

## ***Streptococcus thermophilus* in Cheddar cheese – production and fate of galactose**

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**SUMMARY.** The behaviour of *Streptococcus thermophilus* in combination with *Lactococcus lactis* subsp. *cremoris* or subsp. *lactis* mesophilic starters in experimental Cheddar cheese is reported. In a standard manufacturing procedure employing a 38 °C cook temperature, even very low levels (0·007%) of *Str. thermophilus* combined with normal levels of the mesophilic starter (1·7%) resulted in increased rates of acid production, the formation of significant amounts of galactose (~ 13 mmol/kg cheese), and populations nearly equivalent to those of the mesophilic lactic starter in the curd before salting. At a 41 °C cook temperature, the *Str. thermophilus* attained a higher maximum population (~ log 8·2 colony forming units (cfu)/g) than the *Lc. lactis* subsp. *cremoris* (~ log 6·8 cfu/g) and formed more galactose (~ 28 mmol/kg). *Lactobacillus rhamnosus*, deliberately added to a cheese made using *Str. thermophilus* starter and which contained 24 mmol galactose/kg at day one, utilized all the galactose during the first 3 months of cheese ripening. Adventitious non-starter lactic acid bacteria had the potential to utilize this substrate too, and a close relationship was demonstrated between the increase in this flora and the disappearance of the galactose. Some possible consequences for cheese quality of using *Str. thermophilus* as a starter component are discussed.

**KEYWORDS:** Cheddar cheese, cheese ripening, galactose, *Streptococcus thermophilus*.

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The manufacture of cheese varieties characterized by high “cook” temperatures (50–55 °C) (*e.g.* Emmental, Gruyère, and the hard Italian varieties) typically requires the use of thermophilic starter cultures. These starters include a major component of *Streptococcus thermophilus* together with smaller amounts of various lactobacilli (*e.g.* *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis*). The *Str. thermophilus* is responsible for much of the early acid production. As the galactose moiety of lactose is not utilized by *Str. thermophilus* to any significant extent, the thermophilic lactobacilli play an important role in utilizing this galactose and completing the acid production (Tinson *et al.* 1982*a*; Turner *et al.* 1983).

The manufacture of Cheddar cheese is characterized by the use of lower “cook” temperatures (38 °C) and the use of mesophilic *Lactococcus* starter strains. In the so-called “Short-Method” of Cheddar manufacture (Bley *et al.* 1985) very rapid rates of acid production were achieved by using higher manufacturing temperatures

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(42–43 °C) and a starter comprising *Str. thermophilus* together with the normal mesophilic *Lactococcus lactis* subsp. *cremoris* starter. Reduced manufacturing costs were a major advantage sought by this manufacturing process. Some of the problems that became evident with this type of manufacture were attributed to the presence in the cheese of up to 33 mmol of galactose/kg and the production of CO<sub>2</sub>, probably by non starter lactic acid bacteria (NSLAB), leading to the development of slits and fractures in the cheese (Tinson *et al.* 1982b).

Galactose is a major contributor to the browning that occurs when cheese is heated in applications such as the manufacture of pizzas or processed cheese (Bley *et al.* 1985; Mukherjee & Hutkins, 1994). Excessive browning may lead to rejection of product by the consumer.

We have reported (Martley & Michel, 2001) that a high proportion of mature Cheddar cheeses exhibiting a pinkish colouration at or just beneath the surface contained significant levels of galactose. This galactose was produced by adventitious *Str. thermophilus* during cheesemaking, and was believed to be a key to subsequent reactions leading to the formation of pinkish coloured Maillard-type products.

Commercial starters comprising *Str. thermophilus* together with the mesophilic *Lactococcus* strains have increasingly been promoted for use in the manufacture of Cheddar and other pressed cheese varieties which are not characterized by high cooking temperatures (Anonymous, 1996; Stanley, 1996; Elsborg, 1997), but little information is given about the consequences of such combinations in terms of the growth of the different starter strains and the galactose levels in cheese.

In the present paper we report the results of experiments undertaken to provide an understanding of the behaviour of *Str. thermophilus* in Cheddar cheese, especially with regard to the production and fate of galactose. Trials were carried out using different inoculum levels of *Str. thermophilus* together with mesophilic *Lc. lactis* subsp. *cremoris* and subsp. *lactis* starter strains; cooking at standard (38 °C) or high (41 °C) temperatures; and adding a culture of *Lactobacillus rhamnosus*. Some possible consequences for cheese quality of using *Str. thermophilus* as a component of the starter are discussed.

