Lait (2006) 373–386
© INRA, EDP Sciences, 2006
DOI: 10.1051/lait:2006014

Original article

Growth of proteinase-positive and proteinase-negative lactococci strains in reconstituted goat and cow milks

Daniel ST-GELAIS*, Sylvie HACHÉ

Agriculture and Agri-Food Canada, Food Research and Development Centre,
3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec J2S 8E3, Canada

Received 30 March 2006 – Accepted 16 August 2006

Abstract – The growth of proteinase-positive *Lactococcus lactis* strains and the proteinase-negative variants was studied in reconstituted goat and cow milks at 90, 120 and 150 g·kg^{-1} total solids. pH change and lactic acid production were also compared in the two milks. Goat milk showed a higher buffering capacity than cow milk. The buffering capacity increased with the total solid contents in reconstituted milk. The proteinase-positive strains exhibited a higher maximum specific growth rate (μ_{max}) and a higher acidification rate than the proteinase-negative variants. The bacterial count and the lactic acid concentration after 15 h of incubation were also higher with the proteinase-positive strains. The acidification rate and the lactic acid concentration after 15 h of incubation for all lactococci were significantly higher in reconstituted goat milk than in cow milk and increased with the total solid contents in reconstituted milk. The μ_{max} and the viable counts obtained after 15 h of incubation for lactococci were relatively close in reconstituted goat and cow milks, with the exception of the Wg2S and Wg2L strains. For these strains, the μ_{max} values were significantly higher in goat milk, but their bacterial counts after 15 h of incubation were lower in goat milk. An uncoupling was observed for these strains in goat milk. In general, reconstituted goat milk was an appropriate medium for the production of mesophilic lactic starters. However, to prevent an uncoupling with some strains such as the Wg2S and Wg2L strains, incubation in reconstituted goat milk at 21 °C should be shorter than incubation in reconstituted cow milk.

goat milk powder / cow milk powder / growth of lactococci / reconstituted milk / proteinase-negative variants

摘要 – 蛋白酶阳性乳球菌和蛋白酶阴性变异体菌株在还原山羊奶和牛奶中的生长特性。 本文研究了蛋白酶阳性乳球菌 (*Lactococcus lactis*) 蛋白酶阴性变异体在总固性物含量分别为 90，120 和 150 g·kg^{-1} 的还原羊奶和牛奶中生长特性。对菌株在两种还原奶中的产乳酸能力和还原乳 pH 变化进行了比较。试验结果表明，山羊奶对酸的缓冲能力高于牛奶，而且缓冲能力随着还原奶中总固性物含量的增加而提高。所有蛋白酶阳性乳球菌的最大比生长速率 (μ_{max}) 和产酸速率均高于蛋白酶阴性变异体。培养 15 h 后，蛋白酶阳性乳球菌在还原奶中的细菌总数和乳酸浓度均高于蛋白酶阴性变异体；所有菌株在还原山羊奶中的产酸速率和乳酸浓度显著地高于在还原牛奶中，并且这些指标随着还原奶中总固性物含量的增加而提高。所有菌株在还原山羊奶和还原牛奶中培养 15 h 后，除了蛋白酶阳性乳球菌 Wg2S (*L. lactis* ssp. *cremoris*) 和其蛋白酶阴性变异体 Wg2L 外，其他菌株的 μ_{max} 值和菌落总数非常相近。这些菌株共同的特点是在还原山羊奶中 μ_{max} 值高于还原牛奶，但是在还原山羊奶中的

* Corresponding author (通讯作者): stgelaisd@agr.gc.ca

细菌总数则低于还原牛奶。这些菌株在还原山羊奶中发生了解偶联现象。一般来讲，还原山羊奶适合嗜热乳酸菌的生长。然而为了防止一些菌株如 Wg2S 和 Wg2L 发生解偶联，建议这些菌株在 21 °C 山羊奶中的培养时间应该少于在还原牛乳中的培养时间。

山羊奶粉 / 牛奶粉 / 乳球菌的生长 / 还原奶 / 蛋白酶阴性变异体

Résumé – Croissance de lactocoques de souches protéinase-positive et protéinase-négative dans du lait de chèvre et de vache réhydraté. La croissance de souches protéolytiques de *Lactococcus lactis* et leurs variants non protéolytiques a été étudiée dans les laits de chèvre et de vache reconstitués à 90, 120 et 150 g·kg^{-1} d'extrait sec. L'évolution du pH et la production d'acide lactique dans les deux laits ont également été comparées. Le lait de chèvre avait un pouvoir tampon plus élevé que le lait de vache. Ce pouvoir tampon augmentait avec l'extrait sec du lait reconstitué. Toutes les souches protéinase-positives ont eu un taux de croissance spécifique (μ_{max}) et un taux d'acidification plus élevés que les variants protéinase-négative. Le nombre de bactéries et la concentration en acide lactique, après 15 h d'incubation, étaient également plus élevés avec les souches protéinase-positives. Pour toutes les souches, le taux d'acidification et la concentration en acide lactique après 15 h d'incubation étaient significativement plus élevés dans le lait de chèvre reconstitué que dans celui de vache et augmentaient avec la teneur en extrait sec des laits reconstitués. Le μ_{max} et le nombre de bactéries viables obtenus après 15 h d'incubation étaient relativement proches dans les laits de chèvre et de vache reconstitués, excepté pour les souches Wg2S et Wg2L. Pour ces dernières, les valeurs de μ_{max} étaient significativement plus élevées dans le lait de chèvre, mais leur nombre après 15 h d'incubation était inférieur dans le lait de chèvre comparativement au lait de vache. Un découplage entre la croissance bactérienne des souches Wg2S et Wg2L et la production d'acide lactique a été observé dans le lait de chèvre. Globalement, le lait de chèvre reconstitué constitue un milieu approprié pour produire des ferments lactiques mésophiles. Cependant, pour prévenir le découplage avec certaines souches de lactocoques comme Wg2S et Wg2L, la durée d'incubation à 21 °C devrait être plus courte pour le lait de chèvre que pour le lait de vache reconstitué.

poudre de lait de chèvre / poudre de lait de vache / croissance de lactocoques / lait reconstitué / protéinase-négative

List of abbreviations: Prt$^+$: proteinase-positive; Prt$^-$: proteinase-negative; NCN: non-casein nitrogen; NPN: non-protein nitrogen; TN: total nitrogen; ΔB/ΔpH: buffer index; μ_{max}: maximum specific growth rate; LAB : lactic acid bacteria.

