

THE INFLUENCE OF STARTER AND ADJUNCT LACTOBACILLI CULTURE ON THE RIPENING OF WASHED CURD CHEESES

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Abstract - Ten strains of lactobacillus from the CNRZ collection were tested as adjunct culture in miniature washed curd cheeses manufactured under controlled bacteriological conditions with two different starters, *Lactococcus lactis* subsp. *lactis* IL 416 and *Lactococcus lactis* subsp. *cremoris* AM2. Lactobacilli growth seemed to be dependent on the *Lactobacillus* strain but was not influenced by the starter strain or counts. Lactococci counts were higher in the miniature cheeses with AM2 starter and added lactobacilli than in the control cheeses without lactobacilli. Gross composition and hydrolysis of κ s1 casein were similar for miniature cheeses with and without lactobacilli. In the miniature cheeses manufactured with IL416 starter, the lactobacilli adjunct slightly increased the soluble nitrogen content, but that was not verified in the AM2 miniature cheeses. Phosphotungstic acid nitrogen content increased in miniature cheeses manufactured with IL416 when *Lactobacillus plantarum* 1572 and 1310 adjunct cultures were added. That was also verified for several *Lactobacillus* strains, specially *Lactobacillus casei* 1227, for miniature cheeses manufactured with AM2 starter. Free fatty acid content increased in miniature cheeses made with lactobacilli adjuncts 1310, 1308 and 1219 with IL416 starter, and with strains 1218, 1244 and 1308 for miniature cheeses with AM2 starter. These results indicate that production of soluble nitrogen compounds as well as free fatty acid content could be influenced by the lactobacilli adjunct, depending on the starter strain.

Keywords: NSLAB, cheese ripening, biochemistry, proteolysis, lipolysis.

INTRODUCTION

Cheese ripening is a complex process involving proteolysis, lipolysis and metabolism of lactose, citrate and lactate (Fox et al., 1998). These transformations are produced by microbiological organisms and their enzymes, as well as residual chymosin and indigenous milk proteases (and lipase in raw-milk cheeses). The influence on biochemical transformation of many of these factors have been extensively studied and reviewed. However, the

contribution to cheese ripening of nonstarter lactic acid bacteria (NSLAB) still remains uncertain. In cheeses made with pasteurised milk, NSLAB are adventitious lactic acid bacteria that contaminate cheese, generally after pasteurisation of milk, or that survive the heating (Turner et al., 1986). The main sources of contamination are probably a resident flora in the dairy plant and the raw milk itself (Naylor & Sharpe, 1958). NSLAB are able to grow from very low numbers to 10^7 - 10^8 CFU g⁻¹ during the first weeks of ripening and dominate the cheese

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microflora after starter cell death (Fox et al., 1998). NSLAB are mainly mesophilic lactobacilli in cheeses made with pasteurised milk (McSweeney et al., 1993). The lactic starter strain used in cheese manufacture is supposed to influence the rate of growth and final count of NSLAB during ripening. Some authors have suggested that autolytic starters could enhance NSLAB growth (Thomas, 1987; Martley & Crow, 1993; Crow et al., 1995), though cheese-making studies have not confirmed these results so far (Lane et al., 1997; Hynes et al., 2001).

The objective of this work was to study the expression (growth and biochemical activities) of ten adjunct cultures of mesophilic lactobacilli during the ripening process of miniature washed curd cheeses manufactured with two different starter strains.

MATERIALS AND METHODS

Cultures

a) Starter Cultures

Two types of starter cultures were used in cheese-making: *L. lactis* subsp. *lactis* IL 416 and *L. lactis* subsp. *cremoris* AM2. IL416 was selected for its high resistance to phage infection, while on the contrary, AM2 was selected for its autolytic ability due to the presence of a prophage. Each strain was inoculated in sterilised (110°C - 10 min), reconstituted (10% w/v), low-heat skim milk powder and incubated overnight at 25°C.

b) Adjunct Cultures

Five strains of *Lactobacillus plantarum*, identified by the numbers 1228, 1245, 1310, 1311 and 1572; one of *Lactobacillus pentosus* (1218) and four of *Lactobacillus casei* (1219, 1227, 1244 and 1308) were tested as adjunct cultures. All the strains were obtained from the CNRZ collection and had been isolated from cheese. Strains were grown individually in MRS broth at 30°C overnight.

Cheese Manufacture

Two experimental cheeses (with lactobacilli adjunct culture) and two control cheeses (without lactobacilli) of approximately 40 g were made simultaneously per cheese-making day. The two strains of starter were alternated from one cheese-making day to another. Each lactobacilli adjunct was tested with both starters. The cheeses were made

under controlled bacteriological conditions, according to a previously described protocol (Hynes et al., 2000).