## MATERIALS AND METHODS

### *Cultures*

All cultures used in this study were from the New Zealand Dairy Research Institute's (NZDRI) culture collection. The mesophilic starters used were either a triplet of three single strains of *Lc. lactis* subsp. *cremoris*, or a single strain of *Lc. lactis* subsp. *lactis* NCFB712. The thermophilic starter was *Str. thermophilus* ST5101.

Mesophilic starters were grown in reconstituted skim milk (100 g powder/l) at 22 °C to pH 4.6, then chilled and held for inoculation into the pasteurized cheesemilk at 1.7% (w/v; giving initial counts of log 7.0 cfu/ml of cheesemilk for the *Lc. lactis* subsp. *cremoris*, and log 7.4 cfu/ml for the *Lc. lactis* subsp. *lactis*). Thermophilic starter was prepared in the same way except that it was grown at 42 °C; it was inoculated into the cheesemilk at three different levels: 0.007, 0.034 or 0.17% (w/v; giving initial counts of log 4.4, log 5.1, and log 5.7 cfu/ml respectively). In some experiments, an overnight (30 °C) culture of *Lactobacillus rhamnosus* B3083 in MRS broth (Merck, D-64271 Darmstadt, Germany) was also inoculated to give ~ log 2.2 cfu/ml of cheesemilk.

### *Cheesemaking*

Cheddar cheese was made in the NZDRI pilot plant, producing four rindless blocks (nominal dimensions 36 × 27 × 18 cm) of ~ 10 kg each per vat of 375 litres of pasteurized (72 °C/15 s) milk. The composition of the milk used was (means ± SD): 44.5 ± 0.2 g fat/l; 34 ± 0.4 g total protein/l; 49.7 ± 0.4 g lactose/l. The microbiological quality of the pasteurized milk was: aerobic plate count < 100 cfu/ml; coliforms < 10 cfu/ml; NSLAB < 10 cfu/ml.

Milk at 32.5 ± 0.5 °C was inoculated with starter, then renneted 30 min later (using 10.6 ml rennet per 100 litres of milk; liquid calf rennet, ≥ 92% chymosin, ≤ 8% pepsin, strength 280 International Milk Clotting Units; NZ Dairy Meats, Eltham, New Zealand). The curd was cooked at 38 °C (control) or 41 °C, drained at pH 6.2, and salted at pH 5.3. Cheeses were pressed overnight at 15 °C, bagged and ripened at 10 °C. An experimental trial comprised four vats of cheese made on one day from the same batch of pasteurized milk. All experiments were carried out at least twice.

### *Sampling and microbial analysis*

Samples were taken from each trial vat at the following stages: milk after addition of the starters, curd before draining the whey, curd immediately before salting, the young cheese 24 h after the start of cheesemaking (day-1 cheese), and then monthly. The mesophilic starters were enumerated on M17 agar (M17 broth + 12.5 g agar/l; Difco Laboratories, Detroit, MI48223-7058, USA) incubated at 30 °C for 48 h, conditions which adequately enabled selective recovery of the organisms without interference from the thermophilic *Str. thermophilus*. In our experience batch-to-batch variations of M17 medium were responsible for inconsistent recovery of *Str. thermophilus* on M17 agar, even at its optimum temperature of 42 °C. Instead we enumerated *Str. thermophilus* on Milk Plate Count agar (Oxoid, Basingstoke RG24 0PW, UK) incubated at 42 °C for 2 d. Mesophilic lactococci recover poorly on this medium at 30 °C and not at all at 42 °C, and consequently did not interfere with the count of thermophilic organisms. Adventitious NSLAB and deliberately-added *Lb. rhamnosus* were enumerated on LBS agar (BBL, Cockeysville, MD21030, USA) incubated at 30 °C for 5 d under anaerobic conditions (Anaerocult A; Merck).

### *Chemical/biochemical analyses*

Samples of day-1 cheeses were analysed for fat, moisture, salt and pH according to standard methods (Johnston *et al.* 1994).

Chemical analyses were carried out on water soluble fractions prepared from each cheese sample (Crow *et al.* 1995). Lactose and glucose levels were measured with the glucose-GOD-PAP kit of Boehringer-Mannheim NZ Ltd (Auckland, New Zealand). Galactose, D- and L-lactate, acetate and citrate levels were determined enzymically as described by Crow *et al.* (1995). Statistical differences in these data were determined by analysis of variance (SAS Institute Inc., Cary, NC, USA).