1. INTRODUCTION

Lactic acid bacteria (LAB) are widely used as starters to produce numerous fermented dairy products. The main function of these starters is the acidification of milk by the formation of lactic acid from lactose, a process that modifies the texture and flavor of dairy products. For most purposes, the ability to produce acid at a high and predictable rate is essential in cheese starters [8]. However, although the acidifying activity of different LAB has been studied worldwide in cow milk, very little scientific information is available on the growth of LAB in goat milk [2], especially with respect to reconstituted powders. The majority of the studies that have been carried out refer to the final acid production in goat milk. Dutta et al. [9] showed that bacterial growth and lactic acid production in goat and cow milks were similar. Casalta et al. [5] showed that the origin of the milk (cow, goat or ewe) influenced the acidification rate, which was generally higher in goat milk. Masle and Morgan [16] showed that lactic acid starter activity in goat milk was influenced by the composition of the milk, and notably by the buffering components such as proteins and minerals. Thus, improved activity of mesophilic lactic starters was observed in goat milks as a result of high protein and mineral levels. Ibrahim et al. [13] showed that after one day of incubation a cow milk culture had higher bacterial counts than goat milk culture, but that

after three days of incubation the bacterial count of the cow milk was markedly reduced, whereas no changes were observed in the goat milk. Ibrahim et al. [13] concluded that given that the death rate was higher in cow milk cultures than in goat milk cultures, goat milk is more suitable for the growth of lactic acid starters, as well as for the manufacture of fermented dairy products. Portmann and Pierre [19] showed that the growth of a lactic acid starter was similar in cow and goat milks heated between 70 and 90 °C. They recommended using LAB grown in goat milk to produce pure goat cheeses. These studies showed that mesophilic lactococci grew well in goat milk and that starters could be prepared on this substrate. However, the majority of the studies in the literature were conducted using fresh goat milk. No systematic study of the production of mesophilic starters in reconstituted goat milk has been reported. The use of reconstituted milk is strongly recommended for the production of starters [6]. Thus, there is a need for better knowledge about the growth of lactococci in reconstituted goat milk.

In Canada, the production of goat milk has increased by over 20% per year since 1995 and this is mainly linked to the production of goat cheeses [12]. Normally, to obtain a fermented goat milk product that can be marketed as a pure goat milk product, the manufacturer must prepare the starter in goat milk [1, 19]. Before the starter cultures became available as frozen concentrates or as dried preparations for direct vat inoculation, they were traditionally produced and maintained by propagation in reconstituted milk powder. Today, many Canadian dairy industries still use skim milk powder to produce lactic acid starters.

Among LAB, there are proteinase-positive (Prt^+) strains and proteinase-negative (Prt^-) variants. As Prt^- variants do not have proteinase to hydrolyze proteins, those variants stop growing in milk when the free amino acids and peptides have been consumed; in contrast, Prt^+ strains can continue to grow as a result of their proteolytic activity [17, 28]. The specific growth rate of Prt^+ strains and Prt^- variants is apparently associated with the composition of the culture medium [26]. The composition of goat milk is different from that of cow milk [15, 27]. Casalta et al. [5] showed that acidification in reconstituted goat, cow and ewe milks after 6 hours of incubation at 30 °C was faster with Prt^+ strains than with Prt^- variants. However, it is not known whether total solids have an influence on growth.

The objective of this study was to compare the growth and the lactic acid production of some Prt^+ and Prt^- strains of *Lactococcus lactis* in reconstituted goat and cow milks at different total solids contents in order to determine whether goat milk is an appropriate medium for the production of mesophilic LAB starters.

2. MATERIALS AND METHODS

2.1. Milk preparation

The skim cow milk powder was obtained from Agropur (Granby, Quebec, Canada). The goat milk powder was produced from bulk goat milk (Nubian, LaMancha, Saaneen, Alpine and Toggenburg breeds) purchased from La Laiterie Tournevent (Drummondville, Quebec, Canada) during the summer of 2005. The goat milk was skimmed by using a centrifuge (Alfa-Laval, Type 62181 m-60/1954, Uppsala, Sweden), evaporated to 400 $g \cdot kg^{-1}$ of total solids by means of an APV Anhydro evaporator type JPE (APV, Goldsboro, NC, USA) and dried using a rotary spray dryer (Niro Atomizer, P6,3, modelV, Copenhagen, Denmark). The dryer inlet and outlet temperatures were 185 and 85 °C, respectively. Both milk powders were vacuum packed and stored at 4 °C prior to use.

The cow and goat milk powders were rehydrated in water at 23 °C for a 1-h period to obtain 3 reconstituted cow milks at 90, 120 and 150 $g \cdot kg^{-1}$ dry matter and 3 reconstituted goat milks at 90, 120 and 150 $g \cdot kg^{-1}$ total solids. All the reconstituted milks were heated at 85 °C for 30 min, cooled in ice at 4 °C and distributed in 14-mL sterilized tubes. All the tubes were stored at 4 °C for 18 h prior to use for bacterial growth.

2.2. Analytical methods

The composition of the cow and goat milk powders was determined in duplicate. Dry matter was determined by air-drying at 100 °C [3], fat content was determined using the Mojonnier extraction procedure [4] and ash was determined by heating samples at 550 °C for 16 h in a muffle furnace [3]. Total nitrogen (TN) and non-casein nitrogen (NCN) were measured using the macro-Kjeldahl method [3]. The NCN was measured on the filtrate after casein precipitation at pH 4.6, whereas non-protein nitrogen (NPN) was determined on the filtrate obtained after milk protein removal (1:1) with 240 $g \cdot kg^{-1}$ trichloroacetic acid solution. Casein nitrogen was obtained by means of substraction (TN-NCN). A nitrogen-to-protein conversion factor of 6.38 was applied. The relative proportion of α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein in every powder was determined by high-performance liquid chromatography (HPLC) according to the method described by Jaubert and Martin [14]. Calcium and phosphorus were determined with an inductively coupled plasma spectrometer (model 3510 ICP Spectrometer, Applied Research Laboratories, Sunland, California, USA) according to the method described by St-Gelais et al. [24].

