Effect of Continuous Prefermentation of Milk with an Immobilized Cell Bioreactor on Fermentation Kinetics and Curd Properties

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ABSTRACT

The production of fresh cheese involves a complex fermentation of milk with a mixed culture of mesophilic lactic acid bacteria. The aim of this work was to compare the performance of the traditional batch fermentation process with a new process integrating a continuous prefermentation step with an immobilized cell bioreactor. Acidification and coagulation kinetics, as well as microbiological composition and rheological and sensory properties of the final curd, were studied for the two processes during experiments conducted at 22, 26, and 30°C. At 26°C, the prefermentation step reduced the time to reach the draining pH by 10%, and coagulation time was reduced by 30%. The microbiological composition of the inoculum was more reproducible using an immobilized starter culture than a bulk starter culture. However, one strain of citrate-utilizing *Lactococcus lactis* ssp. *lactis* dominated, representing 80% of the bacterial population in the prefermented milk and in the resulting curd and 60% of the total population in the curd made from batch fermentation. Compared with the batch process, the continuous prefermentation of milk using an immobilized cell bioreactor had no significant effect on the rheological properties of the curd (susceptibility to syneresis, firmness, and modulus of elasticity) or on sensory properties of the final fresh cheese.

(**Key words**: continuous fermentation, immobilized cells, fresh cheese production)

Abbreviation key: **Cit$^+$** = citrate-utilizing.

INTRODUCTION

The manufacture of fresh cheese is a multiple-stage process with some continuous and some batch operations. First, milk is continuously standardized, pasteurized, and filled into large tanks (10,000 to 50,000 L) to which bulk starter culture and rennet are added. The starter culture is a mixture of acidifying and aromatic strains of mesophilic lactic acid bacteria containing different species of lactococci and leuconostoc. Fermentation occurs in batches, usually during the night because of the long incubation times (typically from 16 to 24 h) required to obtain the final lactic curd. After batch fermentation, the lactic curd is then continuously drained by centrifugation (15).

Immobilized cell technology has been proposed for the continuous inoculation and preacidification of cheese milk. Prematuration of milk for fresh cheese manufacture using an immobilized cell bioreactor has been studied by various researchers (3, 6, 7, 14, 17, 19, 20). An immobilized cell bioreactor can be used for a simple milk preacidification at pH ranging from 6.2 to 5.6. Milk inoculation is then performed with a bulk starter culture (3, 6, 7). An immobilized cell bioreactor may also be used to inoculate and acidify milk simultaneously because of the growing activity of the immobilized culture and the resulting cell release into the bulk medium (14, 17, 19, 20). Such a continuous process presents some advantages, such as shorter fermentation times (17, 20) and reduced risks of contamination with undesirable microorganisms and bacteriophages (2, 19, 21). Furthermore, this process eliminates the preparation of bulk starter cultures.

A previous report (20) studied continuous inoculation and milk acidification using immobilized lactic acid bacteria with four strains of mesophilic lactic acid bacteria that had been separately entrapped. The immobilized cell bioreactor had very high productivity and good microbiological stability when operated with UHT milk. At pH 6.2 and at 30°C, the residence time of milk in the bioreactor was very short (less than 2 min), and a massive inoculation of milk with the starter culture (more than 10^8 cfu/ml) was observed (20). Productivity increased further by 70% when pH was controlled at 6.4 by adding fresh milk than when

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pH was controlled at 6.2. This study specially studied the fermentation step following the continuous milk preacidification step. Fermentation, coagulation kinetics, microbiological composition, and rheological and sensory properties of the curd prepared from pasteurized cheese milk were evaluated at different temperatures and compared with those of batch cultures.

MATERIALS AND METHODS

Chemicals

κ-Carrageenan (Satiagel MR150) and locust bean gum were obtained from Sanofi Bio Industries (Villacoublay, France). Sunflower oil was commercial grade product. Pasteurized (75°C for 15 s) skim milk (Ferme Expérimentale, Institut National Agronomique, Grignon, France) was enriched with retentate powder from whey ultrafiltration (Proseca, Epinay sur Orge, France). Calf rennet of active chymosin at 520 mg/L (Chr. Hansen, Arpajon, France) was added to milk for coagulation.