## RESULTS

### *Effect of Str. thermophilus on rate of acid production during cheese manufacture*

The total make time (time from addition of the starters to the milk to salting of the curd at pH 5.3) decreased with increasing amounts of *Str. thermophilus* starter. At the standard 38 °C cook temperature the use of only 0.007 % of *Str. thermophilus*

Table 1. Log starter counts ( $\pm$ SD) during the manufacture of experimental Cheddar cheese. Cheeses were made using 1.7% of mesophilic *Lc. lactis* subsp. *cremoris* together with 0, 0.007, 0.034 and 0.17% of thermophilic *Str. thermophilus*. Cooking temperatures were 38 and 41 °C

Stage of manufacture	Cook temperature (°C)	<i>Lc. lactis</i> ssp. <i>cremoris</i> (counts†)	<i>Str. thermophilus</i> (counts)		
			0.007%	0.034%	0.17%
Milk at inoculation (log cfu/ml)	38	6.96 ± 0.19 (n = 27)	4.39 ± 0.18 (n = 8)	5.16 ± 0.36 (n = 12)	5.71 ± 0.43 (n = 3)
	41	7.08 ± 0.09 (n = 6)	4.37 ± 0.07 (n = 6)	5.07 ± 0.09 (n = 3)	nt
Curd at draining (log cfu/g)	38	8.27 ± 0.45 (n = 27)	7.38 ± 0.56 (n = 8)	7.25 ± 0.49 (n = 12)	7.49 ± 0.67 (n = 3)
	41	7.53 ± 0.14 (n = 6)	7.74 ± 0.23 (n = 6)	7.44 ± 0.14 (n = 3)	nt
Curd pre-salting (log cfu/g)	38	8.11 ± 0.67 (n = 27)	7.79 ± 0.23 (n = 8)	8.18 ± 0.48 (n = 12)	8.48 ± 0.81 (n = 3)
	41	6.77 ± 0.51 (n = 6)	8.23 ± 0.34 (n = 6)	8.29 ± 0.51 (n = 3)	nt
Day-1 cheese (log cfu/g)	38	7.96 ± 0.42 (n = 27)	7.79 ± 0.15 (n = 8)	8.13 ± 0.51 (n = 3)	8.11 ± 0.77 (n = 3)
	41	6.50 ± 0.25 (n = 6)	8.29 ± 0.07 (n = 6)	8.46 ± 0.25 (n = 3)	nt

† Data from cheeses made with no added *Str. thermophilus* as well as from those made with *Str. thermophilus* at the three different levels.

nt, not tested in combination with *Lc. lactis* subsp. *cremoris*.

together with 1.7% of the mesophilic *Lc. lactis* subsp. *cremoris* triplet starter decreased the total make time by 30 min from 6.17 h to 5.67 h. When 0.17% of *Str. thermophilus* was used together with 1.7% of mesophilic starter, the total make time was reduced by nearly 2 h.

#### Starter population changes during cheese manufacture

Maximum populations of the mesophilic *Lc. lactis* subsp. *cremoris* were attained in the curd at draining (log 8.27 cfu/g in the 38 °C-cook cheeses; log 7.53 in the 41 °C-cook cheeses; Table 1). These populations then remained static or tended to decline in the 38 °C-cook cheeses, or declined ~ 1-log by day 1 in the 41 °C-cook cheeses. The more temperature-tolerant *Lc. lactis* subsp. *lactis* strain reached maximum population of log 8.3 ± 0.1 cfu/g in the 41 °C-cook cheeses (results not shown).

In contrast, the thermophilic *Str. thermophilus* continued to increase in the curd between draining and salting, reaching log 7.79 to log 8.48 cfu/g in standard 38 °C-cook cheeses from initial inoculation levels of 0.007 to 0.17% (Table 1). In the 41 °C-cook cheeses, growth of *Str. thermophilus* was enhanced, with counts for curd at draining, curd pre-salting and day 1 tending ~ 0.3 log higher than those at the equivalent points in the standard 38 °C-cook cheeses.