The buffering capacity was determined by titration with 0.36 g $HCl \cdot L^{-1}$ until a final pH of 4.6 as described by Van Slyke [29]. The buffer index was calculated by using the following equation:

$$\Delta B/\Delta pH = \frac{(\text{mL of HCl}) \times (\text{normality factor})}{(\text{volume of sample}) \times (\Delta pH)}. \quad (1)$$

2.3. Lactococci strains

A Prt^+ strain of *Lactoccocus lactis* ssp. *lactis* CNRZ-1076 (1076), and its Prt^- variant, CNRZ-1075 (1075), were obtained from the Centre de Recherches de Jouy-en-Josas (Institut National de Recherche Agronomique, Domaine de Vilvert, France). Two Prt^+ strains of *L. lactis* ssp. *cremoris*, Wg2S and E8S, and their Prt^- variants, Wg2L and E8L, were obtained from the Netherlands Institute for Dairy Research (NIZO, Ede, the Netherlands). Each lactococcal culture was kept in reconstituted skim milk (200 $g \cdot kg^{-1}$ dry matter) containing 50 $g \cdot kg^{-1}$ sucrose and 1.7 $g \cdot kg^{-1}$ ascorbic acid and was stored at –40 °C.

2.4. Culture and bacterial growth

Working cultures were propagated (inoculum size 100 $g \cdot kg^{-1}$) in reconstituted cow milk (120 $g \cdot kg^{-1}$ dry matter) supplemented with 2.0 $g \cdot kg^{-1}$ yeast extract (Difco Laboratories, Detroit, MI, USA) and sterilized at 110 °C for 10 min; incubation was performed at 21 °C for 15 h. For the second transfer, growth was performed in reconstituted cow milk without yeast extract for the Prt^+ strains and with 2.0 $g \cdot kg^{-1}$ yeast extract for the Prt^- variants at 30 °C for 6 h. The last transfer for all the strains was performed in M17 broth (Difco Laboratories, Detroit, MI, USA), which was incubated at 21 °C for 15 h. Active cultures were then centrifuged at 3000× *g* at 23 °C for 10 min (Centra-GP8R, International Equipment Company, Needham Heights, MA, USA). The pellet (bacteria) was diluted in a sterile saline solution (9.0 g $NaCl \cdot kg^{-1}$) to obtain an optical density of 1.40 (λ = 635 nm) using a Beckman spectrophotometer (DU Series 60 Spectrophotometer; Beckman Instruments Inc., Fullerton, CA, USA). The lactococci population in the diluted saline solution was approximately 8×10^8 $cfu \cdot mL^{-1}$ for all the strains.

All the reconstituted milks were inoculated with each diluted saline solution to obtain approximately 2×10^7 $cfu \cdot mL^{-1}$ and all the tubes were incubated at 21 °C for 15 h. This temperature is generally used by manufacturers to produce mesophilic lactic acid starters [6]. The population of the Prt^+ and Prt^- strains was determined after 0, 3, 6, 9, 12 and 15 h on M17 agar (Difco Laboratories, Detroit, MI, USA), which was incubated anaerobically at 30 °C for 48 h. Milk samples were diluted in 1.0 $g \cdot kg^{-1}$ peptone water. However, because some lactococci grow in long chains [10], 3 g of 4-mm solid glass beads were added to each

dilution bottle. To ensure disintegration of the long chains, all the dilution bottles were vigorously shaken 40 times before inoculation on M17 agar plates [25]. The μ_{max} were calculated during exponential growth using the following equation:

$$\ln X = \ln X_0 + \mu_{max} \times T \qquad (2)$$

where X_0 (intercept) is the biomass (cfu·mL^{-1}) when time T (h) = 0. The plot of ln$X$ against time is a straight line with a slope of μ_{max}.

Titratable acidity was determined with 4.4 g·L^{-1} NaOH and reported as grams of lactic acid per liter. The acidification rate for the Prt$^+$ strains was calculated using the following sigmoid equation:

$$\Delta LA = \frac{(a-d)}{\left(1+\left(\frac{t}{c}\right)^b\right)^e} + d \qquad (3)$$

where ΔLA = lactic acid produced (g·L^{-1}), a = minimum lactic acid concentration (which is 0), b = slope coefficient, c = inflection point coefficient, d = maximum lactic acid concentration at infinite time, e = a symmetric parameter, and t = time (h). These parameters (a, b, c, d and e) were obtained by curve fitting to the above model using SigmaPlot software version 8 (SPSS Inc., Chigaco, IL, USA). Equation (3) and its first and second derivatives [18] are used to determine the acidification rate at the inflection time. Because lactic acid production with the Prt$^-$ variants was linear (Fig. 2D), the acidification rate of those variants was calculated by using the following linear equation:

$$\Delta LA = a + bt \qquad (4)$$

where ΔLA = lactic acid produced (g·L^{-1}), a = concentration of lactic acid when t = 0 h, b = acidification rate, and t = time (h).

2.5. Statistical methods

Analysis of variance according to a split-plot design was applied to determine the effects of the types of milk, the concentration of milk total solids and the different strains of *L. lactis* on the maximum specific growth rate (μ_{max}), the population after 15 h of incubation, the acidification rate and the lactic acid concentration after 15 h of incubation. The experiment was performed in triplicate. In this design, types of milk, milk solids contents and replicates were the main plots, and strains were the subplots. These statistical analyses were performed with the general linear models procedure of SAS [22].

Table I. Composition of cow and goat milk powders (g·kg^{-1}).

Constituent[1]	Type of powder	
	Cow	Goat
Dry matter	960	962
Total proteins	348	381
Casein	270	273
α_{s1}-CN	119	46
α_{s2}-CN	18	51
β-CN	109	142
κ-CN	22	33
Whey protein	58	80
α-Lb	19	22
β-Lg	37	55
Non-protein nitrogen	21	28
Fat	8	11
Ash	79	94
Calcium	12	15
Phosphorus	8	12
Lactose	504	467

[1] CN = casein; α-Lb = α-lactalbumin; β-Lg = β-lactoglobulin.

3. RESULTS

3.1. Composition of milk powders

The composition of goat and cow milk powders is presented in Table I. Dry matter contents were close in both types of milk powder. Total protein content was higher in goat milk powder than in cow milk powder, mostly due to the higher whey protein and NPN contents of goat milk powder. In goat

Table II. Probability values[1] for the effect of types of milk (cow and goat) and levels of solids (90, 120 and 150 g·kg^{-1}) on the buffering capacity of reconstituted milks.

	Buffering capacity
Main factors	
Milks	0.0002
Solids	0.0001
Interaction	
Milks × Solids	0.03

[1] Probability values ≤ 0.05 are considered statistically significant.

milk powder, the concentration of fat, ashes, calcium and phosphorus was higher, whereas the lactose content was lower. The casein contents in both types of milk powder were similar, although the α_{s2}-casein, β-casein and κ-casein contents were higher and the α_{s1}-casein content was lower in goat milk than in cow milk. The α-lactalbumin and β-lactoglobulin contents were also higher in the goat milk powder.