Bacteria and Culture Conditions

The starter culture was composed of four strains of mesophilic lactic acid bacteria: *Lactococcus lactis* ssp. *lactis* CNRZ 144 and *Lactococcus lactis* ssp. *cremoris* CNRZ E8 (Centre National de Recherches Zootechniques, Jouy-en Josas, France), *Leuconostoc mesenteroides* ssp. *mesenteroides* X2 (Moorepark Research Center, Cork, Ireland), and citrate-utilizing (**Cit$^+$**) *Lactococcus lactis* ssp. *lactis* CDI1 (Centre de Recherches International André Gaillard, Yoplait, Ivry-sur Seine, France). The strains were kept frozen in skim milk at –20°C and then were reactivated and cultured for 8 h at 30°C before use. Lactococci were routinely grown in M17 broth (Biokar, Beauvais, France), and *Leu. mesenteroides* ssp. *mesenteroides* X2 was grown in MRS broth (Biokar).

Cell Immobilization

Each strain of the mixed culture was immobilized separately in κ-carrageenan and locust bean gum gel beads using a two-phase dispersion technique (9), as has been previously described by Sodini-Gallot et al. (20). For each strain, the beads were incubated in M17 broth (for lactococci) and MRS broth (for leuconostoc) with added KCl (0.2 *M*) and lactose or glucose (50 g/L), respectively, to increase the entrapped population. Two batch cultures of 16 and 6 h were performed in a 2-L stirred bioreactor (Biolafitte, Saint-Germain en Laye, France) at 30°C. The pH was controlled at 6.5 using 6 *N* NaOH. The immobilized population increased from 5.7×10^8 to 2.3×10^{11} cfu/g during this operation. Colonized gel beads in the range of 1.0 to 2.0 mm were recovered by wet-sifting and were used to inoculate the immobilized cell bioreactor.

Continuous Milk Prefermentation

A 2-L stirred bioreactor (Biolafitte, Saint-Germain-en-Laye, France), with a useful volume of 1 L, temperature controlled at 30°C, and agitation rate of 120 rpm, was used to preacidify and inoculate the milk continuously (20). A 250-ml volume of colonized gel beads was used to inoculate the bioreactor; this inoculum consisted of 35% (vol/vol) of *L. lactis* ssp. *lactis* CNRZ 144, 35% of *L. lactis* ssp. *cremoris* CNRZ E8, 25% of Cit$^+$ *L. lactis* ssp. *lactis* CDI1, and 5% of *Leu. mesenteroides* ssp. *mesenteroides* X2. The pH was measured by a pH electrode (Ingold, Paris, France) connected to a pH controller (Setric GI, Toulouse, France). The reactor was rinsed with 4 L of milk to eliminate the bead storage solution. Then, a proportional integrated derived regulation maintained the pH at 6.4 in the reactor by controlling the milk feeding. A consistent flow rate of 41 L of milk/h was obtained after about 1 h of operation. Effluent milk was transferred to a 15-L fermenter, and the temperature was controlled at different set points for the final fermentation. The immobilized cell bioreactor was interrupted after 3 h of operation by decreasing the temperature from 30 to 4°C and rinsing the system with 10 L of KCl solution (0.2 *M*). The beads were then stored 2 or 4 d at 4°C in a solution of KCl (0.2 *M*) and citrate buffer (0.03 *M*) at pH 5.6 until the next prefermentation experiment.

Bulk Starter Production

The four strains were first grown in tubes at 30°C overnight. Milk was used as the media for lactococci, and MRS broth was used for *Leu. mesenteroides* ssp. *mesenteroides* X2. The cultures were then transferred at 1% (vol/vol) to flasks containing 150 ml of milk homogeneously that was enriched with 1.5% (wt/vol) of yeast extract for *Leu. mesenteroides* X2 and incubated at 30°C for 8 h (first transfer). The resulting cultures were used to inoculate (1%, vol/vol) a flask containing 150 ml of milk to prepare the mixed starter culture (second transfer) using the same composition as for the bead inoculum: 35% (vol/vol) of the culture of *L. lactis* ssp. *lactis* CNRZ 144, 35% of *L. lactis* ssp. *cremoris* CNRZ E8, 25% of Cit$^+$ *L. lactis* ssp. *lactis* CDI1, and 5% of *Leu. mesenteroides* ssp. *mesenteroides* X2.