Increases of starter populations due to growth can be distinguished from apparent increases due to simple physical concentration of organisms trapped in the curd. This can be done by taking into account the changing composition of the samples during manufacture – in the present trials the total solids contents of the milk at inoculation, the curd at draining, the curd pre-salting, and the day-1 cheeses were 140, 470, 600 and 660 g/kg respectively. Thus, it was estimated that *Str. thermophilus* underwent about 7 to 9 population doublings from inoculation to

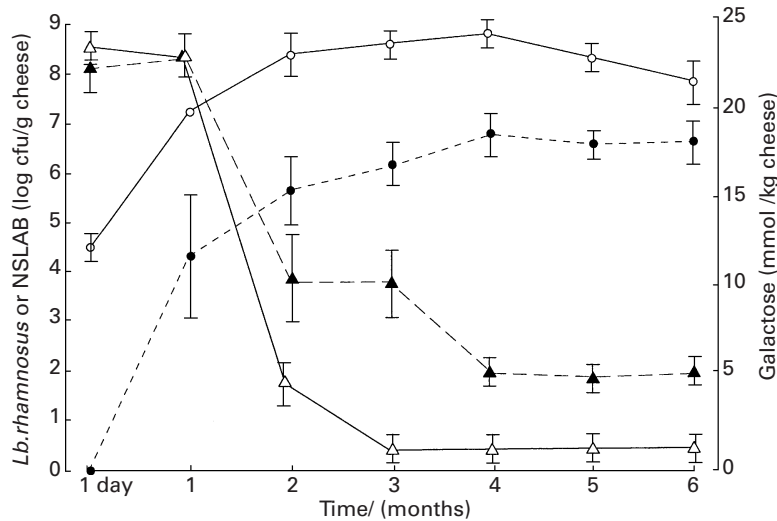


Fig. 1. Growth of deliberately-added *Lactobacillus rhamnosus* (○) and adventitious non-starter lactic acid bacteria (NSLAB) (●) in experimental Cheddar cheese ripened at 10 °C; galactose decrease in the corresponding cheeses (△, ▲). Cheeses were made with mesophilic *Lactococcus lactis* subsp. *cremoris* starter (1.7%) together with thermophilic *Streptococcus thermophilus* (0.17%) and a standard (38 °C) cook temperature. *Lb. rhamnosus* was added to the cheesemilk to give log 2.2 cfu/ml. Values are means of three repeats with SD indicated by vertical bars.

maximum viable counts during manufacture compared with no more than 2–3 doublings for *Lc. lactis* subsp. *cremoris* in the 38 °C- and 41 °C-cook cheeses.

The overall picture presented was of *Str. thermophilus* able to grow rapidly and from low initial levels in the milk to attain populations at least comparable to, and often greater than, the maximum populations of the mesophilic *Lc. lactis* subsp. *cremoris* starter in the same cheese, with the increased cook temperature favouring the overgrowth of the temperature-sensitive *Lc. lactis* subsp. *cremoris* strains by the thermophilic *Str. thermophilus*.

#### Starter population changes during ripening

The population of the mesophilic *Lc. lactis* subsp. *cremoris* starter decreased during ripening by > 1 log in 2 months, while populations of *Lc. lactis* subsp. *lactis* and of *Str. thermophilus* remained constant during this period (results not shown).

#### Non-starter lactic acid bacteria and deliberately-added *Lactobacillus rhamnosus*

Adventitious NSLAB in the milk at the start of cheesemaking and in the day-1 cheeses were undetectable (<10 cfu/ml, and <10 cfu/g), but increased during ripening to ~ log 6 cfu/g (Fig. 1).

Three vats of cheese were made with *Lb. rhamnosus* deliberately added to the milk at log 2.2 cfu/ml at the start of cheesemaking. This organism increased during manufacture to log 4.5 ± 0.21 ( $n = 3$ ) cfu/g at day-1, and to ~ log 8.5 at 2 months, remaining at about this level until 6 months (Fig. 1). As the adventitious NSLAB were present at levels much lower than the deliberately-added *Lb. rhamnosus*, especially in the very young cheeses, they would not be expected to cause problems with enumeration and recovery by the plating procedure used.

Table 2. Biochemical features of day-1 experimental Cheddar cheeses made with different starter combinations and inoculation levels (1.7% (w/v) *Lc. lactis* and 0 to 0.17% *Str. thermophilus*)