3.2. Buffering capacity

Table II presents the significant effects of types of milk and total solid contents on the buffering capacity of reconstituted milks. All the main factors had a significant effect on the buffering capacity, and a significant interaction between types of milk and total solid contents was also observed. Buffering capacity increased significantly with the milk total solids and was higher for goat milk, especially at 120 and 150 g·kg^{-1} total solids (Fig. 1).

3.3. Growth, pH evolution and lactic acid production

Table III presents the significant effects of the main factors as well as of the various significant interactions between types of milk, total solid contents and strains on μ_{max}, total lactococci population after 15 h of incubation, acidification rate for the Prt$^+$ and Prt$^-$ strains and the lactic acid concentration for the Prt$^+$ and Prt$^-$ strains obtained after 15 h of incubation. The types of milk and strains had a significant effect on all the measured parameters. The level of solids also had a significant effect on all the parameters, with the exception of the μ_{max}. A significant interaction between strains and types of milk was observed for all the measured parameters. In addition, a significant interaction between strains and level of solids was observed for the acidification rate and the lactic acid concentration after 15 h of incubation for the Prt$^-$ variants. However,

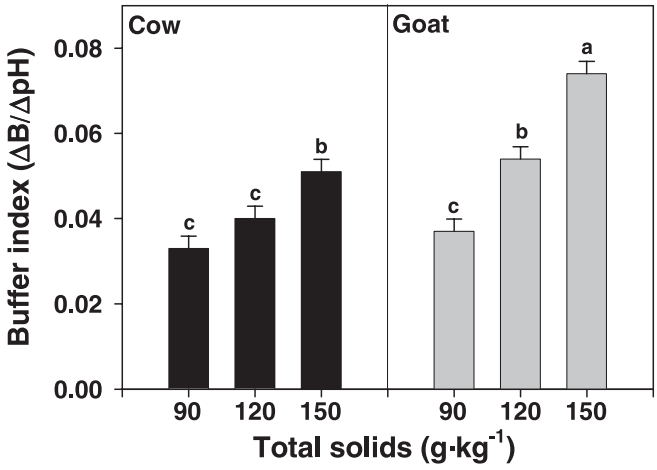

Figure 1. Buffer index of cow and goat milk reconstituted at 90, 120 and 150 g·kg^{-1} total solids.

Table III. Probability values[1] for the effect of types of milk (cow and goat), levels of solids (90, 120 and 150 g·kg^{-1}) and strains of lactococci (Prt$^+$ and Prt$^-$) on different measured parameters.

	μ_{max}	Population count after 15 h	Prt$^+$ acidification rate	Prt$^-$ acidification rate	Prt$^+$ acid lactic content after 15 h	Prt$^-$ acid lactic content after 15 h
Main factors						
Milks	0.03	0.0001	0.0001	0.0001	0.0001	0.0001
Solids	0.25	0.0001	0.0001	0.0001	0.0001	0.0001
Strains	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interactions						
Milks × Solids	0.11	0.28	0.27	0.52	0.10	0.35
Strains × Milks	0.02	0.02	0.006	0.0001	0.002	0.01
Strains × Solids	0.49	0.15	0.44	0.0001	0.53	0.002
Strains × Milks × Solids	0.30	0.11	0.70	0.43	0.42	0.32

[1] Probability values ≤ 0.05 are considered statistically significant.

no significant double interactions between the types of milk and level of solids or triple interactions between strains, types of milk and level of solids were observed for any of the measured parameters.

The growth of the Prt$^+$ and Prt$^-$ strains in reconstituted cow and goat milk powders is shown in Figure 2A. The curves correspond to the combined means of the 3 levels of solid contents. The growth of all lactococci in reconstituted cow and goat milks at 90, 120 and 150 g·kg^{-1} total solids is shown in Figure 3A. The curves correspond to the combined means of the 3 Prt$^+$ and 3 Prt$^-$ strains. All the lactococci grew in all the reconstituted cow and goat milks. However, the growth of all the Prt$^-$ variants was slower than that of the Prt$^+$ strains (Fig. 2A). The stationary phase for the Prt$^+$ strains was reached between 9 and 12 h, while it was reached after 6 h for the Prt$^-$ variants. The lactococci population increased as the concentration of milk total solids increased (Fig. 3A).

The μ_{max}, calculated by using equation (2), and the total lactococci population after 15 h of incubation according to the type of milk, concentration of total solids and strain are presented in Table IV. The μ_{max} for the Prt$^+$ strains was significantly higher than the μ_{max} for the Prt$^-$ variants. The μ_{max} was also higher in goat milk than in cow milk, but only for the Wg2S and Wg2L strains. For the other strains, the μ_{max} was similar in both types of reconstituted milk. In addition, the μ_{max} increased significantly as the total solid contents increased in the milk, but only for the 1076 and 1075 strains. For the other strains (E8S, E8L, Wg2S and Wg2L) the μ_{max} was similar whatever the concentration of total solids.

The populations of all the Prt$^+$ strains after 15 h of incubation were significantly higher than those for the Prt$^-$ variants (Tab. IV). The populations for the Wg2S and Wg2L strains were significantly lower in reconstituted goat milk than in cow milk, while the populations for the other strains were not significantly different in either type of milk. In addition, the populations increased significantly with the milk total solid contents, with the exception of the populations for the Wg2S and Wg2L strains, which stayed relatively constant.