Batch Fermentation for Curd Production

A 15-L reactor was filled with pasteurized fresh milk or prefermented milk supplemented with whey retentate powder (0.1%, wt/vol). The temperature was kept constant by continuously circulating water through the reactor jacket from a thermostated water bath. The time that was required to fill the reactor with prefermented milk from the continuous immobilized cell bioreactor was about 25 min. The milk was stirred by the fermenter impellers (15 min) to achieve the desired fermentation temperature and to solubilize the whey retentate powder. Fresh milk was inoculated with 1% (vol/vol) of the bulk starter culture. Then, 30 ml of calf rennet diluted 100 times were added. The milk was stirred again for about 5 min to allow convenient mixing, and the fermentation recording was started. Three different incubation temperatures (22, 26, and 30°C) were studied using the inoculated fresh milk and the prefermented milk during 24-h trials. Each trial was replicated.

For pH measurement, the reactor was equipped with a combined pH electrode (DPAS Ingold, Paris, France) connected to a transmitter (Demca 3B 1015; Alfortville, France) and with a hot wire sensor for the on-line measurement of rennet coagulation (16). The hot wire sensor was composed of a stainless steel tube containing a platinum resistance (100Ω at 0°C) and an electronic board for the signal treatment (10). The acquisition period was 10 min.

Microbial Enumeration

Bacterial counts in milk (expressed in colony-forming units per milliliter) were obtained by plating appropriate dilutions. The samples were first treated with an Ultra-Turrax (IKA-Labortechnik, Staufen, Germany) for 30 s at 20,000 rpm to break the lactococcal chains. The plating was carried out with a spiral-plater (Interscience, Saint-Nom-La-Bretèche, France) using 14.5-cm diameter Petri dishes. Specific lactic acid bacteria were enumerated using selective agar media: M16 agar (23), Kempler and McKay agar (5), and M17 agar supplemented with 50 mg/L of vancomycin, as described by Sodini-Gallot et al. (20). All microbial enumerations were performed in duplicate.

Rheological Properties of the Final Curd

The rheological properties of the lactic curd were evaluated by measuring the susceptibility to syneresis, the firmness, and the modulus of elasticity of the gel. At the end of the fermentation, four tubes connected to the reactor were used to measure syneresis by a centrifugation method (4). These tubes were cooled to 4°C during 1 h and centrifuged at $550 \times g$ for 10, 20, 30, and 60 min. The weight of serum obtained after each centrifugation time was measured. The model curve for syneresis over time is expressed by a nonlinear regression:

$$W = W_f \times (1 - e^{-Kt})$$

where W_f = percentage of serum obtained for an infinite time, and K = time constant. These two values were selected to characterize the susceptibility of the gel to syneresis.

Fermentation was also conducted in six 400-ml centrifugation bottles using the same conditions as in the 15-L reactor. Rheological analysis of penetrometry on a Stevens LFRA Texture Analyser (C. Steven & Son Ltd., Hertfordshire, United Kingdom) was made before and after centrifugation of the bottles (10 min at 1000 rpm) to simulate draining of the lactic curd during the manufacture of fresh cheese. The operating conditions of the texture analyzer were TA3-TFE 105-504 (25 × 35-mm cylinder) cone type, penetration distance of 15 mm, and speed of probe 0.5 mm/s (22). The analyzer was interfaced to a computer, which collected the signal produced by the probe entering the curd. The rheological characteristics of the curd are the maximum force recorded during penetration (i.e., firmness, in Newtons) and the slope of the linear part of the curve expressing apparent stress as a function of the compression (i.e., the modulus of elasticity, in Pascals) (8).

Sensory Evaluation of the Curd

The effect of the prefermentation step on the sensory qualities of the curd drained by centrifugation (10 min at 1000 rpm) was evaluated by a triangle difference test by 21 panelists. The curds obtained at 22°C with inoculated fresh milk or prefermented milk were compared.

Statistical Analysis

Fermentations were conducted in duplicate. Analysis of variance was carried out to study the effect of the temperature and the prefermentation step on fermentation kinetics and curd rheological properties. Differences between means were considered to be highly significant at $P < 0.01$ and significant at $P < 0.05$.

RESULTS

Fermentation Kinetics

During the final batch fermentation, the changes in pH as a function of the different temperatures (22, 26, and 30°C) of the prefermented milk from the immobilized cell bioreactor, operated at 30°C and pH 6.4, were compared with those of fresh milk inoculated by batch with the bulk starter culture (Figure 1). Milk acidification occurred faster at higher temperature than at lower temperature and pH values were consistently lower in prefermented milk than in fresh milk. The difference in acidification kinetics between the two replicates at 22°C with inoculated fresh milk was probably caused by a slight difference in fermentation temperature (21.4 and 22.2°C, respectively). This result shows the importance of temperature control at low temperature fermentations.