Analysis

a) Gross Composition of Cheeses

pH, dry matter and NaCl content were analysed by standard methods (FIL-IDF).

b) Bacteriological Analysis and Phage Detection

The population of starter lactococci in cheeses after 1, 14 and 28 days of ripening was determined by plating sample dilutions on M17 agar after 48 hours of incubation at 30°C. To determine the population of NSLAB, the samples were also plated on lactobacillus selection agar and incubated for 72 hours under anaerobic conditions. The absence of lytic phages during cheese-making was verified as described by Chapot-Chartier et al. (1994).

c) Proteolysis Assessment

Cheese samples were treated to obtain crude citrate extraction and then soluble fractions at pH 4.4 and in phosphotungstic acid (PTA) (Gripon et al., 1975). The nitrogen content of the fractions was determined by the Kjeldahl method. The insoluble fraction at pH 4.4 was analysed by reverse-phase liquid chromatography (RP-HPLC), and the relation between peak area for α s1 casein and the peptide α s1-I, resulting from chymosin activity, was calculated as an index of primary proteolysis (Hynes et al., 1999).

d) Lipolysis Assessment

Lipolysis in the miniature cheeses was determined by titration with tetra-n-butyl ammonium hydroxid (IDF 1987, Doc. 288) of cheese fat separated by centrifugation (Delacroix-Buchet et al., 1996). Fat acidity was expressed in μ moles of free fatty acids per g of fat, μ mol g⁻¹.

RESULTS AND DISCUSSION

Microbiological Analysis

Examples of growth curves for lactococci and lactobacilli in cheeses with and without an adjunct culture for the two starters tested are shown in Figs. 1 and 2. NSLAB ("wild" lactobacilli) accounted for

< 200 CFU g⁻¹ in all control cheeses during the ripening period.

The CFU g⁻¹ of *L. lactis* IL416 did not decrease during the 28-day ripening period in either control or experimental cheeses. The starter population remained above 10⁹ CFU g⁻¹ and lactobacilli did not dominate the microflora of the cheeses at any ripening stage. The number of lactobacilli changed from ~10⁶ CFU mL⁻¹ in the milk to ~10⁷-10⁸ CFU mL⁻¹ in the curd during cheese-making, moulding and ripening, depending on the strain. This could be a consequence of concentration of lactobacilli cells, but also some growing that compensated for the loss of lactobacilli during whey removal and washing. *Lactobacillus pentosus* 1218 grew a cycle during cheese-making and moulding (from ~10⁶ to ~10⁷ CFU g⁻¹) and then remained constant. *Lactobacillus plantarum* 1245 showed a similar development, but it continued to grow to almost 10⁹ CFU g⁻¹ in the final phase of ripening, achieving the most elevated count of any of the lactobacilli strain tested. The other strains did not increase during cheese-making and moulding. *Lactobacillus*

plantarum 1310, 1572 and 1245 populations gained approximately 1 log cycle during ripening.

In contrast, viable cell counts of the AM2 population decreased to ~10⁷ CFU g⁻¹ in the control cheeses, as was expected because of its autolytic ability. In most experimental cheeses (with lactobacilli), the starter population remained higher than in control cheeses (without lactobacilli). This effect was verified for six lactobacillus strains and as far as the authors know, it has not been reported to date. The result suggests that lactobacillus culture has improved the survival of AM2. Lane et al. (1997) have considered that lactococcal counts on M17 solid medium may be erroneous in cheeses with lactobacilli added, as growth of the latter on M17 would give rise to colonies that might be mistakenly considered to be lactococci. In experimental cheeses prepared with AM2 starter, this overestimation is not possible, as lactobacillus counts are ten times lower than lactococci and therefore negligible. The growth of lactobacilli has not been enhanced by a decrease in the number of viable starter cells, as had been suggested in earlier work (Thomas, 1987).

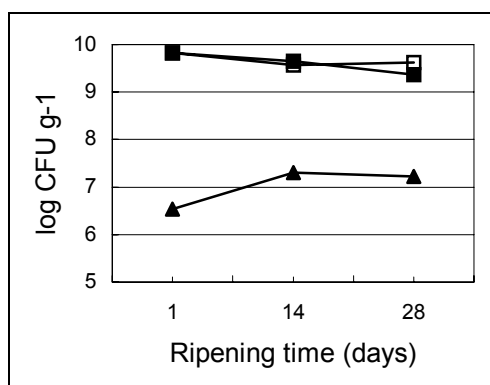


Figure 1: Growth curves for lactococci and lactobacilli in control and experimental cheeses prepared with *L. lactis* IL416. □ Lactococci (control cheese) ■ Lactococci (exp. cheese) ▲ Lactobacilli (exp. cheese). Lactobacilli in control cheeses were not quantifiable.