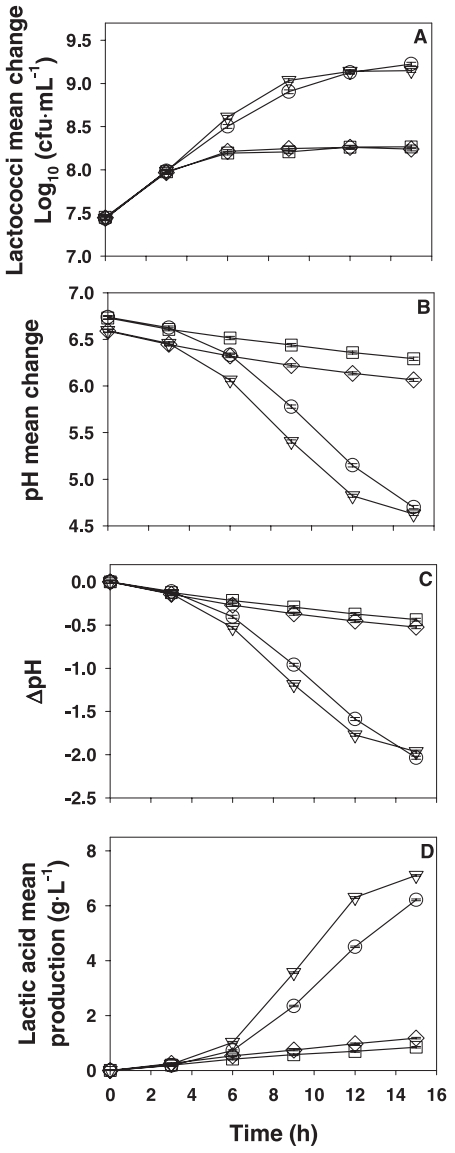

Figure 2. Evolution of population mean (A), pH mean (B), ΔpH mean (C) and lactic acid concentration mean (D) of proteinase-positive (Prt^+) and proteinase-negative (Prt^-) strains of lactococci in rehydrated goat and cow milk powders. Prt^+ strains in cow milk (○), Prt^+ strains in goat milk (▽), Prt^- variants in cow milk (□), and Prt^- variants in goat milk (◇). The curves correspond to the means of the three levels of solid contents. Error bars represent the standard error of the means.

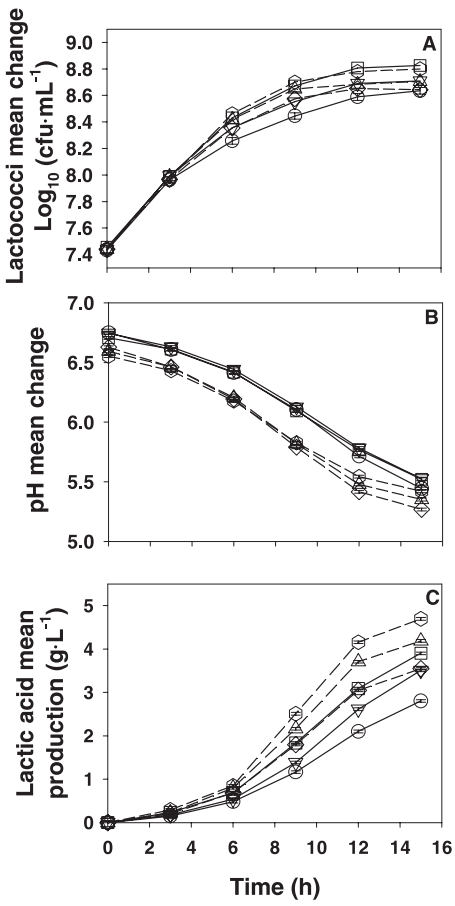

Figure 3. Evolution of population mean (A), pH mean (B) and lactic acid concentration mean (C) of lactococci in goat and cow milk reconstituted at 90, 120 and 150 $g \cdot kg^{-1}$. Cow milk at 90 $g \cdot kg^{-1}$ total solids (○), cow milk at 120 $g \cdot kg^{-1}$ total solids (▽), cow milk at 150 $g \cdot kg^{-1}$ total solids (□), goat milk at 90 $g \cdot kg^{-1}$ total solids (◇), goat milk at 120 $g \cdot kg^{-1}$ total solids (△), and goat milk at 150 $g \cdot kg^{-1}$ total solids (⬡). The curves correspond to means of the three Prt^+ and three Prt^- strains. Error bars represent the standard error of the means.

The pH evolution with the Prt^+ and Prt^- strains in reconstituted cow and goat milks is presented in Figure 2B. The curves correspond to the combined means of the 3 levels of solid contents. The pH evolution in reconstituted cow and goat milks at 90, 120 and 150 $g \cdot kg^{-1}$ total solids is presented in

Table IV. Maximum specific growth rate means and total lactococcal population means after 15 h of incubation of different lactococci in reconstituted cow and goat milks.

Lactococci[1]	Type of milk			Total solids ($g \cdot kg^{-1}$)			
	Cow	Goat	SEM[2]	90	120	150	SEM
	Maximum specific growth rate						
E8S	0.52^{a}	0.53^{a}	0.02	0.52^{ab}	0.54^{a}	0.52^{ab}	0.02
Wg2S	0.44^{de}	0.51^{ab}	0.02	0.46^{cde}	0.48^{bcd}	0.48^{bcd}	0.02
1076	0.51^{ab}	0.50^{abc}	0.02	0.46^{cde}	0.50^{abc}	0.52^{ab}	0.02
E8L	0.44^{de}	0.47^{bcd}	0.02	0.45^{cde}	0.44^{def}	0.47^{cde}	0.02
Wg2L	0.37^{f}	0.45^{cd}	0.02	0.43^{def}	0.42^{efg}	0.40^{fg}	0.02
1075	0.43^{de}	0.40^{ef}	0.02	0.37^{g}	0.42^{efg}	0.44^{def}	0.02
	Lactococcal population ($\log_{10}$ ($cfu \cdot mL^{-1}$))						
E8S	9.13^{c}	9.15^{bc}	0.03	9.02^{f}	9.17^{cd}	9.23^{bc}	0.03
Wg2S	9.21^{b}	8.99^{d}	0.03	9.11^{de}	9.13^{de}	9.06^{ef}	0.03
1076	9.35^{a}	9.30^{a}	0.03	9.27^{b}	9.31^{ab}	9.39^{a}	0.03
E8L	8.30^{f}	8.31^{f}	0.03	8.21^{i}	8.33^{h}	8.38^{h}	0.03
Wg2L	8.15^{g}	8.04^{h}	0.03	8.05^{j}	8.12^{ij}	8.13^{ij}	0.03
1075	8.43^{e}	8.37^{ef}	0.03	8.30^{h}	8.40^{gh}	8.48^{g}	0.03

[1] E8S, Wg2S and 1076 = proteinase-positive strains; E8L, Wg2L and 1075 = proteinase-negative variants.
[2] SEM = standard error of the means.
Means with different superscripts differ significantly ($P \leq 0.05$).

Figure 3B. The curves correspond to the combined means of the 3 Prt^+ and 3 Prt^- strains. The pH value of reconstituted goat milk before inoculation was 6.59, compared with 6.73 for reconstituted cow milks. During milk incubation the pH values for goat milk were always lower than those for cow milk. The pH evolution for both types of milk seemed to be relatively close. In fact, when the pH is expressed as delta pH (Fig. 2C), the curves for goat milk decreased slightly faster than those for cow milk. In addition, after 9 h (Fig. 3B), the pH decreased more slowly as the total solid contents increased in the milk, especially in goat milk. The pH evolution was also faster with Prt^+ strains than with Prt^- variants (Fig. 2B).