To compare acidification kinetics, different parameters were calculated from the acidification curves (Table 1): the maximum acidification rate, the pH at maximum acidification rate, and the time to reach maximum acidification rate. The effect of temperature on acidification kinetics was highly significant ($P < 0.01$). The maximum acidification rate increased by about 47% between 22 and 30°C, and the time to reach the maximum acidification rate decreased by 48%. In contrast, the pH at the maximum acidification rate was not influenced by temperature or type of inoculation (pH = 5.17; SD = 0.03).

The times to reach pH 4.75 and 4.5 were also calculated (Table 1). For production of fresh cheese, the fermentation was usually stopped at a pH between these two values. The effect of temperature on these fermentation times was highly significant ($P < 0.01$). For example, the time to reach pH 4.75 increased by about 30 and 80% at 26 and 22°C, respectively, compared with 30°C, for both inoculated fresh milk or prefermented milk. The effect of the prefermentation step was also significant ($P < 0.05$); the time to reach pH 4.75 was reduced 8 to 16% compared with traditional fermentation methods. The effects of temperature and the prefermentation step on coagulation time were also studied (Table 1). The coagulation was improved by high temperatures ($P < 0.05$); for prefermented or inoculated fresh milks, the increase in coagulation time was about 60% at 26°C and 110% at 22°C compared with the coagulation time at 30°C. The prefermentation step reduced coagulation time ($P < 0.05$) by about 36 and 14% at 22 and 30°C, respectively.

Microbiological Composition of Milk

The microbiological composition of the starter culture is crucial for the acidification kinetics and the coagulum composition. To compare these two processes (with or without milk prefermentation), the microbiological composition of milk (total and specific populations) was analyzed during the fermentations (Figure 2). The prefermented milk was inoculated with the mixed culture at a constant level (5.8×10^7 cfu/ml; SD = 1.7×10^7 cfu/ml). The strain equilibria in prefermented milk were remarkably stable: 12.1% (SD = 3.5) of *L. lactis* ssp. *lactis* CNRZ 144, 4.5% (SD = 2.6) of *L. lactis* ssp. *cremoris* CNRZ

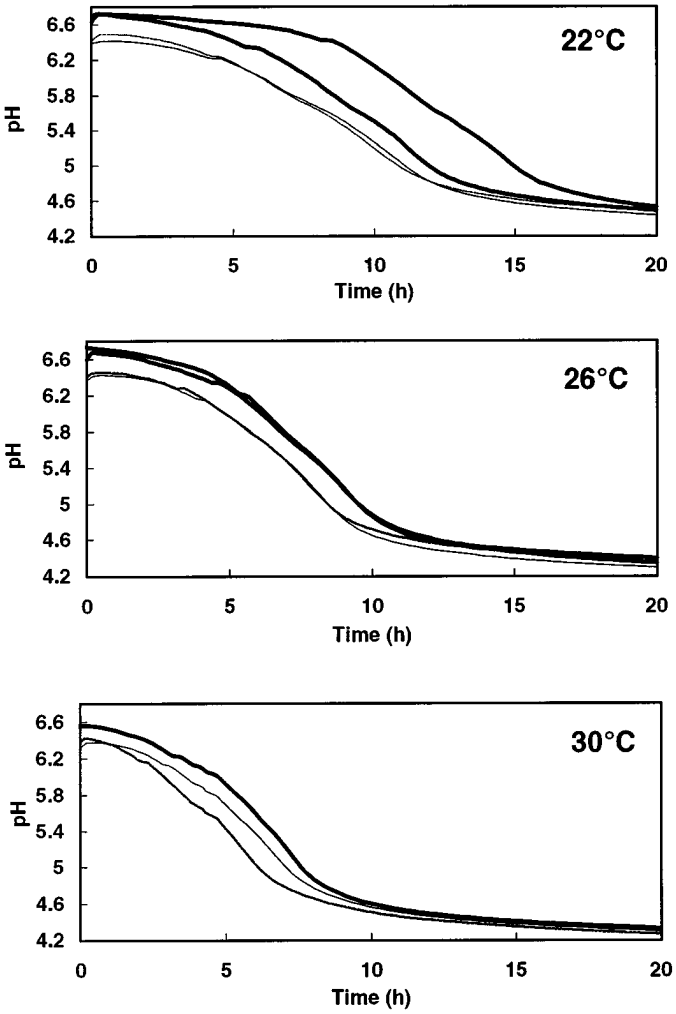

Figure 1. Evolution of pH over time during fermentation at 22, 26, and 30°C of prefermented milk (——) and inoculated fresh milk (▬▬). Time 0 corresponds to time of starter and rennet addition for inoculated fresh milk or time of rennet addition for prefermented milk. All experiments were performed in duplicate.

