2. Materials and methods

2.1 Bacterial strains

Lactobacillus delbrueckii subsp. *bulgaricus* CIDCA 331 (LBB), and *S. thermophilus* CIDCA 321 (CP2) were isolated and identified in our laboratory (2). Strains were maintained at –80 °C in milk. Stock cultures were propagated in UHT skimmed milk (12% milk solids., 2.8–2.9% fat and 3.3% protein) (Parmalat S.A, Buenos Aires, Argentina) at 37 °C for 18 h and then subcultured in milk at 37 °C until pH 5 (6 to 8 h to obtain an active inoculum).

2.2 Media and growth conditions

The growth medium was either UHT skimmed milk or UHT skimmed milk plus SF solution, with a final concentration of 40 ppm (mg. kg^{-1}). 50 ml of milk, in 100 ml hermetically-sealed bottles, inoculated with active inoculum (1%) and incubated at 30 °C were used to measure EPS. Measurements of optical density (OD), Viscosity and EPS were conducted immediately after the desired pH (4.5–4.6) was reached.

Three independent experiments were performed for each single-strain cultures and mixed- starter cultures.

2.3 Determination of turbidity and pH of bacterial cultures

The pH of cultures was determined at 25 °C using a Cole-Parmer (Chicago, IL) combined glass-calomel microelectrode. The turbidity of bacterial cultures was determined at 480 nm in a Shimadzu (Kyoto, Japan) double beam spectrophotometer. Samples were removed from culture and diluted 1:10 (V/V) in 2% (W/V) Na_2EDTA, pH 12, according to KANASAKI *et al.* (9).

2.4 Measurement of EPS

Polysaccharide was measured (as glucose equivalents) in samples (50 ml) of fermented milk after separation of milk protein by centrifugation at 10.000 x g during 15 min. Ethanol was added to the supernatant fluid (equal volumes), then the pellet was resuspended and a second ethanol precipitation was applied. The total carbohydrate in the redissolved pellet (using 1 ml distilled water) was determined by addition of 10 ml Antrona Reactive. Extinction at 620 nm was determined and concentrations were taken from a calibration curve constructed from glucose standards (6). All the samples were tested for the absence of lactose by thin layer chromatography (TLC) on Silica gel G type 60 plates using *n*-propanol–acetic acid-water (70:20:10) as running solvent (5).

2.5 Viscosity

A rotational viscometer of concentric cylinders (Haake Rotovisco RV2, Germany) with a NV sensor (Nieder Viskositat) was used. Rheological parameters were measured at 30 °C. Flow behavior was analyzed plotting shear stress (τ) *vs.* shear rate (D). The following program was performed: an increasing sequence from 0 to 2769.9 s^{-1} in a period of 3 min, followed by 1 min at the maximum value and a corresponding decreasing sequence in 3 min. Apparent viscosity (η_{ap}) was calculated at 256 x 5.41 s^{-1} and expressed in mPa x s.

2.6 Cheese elaboration

Three starter cultures were used: *L. delbrueckii* subsp. *bulgaricus* (LBB) in milk free of SF, LBB in milk with SF (40 ppm) and mixed cultures obtained from *L. bulgaricus* and *S. thermophilus* (LBB+CP2), in a 1:1 ratio, in milk free of SF. Starters were incubated at 30 °C to final pHs of 5.

To prepare Quartirolo cheese (10, 11) lots of 500 l of pasteurized milk (17° D) were brought to 30–32 °C, $CaCl_2$ (150 g) and starter (5 l) were added. This mixture was incubated to increase acidity by 5° D between 40 and 60 min (11). At this point rennet (300 ml) were added and temperature was raised to 32–34 °C for 30–40 min to produce milk coagulation. The whey was removed, and the curd was pressed, salted and finally packaged in low gas permeability films. The cheeses were ripened for 20 d at 10 °C.