	Cook temperature (°C)	<i>Lc. lactis</i> subsp. <i>cremoris</i> +				<i>Lc. lactis</i> subsp. <i>lactis</i> +	
		<i>Str. thermophilus</i>				<i>Str. thermophilus</i>	
		0%	0.007%	0.034%	0.17%	0%	0.007%
Lactose (mmol/kg cheese)	38	17.9 ± 4.6 <sup>a</sup> (n = 6)	7.3 ± 2.5 <sup>bc</sup> (n = 6)	6.2 ± 1.4 <sup>bc</sup> (n = 9)	9.8 ± 4.2 <sup>b</sup> (n = 3)	6.7 ± 0.1 (n = 2)	< 2 (n = 2)
	41	nt	3.9 ± 1.9 <sup>bc</sup> (n = 3)	2.9 ± 1.6 <sup>c</sup> (n = 3)	nt	11.2 ± 4.0 (n = 2)	2.8 ± 0.0 (n = 2)
Galactose (mmol/kg cheese)	38	< 2 <sup>c</sup> (n = 6)	13.6 ± 5.4 <sup>b</sup> (n = 6)	18.2 ± 2.6 <sup>b</sup> (n = 9)	26.2 ± 0.8 <sup>a</sup> (n = 2)	< 2 (n = 2)	12.8 ± 2.8 (n = 2)
	41	nt	28.9 ± 0.5 <sup>a</sup> (n = 3)	28.0 ± 2.5 <sup>a</sup> (n = 3)	nt	< 2 (n = 2)	24.7 ± 8.2 (n = 2)
L-Lactate (mmol/kg cheese)	38	78.0 ± 13.8 <sup>bc</sup> (n = 6)	85.6 ± 13.9 <sup>abc</sup> (n = 6)	104.8 ± 12.0 <sup>a</sup> (n = 9)	99.1 ± 6.8 <sup>ab</sup> (n = 3)	97.1 ± 25.1 (n = 2)	128 ± 19.8 (n = 2)
	41	nt	72.9 ± 9.0 <sup>c</sup> (n = 3)	68.8 ± 8.7 <sup>c</sup> (n = 2)	nt	95.8 ± 13.1 (n = 2)	104 ± 20.7 (n = 2)

<sup>a, b, c</sup> Data sets for lactose, galactose, and L-lactate were each analysed by ANOVA for cheeses made with *Lc. lactis* subsp. *cremoris* together with *Str. thermophilus*. Values with different superscripts (a–c) within each data set are significantly different ( $P < 0.05$ ). There were insufficient points to carry out useful statistical analysis of data for cheeses made with *Lc. lactis* subsp. *lactis* together with *Str. thermophilus*. n, number of experimental cheeses manufactured. nt, not tested.

#### Biochemical features of day-1 cheeses

The composition of the control and the experimental cheeses made during this study were comparable to one another. The day-1 analyses were (means ± SD): 340 ± 11 g moisture/kg, 353 ± 11 g fat/kg, 17.2 ± 1 g salt/kg, pH 5.26 ± 0.07 (n = 46).

The biochemical parameters of the different experimental Cheddar cheeses manufactured are given in Table 2. Galactose was found only in those cheeses where *Str. thermophilus* was used as a component of the starter. Significantly higher levels of galactose were formed in the 41 °C-cook cheeses than in the standard 38 °C-cook cheeses. Increasing the amounts of *Str. thermophilus* inoculum from 0.007 to 0.17% also significantly increased the galactose formed in these cheeses. For the standard 38 °C-cook cheeses, increasing the amount of *Str. thermophilus* inoculum to 0.17% resulted in significantly reduced residual lactose and increased lactate concentrations in day-1 cheeses, reflecting the faster rates of acid production noted during manufacture in the vat.

All the day-1 cheeses had no detectable glucose, D-lactate or acetate (< 2 mmol/kg). The mean citrate level was 7.5 ± 1.5 mmol/kg (n = 39).

#### Biochemical features during cheese ripening

The residual lactose in all the day-1 cheeses disappeared during ripening. In control cheeses made with mesophilic *Lc. lactis* subsp. *cremoris* starter and which contained the highest lactose level at day-1, lactose had completely disappeared by 3 to 5 months of ripening. For cheeses made with both mesophilic and thermophilic starters, disappearance of lactose was more rapid, reflecting the lower initial levels of lactose at day 1 (Table 2). In most of them, lactose was undetectable (< 2 mmol/kg) at 3 months.

The galactose level of cheeses inoculated with *Str. thermophilus* decreased during ripening but with some variability among cheeses. The higher the galactose

concentration in day-1 cheeses, the longer it took to disappear. In most cheeses, the galactose level started to decrease from about 2 months to be nearly undetectable ( $< 2$  mmol/kg) after 5 to 7 months of ripening but in some other cheeses, its level had only decreased to  $\sim 10$  mmol/kg after that period of time. There was a close relationship between this disappearance of galactose and the establishment of the adventitious NSLAB microflora during cheese ripening (Fig. 1). In the cheeses where the deliberately-added *Lb. rhamnosus* reached  $\log 8.3 \pm 0.76$  ( $n = 2$ ) cfu/g at 2 months, the galactose decreased more rapidly to be below the threshold of detection ( $< 2$  mmol/kg) after 3 months.