TABLE 1. Acidification and coagulation characteristics of inoculated fresh milk and prefermented milk during fermentation at different temperatures (22, 26, and 30°C).[1]

	22°C				26°C				30°C			
Probe[2]	Inoculated		Prefermented		Inoculated		Prefermented		Inoculated		Prefermented	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
pH												
V_m, upH/min	0.0049	0.0003	0.0042	0.0001	0.0061	0.0002	0.0058	0.0001	0.0067	0.0003	0.0066	0.0003
pH_m	5.16	0.04	5.15	0.09	5.20	0.02	5.23	0.02	5.22	0.00	5.16	0.08
T_{Vm}, min	775	105	620	30	535	5	465	5	385	25	340	30
$T_{4.75}$, min	903	83	755	5	635	10	570	10	478	18	440	25
$T_{4.5}$, min	1203	38	1083	58	815	25	760	50	635	45	610	30
Hot wire												
T_c, min	415	85	265	5	300	30	215	15	175	15	150	2

[1]All experiments were performed in duplicate.

[2]V_m = Maximum acidification rate, pH_m = pH at V_m, T_{Vm} = time to reach V_m, $T_{4.75}$ or $T_{4.50}$ = time to reach pH 4.75 or pH 4.50, and T_c = time to reach maximum slope of the hot wire probe signal, which corresponds to coagulation time.

E8, 82.1% (SD = 3.6) of Cit$^+$ *L. lactis* ssp. *lactis* CDI1, and 1.4% (SD = 0.7) of *Leu. mesenteroides* ssp. *mesenteroides* X2. Milk that had been inoculated with the bulk starter culture contained 2.2×10^7 cfu/ml (SD = 1.1×10^7), but its strain equilibria varied between the different experiments; the mean was 37.4% (SD = 15.7) for *L. lactis* ssp. *lactis* CNRZ 144, 21.1% (SD = 15.7) for *L. lactis* ssp. *cremoris* CNRZ E8, 36.1% (SD = 7.3) Cit$^+$ *L. lactis* ssp. *lactis* CDI1, and 5.1% (SD = 3.9) for *Leu. mesenteroides* ssp. *mesenteroides* X2.

In the prefermented milk, the mixed culture was largely dominated by strain Cit$^+$ *L. lactis* ssp. *lactis* CDI1, which represented more than 80% of the initial bacterial population. Strain equilibria did not change during the final fermentation. When milk was inoculated with the bulk starter culture, strain equilibrium changed considerably during fermentation in favor of Cit$^+$ *L. lactis* ssp. *lactis* CDI1, which accounted for more than 60% of the bacterial population in the final curd (Figure 2). The changes in bacterial populations during fermentation of prefermented and inoculated fresh milk at 22°C are reported in Figure 3. At 22°C, the change in equilibrium occurred quickly with the inoculated fresh milk; after 6 h of fermentation, at a pH of 6.4, Cit$^+$ *L. lactis* ssp. *lactis* CDI1 became the dominant strain in the culture and represented 58% of the total bacterial population (initial proportion was 27%). The same trend was observed at 26 and 30°C (data not shown). However, the proportions of the different strains in the total population did not change significantly during the 24-h fermentations of the prefermented milk. The total population after 24 h of incubation was not influenced by the type of milk inoculation or by the incubation temperature; mean value was 2.2×10^9 cfu/ml (SD = 0.5×10^9 cfu/ml).

Rheological Properties of Curd

The effects of fermentation temperatures (22, 26, and 30°C) and prefermentation on the rheological properties of the curd were studied. The fermentation temperature and the prefermentation step had no significant effect on the susceptibility of the gel to syneresis. The mean syneresis time constant was equal to 9.0 min (SD = 3.0 min), and the mean percentage of serum at infinite time was 61.3% (SD = 3.3%). The firmness and the modulus of elasticity of the curd, obtGAained at different fermentation temperatures and with prefermented or inoculated fresh milk, were not significantly different. The mean values for firmness and modulus of elasticity of curd were 2.21 N and 22.1 kPa, respectively. Centrifugation of the curd resulted in an increase in dry matter content to 16.3%. Firmness and the modulus of elasticity of curd before and after centrifugation were different ($P < 0.01$), equal to 0.97 N and 9.7 kPa, respectively, which represented decreases of 56 and 19% from values obtained before centrifugation.