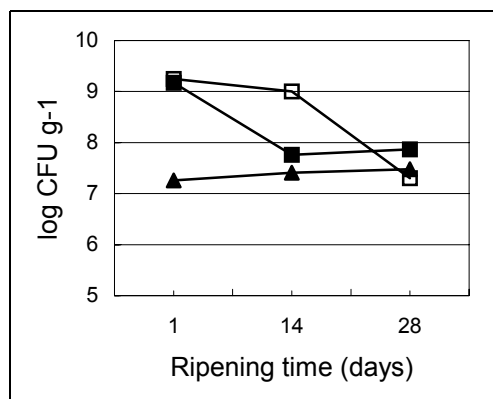


Figure 2: Growth curves for lactococci and lactobacilli in control and experimental cheeses prepared with *L. lactis* IL416. □ Lactococci (control cheese) ■ Lactococci (exp. cheese) ▲ Lactobacilli (exp. cheese). Lactobacilli in control cheeses were not quantifiable.

Compositional Analysis

The composition of one-day-old miniature cheeses showed no significant differences in dry matter and S/M content between starter strain. The average values for dry matter and S/M were respectively 45.03 (standard deviation (s.d.) 0.76, n=80, and 2.44 (s.d. 0.30, n=80). pH was somewhat lower for cheeses made with AM2 starter, 5.08 (s.d. 0.05, n=40), than for those made with IL416 starter, 5.18 (s.d. 0.06, n=40).

Proteolysis Assessment

a) Nitrogen Fractions

Twenty-eight-day-old cheeses made with lactobacilli adjunct showed similar or slightly higher levels of SN than control cheeses (without lactobacilli) for starter IL416 (Fig. 3). The average values were 20.35% (s.d. 1.00, n=10) for control and 21.55 % (s.d. 1.74, n=10) for experimental cheeses. SN levels of cheeses made with AM2 starter with and without lactobacilli were very close: the average SN values were 20.05% (s.d. 1.99, n=10) for control

and 20.52% (s.d. 1.38, n=10) for experimental cheeses (Fig. 3). No general trend could be identified, only *L. plantarum* 1310 with the two starter strains showed a significant increment in SN level (3% with IL 416 and 1.73% with AM2).

Phosphotungstic acid-soluble nitrogen (PTA-N) for control and experimental cheeses for both IL416 and AM2 starters at 28 days of ripening are shown in Fig. 4. The PTA-N levels for control cheeses made with AM2 starter varied more than for those made with IL416. The average PTA-N value for AM2 was 3.25% with an s.d. of 1.99 (n=10), while it was 2.47% with an s.d. of 0.30 (n=10) for IL416 control cheeses. The production of PTA-N was enhanced for *L. plantarum* 1310 and 1572 with IL416 starter. The effect of lactobacilli adjunct in AM2 cheese was less evident, probably because the lytic behavior of the starter was not reproducible during ripening and the consequent availability of intracellular peptidases changed from one cheese-making to another. In some cases control cheeses showed a slightly higher level of PTA-N than experimental ones. This may be due to a lower lysis of the starter cells, as suggested by the higher viability of AM2 starter in several experimental cheeses (See 4.1).