Lactic acid production for the Prt^+ and Prt^- strains of lactococci in reconstituted cow and goat milks is presented in Figure 2D. The curves correspond to the combined means of the 3 levels of solid contents. Mean lactic acid production in reconstituted cow and goat milks at 90, 120 and 150 $g \cdot kg^{-1}$ total solids is presented in Figure 3C. The curves correspond to the combined means of the 3 Prt^+ and 3 Prt^- strains. Lactic acid production was higher with the Prt^+ strains than with the Prt^- variants and was faster and higher in goat milk than in cow milk (Fig. 2D). In addition, the lactic acid production increased with milk total solid contents and was higher in goat milk than in cow milk (Fig. 3C).

Table V. Acidification rate means and lactic acid concentration means after 15 h of incubation of different lactococci in reconstituted cow and goat milks.

Lactococci[1]	Type of milk			Total solids ($g \cdot kg^{-1}$)			
	Cow	Goat	SEM[2]	90	120	150	SEM[2]
	Acidification rate						
E8S	0.92^{c}	1.33^{a}	0.06	0.93^{b}	1.13^{a}	1.31^{a}	0.08
Wg2S	0.50^{e}	0.72^{d}	0.06	0.51^{d}	0.69^{c}	0.64^{cd}	0.08
1076	0.51^{e}	1.14^{b}	0.06	0.71^{c}	0.81^{bc}	0.94^{b}	0.08
E8L	0.08^{g}	0.11^{f}	0.003	0.07^{g}	0.09^{f}	0.13^{e}	0.004
Wg2L	0.05^{h}	0.06^{h}	0.003	0.05^{ij}	0.06^{gh}	0.07^{g}	0.004
1075	0.04^{i}	0.06^{h}	0.003	0.04^{j}	0.05^{hi}	0.07^{g}	0.004
	Lactic acid concentration ($g \cdot L^{-1}$)						
E8S	7.02^{b}	7.93^{a}	0.14	6.22^{de}	7.59^{b}	8.61^{a}	0.23
Wg2S	6.46^{c}	6.82^{b}	0.14	5.52^{fg}	6.77^{cd}	7.70^{b}	0.23
1076	5.30^{d}	7.05^{b}	0.14	5.15^{g}	6.00^{ef}	7.36^{bc}	0.23
E8L	1.15^{f}	1.55^{e}	0.04	1.02^{j}	1.24^{i}	1.78^{h}	0.05
Wg2L	0.89^{h}	1.05^{fg}	0.04	0.76^{l}	0.94^{jk}	1.21^{i}	0.05
1075	0.64^{i}	0.99^{gh}	0.04	0.63^{m}	0.81^{kl}	1.00^{j}	0.05

[1] E8S, Wg2S and 1076 = proteinase-positive strains; E8L, Wg2L and 1075 = proteinase-negative variants.
[2] SEM = standard error of the means.
Means with different superscripts differ significantly ($P \leq 0.05$).

Table V presents the acidification rates and the lactic acid concentrations after 15 h of incubation calculated using equation (3) for the Prt$^+$ strains and equation (4) for the Prt$^-$ variants, allowing for comparison of the effects of the type of milk, concentration of total solids and strain. The acidification rate was higher for the Prt$^+$ strains than for the Prt$^-$ variants and was significantly higher in goat milk than in cow milk. In addition, the acidification rate increased significantly with the milk total solid contents, especially for the Prt$^+$ strains. The E8S strain had the best acidification rate among the Prt$^+$ strains, and E8L had the best acidification rate among the Prt$^-$ variants. Lactic acid production after 15 h of incubation was significantly lower for the Prt$^-$ variants than for the Prt$^+$ strains (Tab. V) and was significantly higher in goat milk than in cow milks. In addition, for all the lactococcal strains, the lactic acid concentration after 15 h increased significantly with the milk total solid contents. The highest contents were obtained with E8S for the Prt$^+$ strains and E8L for the Prt$^-$ variants.

The behavior of the Wg2S strain and its variant was different comparatively to the other Prt$^+$ and Prt$^-$ strains. To demonstrate it, the population and the lactic acid concentration obtained for the E8S and Wg2S strains in reconstituted cow and goat milks at 90, 120 and 150 $g \cdot kg^{-1}$ total solids after 15 h of incubation are presented in Figure 4. Similar comparative observations could be done between Wg2S or Wg2L and the other

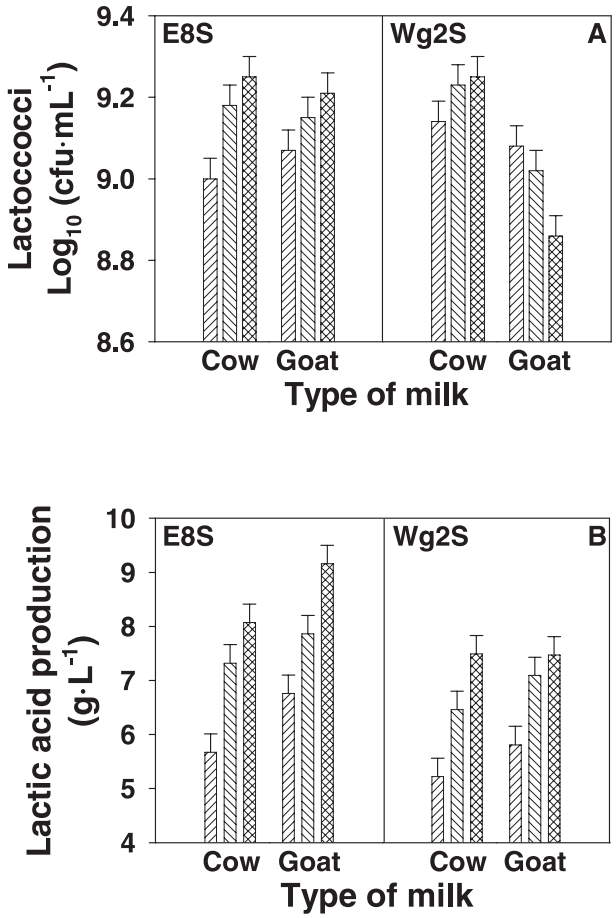

Figure 4. Lactococci count (A) and lactic acid production (B) after 15 h of incubation of the E8S and Wg2S strains in reconstituted cow and goat milks at 90 (▨), 120 (▧) and 150 (▩) $g \cdot kg^{-1}$ total solids. Error bars represent the standard error of the means.

strains (data not shown). The population of the E8S strain increased with the level of total solids in reconstituted cow and goat milks. The bacterial count of the Wg2S strain also increased with the level of total solids, but only in reconstituted cow milk. In reconstituted goat milk, the population of the Wg2S decreased with the level of total solids. However, for the E8S and Wg2S strains the lactic acid concentration increased with the level of total solids in reconstituted cow and goat milks.