Sensory Evaluation of Drained Curd

The drained curds obtained at a fermentation temperature of 22°C with inoculated fresh milk or prefermented milk were compared by a triangle difference test. The two types of curds were not significantly different at a 95% confidence level; only 11 of 21 subjects detected a difference between the samples.

DISCUSSION

This study compared a batch fermentation process with a system that integrates a prefermentation step in an immobilized cell bioreactor. The continuous

release of growing entrapped cells from gel beads allowed for continuous inoculation of milk circulating in the bioreactor. The fermentation is usually stopped at pH between 4.5 and 4.75 during the manufacture of fresh cheese; a compromise must be made between the capacity of draining of coagulum, which is better at low pH, and dry matter losses during centrifugation, which are reduced at high pH (12). Acidification kinetics during mesophilic fermentation are highly dependent on temperature. The fermentation time required to reach pH between 4.5 and 4.75 increased by more than 60% between 30 and 22°C. With a

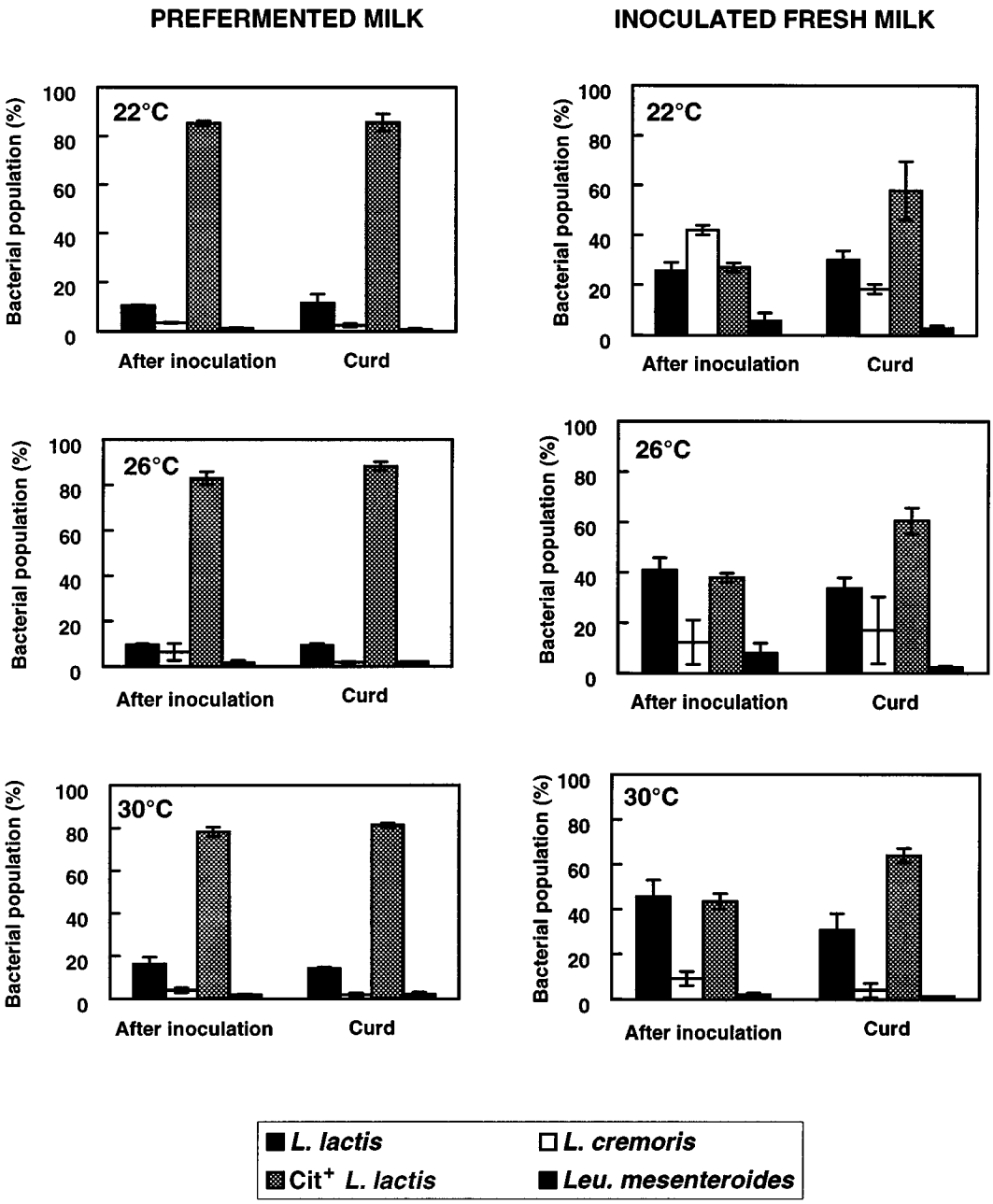

Figure 2. Strain equilibria in prefermented milk (pH 6.4; mean total population of 5.8×10^7 cfu/ml and SD = 1.7×10^7 cfu/ml) and corresponding lactic curd (mean total population of 2.2×10^9 cfu/ml and SD = 0.4×10^9 cfu/ml) and in fresh milk inoculated with the bulk starter (pH 6.6; mean total population 2.2×10^7 cfu/ml and SD = 1.1×10^7 cfu/ml) and corresponding lactic curd (mean total population 2.1×10^9 cfu/ml and SD = 0.7×10^9 cfu/ml) during fermentations conducted at 22, 26, and 30°C. Experimental data are mean values from two replicates. *L. lactis* = *Lactococcus lactis* ssp. *lactis* CNRZ 144, Cit$^+$ *L. lactis* = citrate-utilizing *L. lactis* ssp. *lactis* CD11, *L. cremoris* = *L. lactis* ssp. *cremoris* CNRZ E8, and *Leu. mesenteroides* = *Leuconostoc mesenteroides* ssp. *mesenteroides* X2.