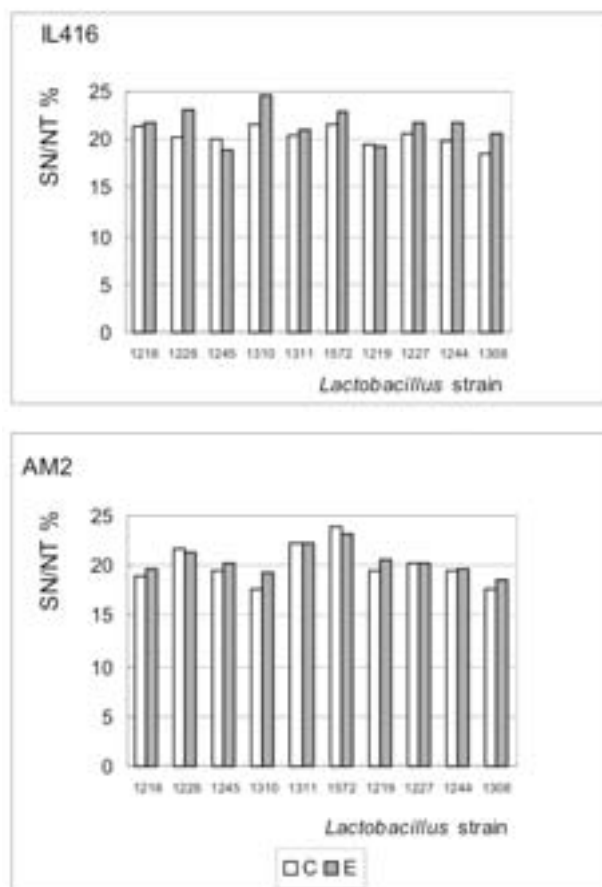


Figure 3: Soluble nitrogen at pH 4.4, expressed as a percentage of total nitrogen, for 28-day-old control and experimental cheeses, with IL416 and AM2 starters. Numbers under x-axis indicate *Lactobacillus* strain.

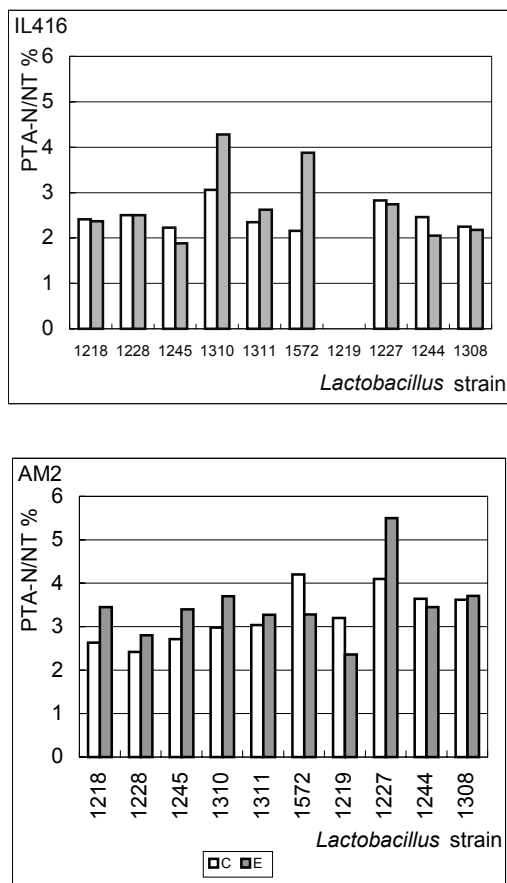


Figure 4: Soluble nitrogen in phosphotungstic acid expressed as a percentage of total nitrogen, for 28-day-old control and experimental cheeses, with IL416 and AM2 starters. Numbers under x-axis indicate *Lactobacillus* strain. (IL416-1219: missed sample).

b) HPLC of Casein

The hydrolysis of α s1 casein to give α s1-I peptide, also called α s1CN(f24-199), caused by chymosin, was taken as an index of primary proteolysis. The ratio $\frac{\alpha s1-I}{\alpha s1-I + \alpha s1}$ was calculated with the areas of the peaks in HPLC chromatograms. The chromatographic profiles were essentially the same for all miniature cheeses (not shown). The average for the ratio of the areas was 40.45% (s.d. 3.43, n=20) for miniature control cheeses and 40.69% (s.d. 2.88, n=20) for miniature cheeses with lactobacilli.

Lipolysis Assessment

The initial level of free fatty acids (FFA) in the fat of miniature cheeses was 19.07 μ moles of free fatty acid per g of fat (s.d. 5.05, n=8). Variability was relatively high (> 25%) as a result of different levels of lipolysis of cheese-making milk. After 28

days of ripening the increase in lipolysis for miniature cheeses ranged from 0.5 to 3.5 μ moles of free fatty acid per g of fat for both control and experimental cheeses.

Levels of fat acidity for cheeses with lactobacilli added were significantly higher than those for controls for five strains of lactobacillus throughout the ripening period: *L. plantarum* 1310 and *L. casei* 1219 for cheeses prepared with IL416 starter and *L. pentosus* 1218 and *L. casei* 1244 and 1308 for those prepared with AM2 starter. AM2 starter with adjunct culture *L. plantarum* 1245 was the only association which gave a lower value for experimental than for control cheese.

CONCLUSIONS

The starter strain did not influence the growth of adjunct culture cells. Lactobacilli did not grow faster or to higher final counts in miniature cheeses made

with the lytic starter. The presence of the adjunct culture also results in altered proteolytic end products, depending on the starter strain. Nevertheless, no general conclusion can be drawn about the influence of lactobacillus adjuncts in the ripening products (SN, PTA-N or FFA). The lactobacillus strains which produced the strongest biochemical changes in comparison to the control cheeses were CNRZ1227, 1572, 1219, 1308, 1244 and 1310.

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