4. DISCUSSION

In this study, the total proteins, whey protein, NPN, calcium and phosphorus contents were higher in goat milk powder than in cow milk powder. These results were similar to those obtained by Ibrahim et al. [13]. The composition of the milk can affect the buffering capacity, which increases with the total solids [21, 25, 27]. Our results confirm the literature in this respect. The buffer index of reconstituted

milks increased with the milk total solid contents and was higher for goat milk than for cow milk. These results seem to differ from those obtained by Casalta et al. [5], who reported that goat milk had a lower buffering capacity than cow milk because of the low casein and mineral contents in goat milk. However, the composition of goat milk depends on the breed of goat [27]. In Canada, there are five goat breeds, and some of them produce milk with buffering capacities similar to or higher than cow milk [27]. In this study, the goat milk powder was obtained from bulk milk (five goat breeds). Srilaorkul et al. [23] determined that the contribution of casein, whey protein and salts to the total buffer system intensity of cow milk is 36.0, 5.0 and 59.0%, respectively. Therefore, even though the casein contents between cow and goat milk powders were close, the fact that the whey protein and mineral contents, and particularly the phosphorus and calcium contents, were higher in goat milk powder than in cow milk powder could explain the higher buffering capacity of reconstituted goat milk at identical total non-fat solid contents.

Among lactococci, there are proteolytic strains and nonproteolytic variants. In this study, the Prt^+ strains (E8S, Wg2S and 1076) grew well in reconstituted cow and goat milks and stopped multiplying after 12 h of incubation, while the Prt^- variants (E8L, Wg2L and 1075) did not grow very well, and their viable counts stopped increasing after 6 h. These results indicate that, in both types of reconstituted milk, the Prt^- variants probably stopped growing when the free amino acids and peptides had been consumed, whereas the proteolytic activity allowed the Prt^+ growth to continue. The lactic acid production and the acidification rate were also higher for the Prt^+ strains than for the Prt^- variants. These results obtained with reconstituted cow and goat milks are in agreement with those obtained by St-Gelais et al. [25], who found that the Prt^- variants E8L, Wg2L and 1075 did not grow very well in reconstituted cow milk, because these nonproteolytic strains could not hydrolyze proteins. Casalta et al. [5] observed that the Prt^- variants they used could acidify goat milk to a pH of 5.5 after 9 h of incubation, whereas cow milk was not acidified. Their results are different from those obtained in this study. The E8L, Wg2L and 1075 variants slightly acidified the reconstituted goat and cow milks. The pH value obtained after 15 h of incubation with those variants was above 6.0. These discrepancies could be due to the types of strains used to acidify the milks, to the varying buffering power of goat milk and to the treatment used to heat reconstituted milks. Casalta et al. [5] isolated their Prt^- variants from goat cheese; those variants were potentially more adapted to growth in goat milk than the variants used in this study. In addition, they heated their reconstituted milks at 63 °C for 30 min instead of 85 °C for 30 min in this study. Chavarri et al. [7] showed that the heat treatment used to heat milk can modify the lactic acid production by mesophilic bacteria.

The composition and the buffering capacity of milk can affect the growth and acidification rate of lactic acid starters [5, 9, 13, 16, 25]. Masle and Morgan [16] showed that the acidification capacity of lactic acid starters in goat milk was influenced by variations in the composition of the milk, mainly the mineral and protein contents. The results obtained in this study confirm this observation. Even though the μ_{max} were similar when the total solids in reconstituted milks increased, the acidification rate and lactic acid concentration measured for the Prt^+ and Prt^- strains after 15 h of incubation increased with the milk total solid contents and with the buffering capacity of the milk. Moreover, the pH fall was slower in reconstituted milks (and in goat milk especially) at 150 $g \cdot kg^{-1}$ total solids than in reconstituted milks at 90 $g \cdot kg^{-1}$, even though the lactic acid concentration was higher in reconstituted milks (and in goat milks especially) at 150 $g \cdot kg^{-1}$.

Normally, the high buffering capacity of milk can act as a protective mechanism for the bacteria [16, 21]. In this study, however, the bacterial growth in reconstituted goat milk slowed down after 9 h of fermentation as compared with cow milk, especially at 90 and 120 $g \cdot kg^{-1}$ total solids, even though the buffer index was higher in the goat milk. This observation was mainly due to the

Wg2S and Wg2L strains, which had lower viable counts after 15 h of incubation in goat milk than in cow milk, when compared with the other strains. Ibrahim et al. [13] observed with their lactococcal strains that cow milk allowed a higher bacterial count than goat milk after one day of incubation. After 3 days of incubation, however, the bacterial count in cow milk was reduced, and no change was observed in goat milk. These observations by Ibrahim et al. [13] were slightly different from those obtained in this study and seem to have been dependent on the types of starters used to inoculate the milk. The populations of the E8S, E8L, 1076 and 1075 strains after 15 h of incubation were not significantly different in reconstituted goat and cow milks, but the bacterial count for the Wg2S and Wg2L strains was significantly lower in goat milk and decreased with the level of total solids. So, an uncoupling was observed with the strains Wg2S and Wg2L in reconstituted goat milks. These strains stopped growing but the lactic acid production continued. In general, uncoupling is not desired during the production of mesophilic lactic acid starter, because the lactic acid accumulation in milk could affect the activities of bacteria (over-ripeness) [11, 20]. The incubation of the Wg2S and Wg2L strains should be shorter in reconstituted goat milk to prevent an uncoupling and over-ripeness [20].