prefermentation step, fermentation time was reduced by 10 to 15% at temperatures between 26 and 22°C. The milk inoculation during the prefermentation step in this experiment was lower (ca. 60%) than that in previous experiments (20) (ca. 5.8×10^7 and 1.5×10^8 cfu/ml, respectively). In this experiment, the intermittent use of the pilot bioreactor and the short time of operation (1 h instead of 20 h) before milk was collected in this experiment were probably not sufficient to reach steady-state conditions. As a consequence, fermentation time (440 min) was longer in this experiment than in a previous report (20) (378 min). Therefore, a prefermentation step operated at steady-state should reduce the fermentation time by 10 to 15% of that of classic batch fermentation at temperatures between 26 and 22°C. The decrease in fermentation time with prefermented milk, compared with milk inoculated with a bulk starter culture, might be explained by a higher inoculation level. Prefermented milk also had a lower pH than did inoculated fresh milk, which contributed to the reduction in the fermentation time. Furthermore, the cells released in the immobilized cell bioreactor are exponentially growing cells for which the lag phase is reduced or absent. In this study, the bulk starter for milk inoculation was used immediately after production, and its activity was not affected by storage. This situation differs from the starter cultures used in fresh cheese production (freeze-dried starters or bulk starter cultures stored at low pH), for which an extensive lag phase may be observed, depending on storage conditions. Therefore, when compared with industrial production, the continuous prefermentation system should lead to much larger savings in fermentation time. Prévost and Diviès (17) reported a reduction of 56% in fermentation time at 25°C for cheese made from prefermented milk compared with that for milk inoculated with a freeze-dried starter.