In the cheeses made with the *Lc. lactis* subsp. *lactis* mesophilic starter together with *Str. thermophilus*, the galactose concentration decreased by  $\sim 6$  mmol/kg during the first month of ripening *i.e.* before the establishment of any significant NSLAB population (results not shown).

Levels of L-lactate increased during the first months of ripening, reflecting lactose decrease. Detectable levels of D-lactate were formed only after at least 4 months. Levels found varied greatly among cheeses and were related to differences in the establishment of NSLAB and probably also to their ability to produce D-lactate.

#### DISCUSSION

The present results provided evidence that very low initial levels of *Str. thermophilus* in the cheesemilk could increase the rate of acid production during the manufacture of standard 38 °C-cook temperature Cheddar, and form significant quantities of galactose. Increasing the initial levels of *Str. thermophilus* and increasing the cooking temperature favoured increased rates of acid production and galactose formation. With a 0.007% inoculum and a 41 °C cook the concentrations of galactose produced were as high as those reported for Short-Method Cheddar where much higher levels of *Str. thermophilus* were used (30–50% as a proportion of the mesophilic starter; Radford & Hull, 1982; Tinson *et al.* 1982*b*).

Sometimes *Str. thermophilus* and related organisms are found in pasteurized cheesemilk (Hup & Stadhouders, 1979; Bouman *et al.* 1982; Martley & Crow, 1993). If these organisms were present in pasteurized milk without the cheesemaker's knowledge at levels sufficient to increase the rate of acid production during Cheddar cheesemaking, his or her response, to slow the acid production rate, would normally be to increase the cook temperature, or to reduce the amount of normal (*i.e.* mesophilic) starter being used in later vats (Martley & Michel, 2001). However, both of these responses would only further favour the growth and acid production by *Str. thermophilus* over that of the normal mesophilic lactic starter.

Although the optimum for growth of *Str. thermophilus* is 42–43 °C, there is a lot of variation between individual strains, and small temperature shifts, particularly on the lower side of the optimum, may have big effects on activity (Martley, 1983). The present trials were carried out with only one strain of *Str. thermophilus* – other strains could give different results. The activity of *Str. thermophilus* strains in practical cheesemaking will thus depend on the growth response of a particular strain over the temperature profile of the entire manufacturing process.

We showed that the galactose produced by *Str. thermophilus* may persist for months in cheese, or be utilized during ripening either by adventitious NSLAB or by a deliberately-added lactobacillus adjunct culture. This provides an explanation for the observation that galactose was not recovered from all mature ( $\geq 6$  months old) Cheddar cheeses exhibiting a pinkish colouration (Martley & Michel, 2001).

We confirmed that mesophilic *Lc. lactis* subsp. *lactis* starter could utilize some galactose during about the first month of cheese ripening at low temperature when the viability of *lactis* strains remains high. About 6 mmol galactose/kg were utilized during this time, consistent with values obtained by Radford & Hull (1982). Although *cremoris* strains have the potential to utilize galactose in laboratory conditions (Thomas *et al.* 1980), their greater salt sensitivity, poorer survival and more extensive autolysis in cheese than *lactis* strains probably explains the observation that they cannot utilize galactose in cheese during ripening.

While it may be tempting to add adjunct cultures specifically to remove galactose formed in Cheddar cheeses made with *Str. thermophilus*-containing starters, such cultures may produce gas, and thus favour the development of open texture in the cheese (Tinson *et al.* 1982*b*); in addition they may have negative effects on cheese flavour depending on the products of galactose utilization.

Residual galactose has been implicated in the development of a pinkish colouration in mature Cheddar cheese (Martley & Michel, 2001) and also contributes to browning reactions when cheese is heated (Bley *et al.* 1985; Zehren & Nusbaum, 1992). Thus, the consequences of residual galactose in Cheddar cheese should be considered if cheese is to be matured for extended periods, or is destined to further applications such as the manufacture of processed cheese.

In summary, *Str. thermophilus* is likely to have the potential to affect Cheddar cheese quality for three main reasons: its effects on the rate of acid production during manufacture; the formation of galactose and the consequential formation of pinkish colouration in aged cheese; and unknown effects on the cheese ripening and flavour development processes. We suggest that care should be taken in using this organism as a component of starters for Cheddar cheese manufacture.

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