5. CONCLUSION

The results presented in this study reveal that reconstituted goat milk is an appropriate medium for the production of mesophilic lactic acid bacteria starters. In general, the performance of lactic acid starter strains in reconstituted goat milk seems to be similar to the performance in fresh goat milk that has been reported in the literature. The higher buffering capacity observed in reconstituted goat milk allowed us to obtain a better bacterial growth and lactic acid production than in reconstituted cow milk with the exception of the Wg2S and Wg2L strains. For these strains, an uncoupling was observed in goat milk. To prevent an uncoupling and an over-ripeness with some strains, like the Wg2S and Wg2L strains, incubation at 21 °C could therefore be shorter in reconstituted goat milk than in reconstituted cow milk. Further investigation is required to determine the effect of the origin of the milk on the subsequent specific activity of lactococci in the manufacturing of fresh goat cheese.

Acknowledgments: The authors thank Gaétan Bélanger and Caroline Lapointe for their technical assistance. The authors also extend their appreciation to Claude P. Champagne for his review of this manuscript.

REFERENCES

[1] Abrahamsen R.K., Rysstad G., Fermentation of goats' milk with yogurt starter bacteria: A review, Cult. Dairy Prod. J. 26 (1991) 20–26.

[2] Alonso-Calleja C., Carballo J., Capita R., Bernardo A., Gracia-Lopez M.L., Comparison of the acidifying activity of *Lactococcus lactis* subsp. *lactis* strains isolated from goat's milk and Valdeteja cheese, Letters Appl. Microbiol. 34 (2002) 134–138.

[3] AOAC, Dairy Products, Official Methods of Analysis, Vol II, Chapter 33, 16th edn. Association of Official Analytical Chemist International, Arlington, VA, 1995.

[4] Atherton H.V., Newlander J.A., Tests for fat: Babcock, Gerber, and Mojonnier, in: Chemistry and testing of dairy products, 4th edn., Avi Publishing Co, Inc., Westport, CT, 1977, p. 71.

[5] Casalta E., Vassal Y., Desmazeaud M.J., Casabianca R., Comparaison de l'activité acidifiante de souches de *Lactococcus lactis* isolées de lait et de fromage de Corse, Lebensm. Wiss. Technol. 28 (1995) 291–299.

[6] Champagne C.P., Audet P., Préparation des ferments en usine, in: La Fondation des Gouverneurs and Edisem (Ed.), Production de ferments lactiques dans l'industrie laitière, Saint-Hyacinthe, Québec, Canada, 1998, pp. 117–151.

[7] Chavarri F.J., Numez J.A., Numez M., Behaviour of *Streptococcus lactis* in heat treated (80 °C for 30 min) or sterilised cow's or ewe's milk, J. Dairy Res. 50 (1983) 357–363.

[8] Cogan T.M., Hill C., Cheese starter cultures, in: Fox P.F. (Ed.), Cheese: chemistry, physics and microbiology, Chapman and Hall, London, UK, 1993, pp. 193–255.

[9] Dutta S.M., Kuila R.K., Ranganathan B., Laxminarayana H., A comparative study of the activity of starter cultures in different types of milk, Milchwissenschaft 26 (1971) 158–161.

[10] Emmons D.B., Elliot J.A., Beckett D.C., Effect of lactic streptococcal agglutinins in milk on curd formation and manufacture of cottage cheese, J. Dairy Sci. 49 (1966) 1357–1366.

[11] Harvey R.J., Damage to *Streptococcus lactis* resulting from growth at low pH, J. Bacteriol. 90 (1965) 1330–1336.

[12] Ianni R., L'industrie de la chèvre laitière. Un portrait canadien, Agriculture and Agro Food Canada, Ottawa, Canada, 2002, pp. 1–16.

[13] Ibrahim E.M., Movran M.A., Hanafy N.E., Growth and biochemical behaviour of some lactic acid starters in cow's and goat's milk, Assiut J. Agric. Sci. 21 (1990) 205–219.

[14] Jaubert A., Martin P., Reverse-phase HPLC analysis of goat caseins; identification of α_{s1} and α_{s2} genetic variants, Lait 72 (1992) 235–247.

[15] Jenness R., Composition and characteristics of goat milk: review 1968–1979, J. Dairy Sci. 63 (1980) 1605–1630.

[16] Masle I., Morgan F., Compositional factors involved in the variable acidification capacity of goat milk by lactic starters, Lait 81 (2001) 561–569.

[17] Otto R., de Vos W.M., Gavrieli J., Plasmid DNA in *Streptococcus cremoris* Wg2: Influence of pH on selection in chemostats of a variant lacking a protease plasmid, Appl. Environ. Microbiol. 43 (1982) 1272–1277.

[18] Pandey P.K., Ramaswamy H.S., St-Gelais D., Effect of high pressure processing on rennet coagulation properties of milk, Innov. Food Sci. Emerging Technol. 4 (2003) 245–256.

[19] Portmann A., Pierre A., Culture de ferments lactiques dans le lait de chèvre comparaison avec le lait de vache, Revue Laitière Française 312 (1973) 629–637.

[20] Ross G.D., Observation on the effect of inoculum pH on the growth and acid production of lactic streptococci in milk, Aust. J. Dairy Technol. (1980) 147–149.

[21] Salaun F., Mietton B., Gaucheron F., Buffering capacity of dairy products, Int. Dairy J. 15 (2005) 95–109.

[22] SAS® User's Guide: Statistics, Version 8.2 edition. SAS Inst., Inc., Cary, NC, 1999.

[23] Srilaorkul S., Ozimek L., Wolfe F., Dziuba J., The effect of ultrafiltration on physicochemical properties of retentate, Can. Inst. Food Sci. technol. J. 22 (1989) 56–62.

[24] St-Gelais D., Haché S., Gros-Louis M., Combined effects of temperature, acidification, and diafiltration on composition of skim milk retentate and permeate, J. Dairy Sci. 75 (1992) 1167–1172.

[25] St-Gelais D., Roy D., Haché S., Growth and activities of *Lactococcus lactis* in milk enriched with low mineral retentate powders, J. Dairy Sci. 75 (1992) 2344–2352.

[26] St-Gelais D., Roy D., Haché S., Desjardins M.L., Gauthier S.F., Growth of nonproteolytic *lactococcus lactis* in culture medium supplemented with different casein hydrolyzates, J. Dairy Sci. 76 (1993) 3327–3337.

[27] St-Gelais D., Turcot S., Ould Baba Ali A., Chemical composition and properties of milk from five goat breeds, Milchwissenschaft 60 (2005) 140–143.

[28] Thomas T.D., Pritchard G.G., Proteolytic enzymes of dairy starter cultures, FEMS Microbiol. Rev. 46 (1987) 245–268.

[29] Van Slyke D.D., On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and concentration and reaction of the buffer solution, J. Biol. Chem. 52 (1922) 525–570.