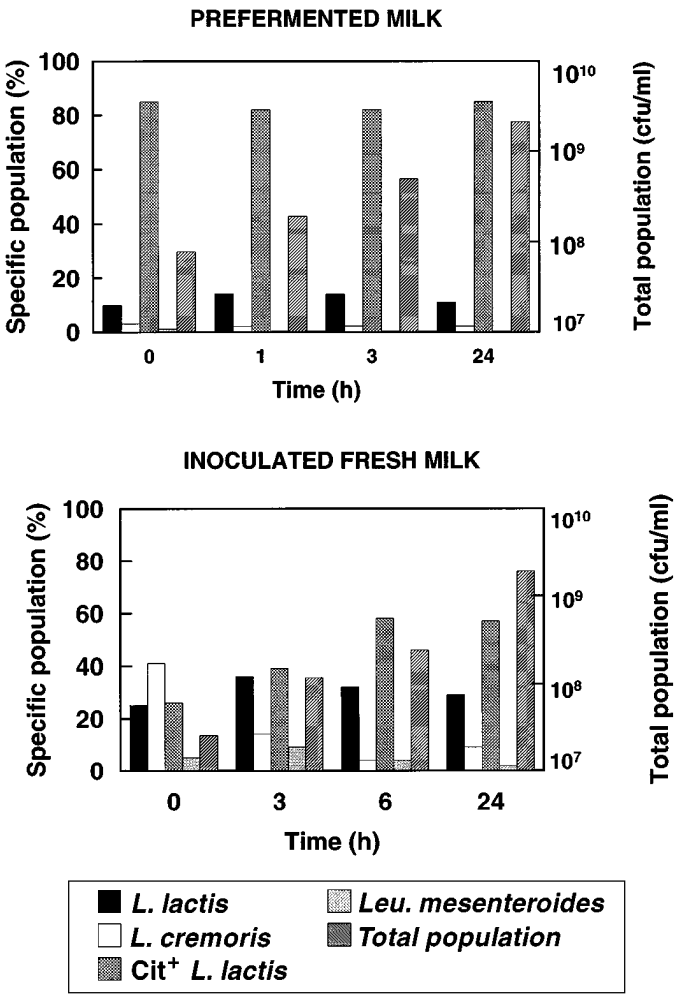

Figure 3. Change in the microbial populations during fermentation at 22°C of prefermented milk and inoculated fresh milk. Experimental data are mean values from two replicates. *L. lactis* = *Lactococcus lactis* ssp. *lactis* CNRZ 144, Cit$^+$ *L. lactis* = citrate-utilizing *L. lactis* ssp. *lactis* CD11, *L. cremoris* = *L. lactis* ssp. *cremoris* CNRZ E8, and *Leu. mesenteroides* = *Leuconostoc mesenteroides* ssp. *mesenteroides* X2.

We observed that coagulation kinetics were highly dependent on the fermentation temperature in the range and the inoculation mode tested. The effect of temperature on coagulation has also been shown by others. Ramet and Weber (18) reported lower flocking time during the enzymatic coagulation of milk when temperature was increased between 20 and 40°C. Zoon et al. (25) showed that firming rate increased as temperature increased from 25 to 35°C. The initial pH of milk had a significant effect on coagulation time; coagulation time was lower by 30% with prefermented milk (pH 6.4) than with inoculated fresh milk (pH 6.7). Noël et al. (13) also showed that at 33°C coagulation time decreased (ca. 60%) as rennetting pH decreased from 6.7 to 6.4. This observed reduction in coagulation time with the continuous milk prefermentation may decrease the risk of phage contamination, which is much lower in solid coagulum than in liquid medium (1).

The prefermentation system presented an interesting microbiological stability compared with the classical batch fermentation. Milk that was inoculated with the immobilized cell bioreactor exhibited more reproducible initial microbiological composition than milk that was inoculated with the bulk starter culture. However, strain Cit$^+$ *L. lactis* ssp. *lactis* CDI1 was dominant in both the released and entrapped cell populations because of better growth at conditions of low pH and high lactic acid concentration in competition with the other strains of the mixed culture, as shown previously (19). This phenomenon could make the system more vulnerable to phage infection than with a more balanced, immobilized mixed culture.

However, risks of phage infection are reduced with immobilized cultures because of the exclusion of phage particles from the gel matrix (21). Our previous study (19) showed the high resistance of the immobilized cell bioreactor to contamination during experimentation over 7-wk periods with pasteurized milk. A rapid and significant change in the proportions of the four strains in favor of Cit$^+$ *L. lactis* ssp. *lactis* CDI1 was observed during incubation at different temperatures of milks inoculated with the bulk starter (Figures 2 and 3), confirming the high competitivity of this strain in the mixed culture in milk. Others (11, 24) have also reported a similar dominance by strains of Cit$^+$ *L. lactis* ssp. *lactis* during starter propagation.

CONCLUSIONS

The present study compared the fermentation kinetics and curd properties during two different processes for the manufacture of fresh cheese. The fermentation time required to reach the draining pH was reduced by more than 10% using the prefermentation step in the immobilized cell bioreactor at temperatures between 26 and 22°C. This reduction is expected to be much greater than the existing fermentation times in the industry in which starters are prepared and stored under less than optimal conditions. With the continuous inoculation system, prefermented milk was collected directly and rennetted without a lag period between inoculation and rennetting, as is current practice. The stability of the inoculum composition was better with the prefermentation system than with the batch inoculation, which used bulk starters. The rheological and sensory qualities of final curds were similar for both processes.

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