Two batches of cheeses with each of the 3 different starter cultures were elaborated. Each batch yielded 16 to 20 cheese loaves. Two loaves from each batch were used to study the ripening stage. In cheeses elaborated with SF, this was added to the milk at a final concentration of 40 ppm and, immediately after the addition of the starter culture, prepared in milk plus SF (12).

2.7 Ripening evaluation

The following determinations were performed in cheeses after 10 and 20 d of ripening.

2.7.1 Proteolytic activity

Cheese samples were taken and diluted 1/10 (W/V) in tryptone 0.1% (W/V) (13). Determination of TCA-soluble nitrogen in cheese was performed according to the method of CITTI (14). Proteins were precipitated with TCA at a final concentration of 8.0 g/100 ml, followed by centrifugation at 7000 x g 15 min. The TCA soluble-N was determined in the supernatant with Folin's reagent and expressed as mg Tyr/100 ml. Absorbance at 650 nm was measured in a Shimadzu (Kyoto, Japan) double-beam spectrophotometer.

2.7.2 Meltability

Meltabilty is defined as the ability of cheese particles to flow together forming a continuous and uniform mass (15). To evaluate this property, 6 disks (5 mm thick and 18.0 mm in diameter) were cut from the inside of a cheese sample using a large cork borer-type cutter. Cold disks (4 °C) were placed in Petri dishes, covered, tempered at room temperature (20±2 °C) for 30 min, and placed in an 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 sample was measured to the nearest 0.01 cm at 4 different angles. The means were calculated and compared to the initial diameter. Results were expressed as % of meltability (16, 17).

2.7.3 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.

2.7.4 Hardness analysis

Cylinders of cheese (o 1.5 cm, height 2.5 cm) were obtained from the inside of each cheese using a cork borer-type cutter. These samples 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, USA); 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 (18). Each test was repeated at least 6 times for two cheese loaves belonging to the same production, Mean values were reported.

2.8 Statistical analysis

Statistical analysis was applied to experimental data using Systat-Software (Systat Version 5-0, Systat, Inc. USA). Analysis of LSD (least significant difference) was performed at $\alpha < 0.05$.

3. Results

3.1 Effects of sodium formate (SF) on EPS production by L. bulgaricus LBB

Many of the investigations cn EPS formation by starter cultures used in the manufacture of yogurt and fermented milks have been carried out in milk. Recently, chemically defined media have been used successfully for EPS production from *L. lactis* subsp. *cremoris* and *L. casei* (19, 20, 21).

The EPS-production capacity, viscosity, optical density (O.D.) and pH of milk fermented with single-strain cultures (LBB and CP2) and of the corresponding mixed starter cultures (LBB+CP2) are presented in Table 1. The effects of SF on EPS- production by single strain (LBB) and mixed cultures (LBB+CP2) is also reported.

Table 1 shows that the lactobacilli (LBB) and the streptococci (CP2) single strains were EPS producing starters. However, if used together in mixed starter culture (LBB+CP2) or if SF was added to lactobacilli single strain (LBB), a stimulus on the amount of EPS production was observed. If the control culture of lactobacilli (LBB) is considered as EPS, then the mixed starter culture (LBB+CP2) and the lactobacilli with added of SF (LBB+SF) can be considered as EPS plus (EPS+).

Significant differences in the viscosity of the bacterial cultures were also found between single strain cultures (LBB) and the mixed starter (LBB+CP2) or the LBB with addition of SF. However, the addition of SF to the mixed starter did not result in significant differences neither in EPS production nor in viscosity over the EPS+ cultures.

As can be seen in Table 1 incubation was carried out to similar final values of pH. The fact that no significant differences were detected in OD values would indicate that similar bacterial masses were produced and the specific EPS production could be evaluated on the same basis. Some errors in the lactobacilli counts (LBB) could be induced by the fact that SF causes the breakdown of lactobacilli chains (22).

Table 1: Exopolysaccharide production, apparent viscosity, optical density (OD) and pH of cultures incubated at 30°C in UHT skim milk

Strain/ Growth condition	EPS (µg/ml)	Viscosity (mPa x seg)	OD	pH
LBB in milk	66.9±2.2^a	9.9±0.8^a	0.239±0.002^a	4.57±0.07^a
CP2 in milk	64.6±1.6^a	10.1±0.5^a	0.228±0.03^a	4.59±0.02^a
LBB in milk+SF	156.9±11.5 ^{b}	17.0±1.12^b	0.235±0.036^a	4.50±0.05^a
LBB+ CP2 in milk	177.2±20.8 ^{b}	19.5±0.4^b	0.236±0.02^a	4.49±0.10^a
LBB+CP2 +SF	190.4±12.5 ^{b}	20.2±1.4^b	0.226±0.04^a	4.45±0.10^a

Data are the average of 3 independent experiments. ^{a,b} In each column numbers with the same letter (for each technological parameter determined) are not significantly different ($\alpha \leq 0.05$).

3.2 Cheese elaboration

Different reports indicated that cheese moisture levels were significantly higher in Mozzarella cheeses made with exopolysaccharide-positive *vs* exopolysaccharide-negative streptococci, and melt properties were better in the higher moisture cheeses (23). PERRY *et al.* (24, 25) and LOW *et al.* (26), found significant differences in moisture and melting properties between cheeses made with or without exopolysaccharide-producing starter cultures. The ability of increasing cheese moisture level (even by only 1%) gives processors an important economic advantage in the highly competitive Mozzarella industry (26).

Meltability, water content, proteolytic activity and hardness of Quartirolo cheeses made with different starters are presented in Table 2 after 10 and 20 d of ripening.

Table 2: Parameters employed to evaluate the ripening of Quartirolo cheese

Parameters	Ripening time	Cheese prepared with starters: LBB	LBB+SF	LBB+CP2
Meltability	10 d	25.0±1.41%^{a}	44.5±2.12%^{b}	48.5±2.12%^{b}
	20 d	26.5±9.19%^{a}	46.5±9.19%^{b}	60.0±14.14%^{b}
Moisture content (g_{water}/100 g_{cheese})	10 d	46.95±1.06%^{a}	50.3±1.06%^{b}	52.60±0.42%^{b}
	20 d	50.10±0.18%^{a}	52.0±0.84%^{b}	53.40±0.56%^{b}
Hardness (N)	10 d	19.4±3.9^a	14.7±0.98^b	10.4±2.2^b
	20 d	17.4±3.6^a	8.9±1.9^b	6.5±0.4^b
TCA-soluble nitrogen (mg tyr/100 ml)	10 d	2.90±0.21^a	2.58±0.12^a	2.77±0.28^a
	20 d	6.89±0.61^a	10.90±0.36^b	9.87±0.24^b

Values of TCA-soluble nitrogen correspond to an average of 4 replicates from 2 cheese loaves belonging to 2 different batch productions. Hardness, meltability and moisture content values correspond to an average of 6 replicates from 2 cheese loaves belonging to 2 different batch productions. ^{a,b}Numbers with the same letter (for each parameter evaluated) are not significantly different ($\alpha \leq 0.05$).

Starters with enhanced EPS production capacity (mixed starter culture, LBB+CP2 and single strain culture plus SF, LBB+SF) produced cheeses with significant higher water content and meltability than lactobacilli single strain starters (LBB). On the other hand, hardness was significantly lower for cheeses made with EPS$^+$ starters (LBB+ CP2 and LBB+SF) compared with control cheese (LBB).

The proteolityc activity measured as TCA-soluble nitrogen was not significantly different ($\alpha \leq 0.05$) after 10 d of ripening but become significantly higher in cheeses made with EPS$^+$ starters (LBB+CP2 and LBB+ SF) by day 20 of ripening.

Finally, water content, meltability, soluble nitrogen levels and textural properties were similar for cheeses made with mixed starters (LBB+CP2) and lactobacilli plus SF (LBB+SF).

4. Conclusions

These results suggest that EPS$^+$ cultures (LBB+ SF and LBB+CP2) could be used by cheese manufacturers to increase cheese moisture content and obtain better melting, textural and proteolytic properties, over those obtained with single LBB cultures.

Application of these results for soft cheese (Quartirolo type) would suggest that the increase in moisture content is a crucial strategy for the manufacture of Quartirolo cheese that will melt adequately when it is baked on a pizza.

5. References

(1) García-Garibay, M., Marshall, V.: J. Appl. Bacteriol. **70** 325–328 (1991)

(2) Moreira, M., Abraham, A., de Antoni, G.: Milchwissenschaft **52** (11) 607–610 (1997)

(3) Rawson, H., Marshall, V.: Internat. J. Food Sci. Technol. **32** 213–220 (1997)

(4) Ricciardi, A., Clementi, F.: It. J. Food Sci. **12** (1) 23–45 (2000)

(5) Rimada, P., Abraham, A.: J. Dairy Res. **68** 125–129 (2001)

(6) Marshall, V., Rawson, H.L.: Internat. J. Food Sci. Technol. **34** 137–143 (1999)

(7) Moreira, M., Abraham, A., de Antoni, G.: Rev. Arg. Lactología **18** 42–53 (1999)

(8) Moreira, M., Abraham, A., de Antoni, G.: J. Dairy Sci. **83** 395–400 (2000)

(9) Kanasaki, M., Breheny, S., Hillier, A., Jago, G.: Aust. J. Dairy Technol. **30** 142–145 (1975)

(10) Marsili, R., Ostapenko, H., Simons, R., Green, D.: J. Food Sci. **46** 52–57 (1981)

(11) AOAC: Official Methods of Analysis, 14th ed. (AOAC 16.023), Arlington, VA (1984)

(12) Moreira, M., Storani, E., Bevilacqua, A., de Antoni, G.: Ital. J. Food Sci. 16 (2)0-0 (2003)

(13) Mor-Mur, M., Pla, R., Carretero, C., Guamis, B.: Alimentaria **236** 55–58 (1982)

(14) Citti, J.; Sandine, W., Elliker, P.: J. Dairy Sci. **46** 337–341 (1963)

(15) Kindstedt, P.: Crit. Rev. Food Sci. Nutr. **33** (2) 167–172 (1993)

(16) Kosikowski, F. Cheese and Fermented Milks Foods. 2nd edn. Edwards Brothers, Arbor, MI (1982)

(17) Bertola, N., Bevilacqua, A., Zaritzky N.E.: J. Dairy Sci. **75** 273–3281 (1996)

(18) Bevilacqua, A.: Sc. D. Thesis. Fac. Ing., Univ. Nac. de La Plata (1997)

(19) Cerning, J.: Lait **75** 463–472 (1995)

(20) Cerning, J., Bouillanne, C., Desmazeaud, M., Landon, M.: Biotechnol. Lett. **10** 255–260 (1988)

(21) Marshall, V.M., Cowie, E.N., Moreton, R.S.: J. Dairy Res. **62** 621–628 (1995)

(22) Suzuki, I., Kato, S., Kitada, T., Yano, N., Morichi, T.: J. Dairy Sci. **69** (4) 971–978 (1985)

(23) Petersen, B., Dave, R., McMahon, D., Oberg, C., Broadbent, J.: J. Dairy Sci. **83** 1952–1956 (2000)

(24) Perry, D., McMahon, D., Oberg C.: J. Dairy Sci. **80** 799–805 (1997)

(25) Perry, D., McMahon, D., Oberg C.: J. Dairy Sci. **81** 563–566 (1998)

(26) Low, D., Ahlgren, J., Horne, D., McMahon, D., Oberg, C., Broadbent, J.: Appl. Environm. Microbiol. June, **64** 2147–2151 (1998)