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Effect of milk pasteurisation temperature on age-related changes in lactose metabolism, pH and the growth of non-starter lactic acid bacteria in half-fat Cheddar cheese

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Abstract

Half-fat Cheddar cheese (~15%, w/w, fat) was manufactured on three occasions from milk pasteurised at 72, 77, 82 or 87 °C for 26 s, and analysed over a 270 day ripening period. Increasing milk pasteurisation temperature significantly increased the levels of moisture (from ~45% at 72 °C to 50% at 87 °C), total lactate, and D(−)-lactate in cheese over the 270 day ripening period. Conversely, the cheese pH decreased significantly on increasing pasteurisation temperature. Increasing the pasteurisation temperature did not significantly affect the populations of starter or non-starter lactic acid bacteria during maturation. The use of higher pasteurisation temperatures would appear particularly amenable to exploitation as a means of producing high-moisture (e.g., 40–41%), short-ripened, mild-flavoured Cheddar or Cheddar-like cheeses.

Keywords: Pasteurisation temperature; Half-fat Cheddar; Lactate; pH; Non-starter lactic acid bacteria

1. Introduction

Cheeses produced with reduced fat content can have inferior flavour, texture, and functionality compared to their full-fat equivalents (Guinee & Law, 2002). Various process modifications have been used to improve the quality of such cheeses (Ardö, 1997; Fenelon & Guinee, 1997). One such approach has been the pasteurisation of cheesemilk at temperatures higher than those used conventionally (i.e., 72 °C for 15 s) (Guinee et al., 1998; Rynne, Beresford, Kelly, & Guinee, 2004). Increasing pasteurisation temperature results in denaturation of whey proteins, and their probable interaction with casein micelles (Law, Banks, Horne, Leaver, & West, 1994). The latter changes coincide with reductions in the ability of the rennet-induced gels from these milks to synerese (Pearse, Linklater, Hall, & Mackinlay, 1985; Pearse & Mackinlay, 1989) and a resultant higher content of cheese moisture (Banks, 1990; Banks, Law, Leaver, & Horne, 1994; Guinee et al., 1998; Rynne et al., 2004). The higher moisture content leads to reductions in firmness and fracture stress (de Jong, 1978; El-Koussy, Amer, & Ewais, 1977; Guinee et al., 1998; Green, 1990a, 1990b; Marshall, 1986; Rynne et al., 2004), which is desirable in reduced-fat Cheddar to moderate its otherwise undesirable texture.

As water is a solvent for lactose, it follows that increasing the cheese moisture content leads to higher levels of lactose in the cheese. Lactose is fermented to lactate, mainly the L(+) isomer, at a rate dependent on the salt-in-moisture level in the cheese and the salt sensitivity of the starter culture strains used (Thomas & Crow, 1983; Turner & Thomas, 1980). Non-starter lactic acid bacteria (NSLAB) have the ability to convert L(+)-lactate to D(−)-lactate, eventually producing a racemic mixture (McSweeney & Fox, 2004). Racemisation of lactate may have consequences

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for cheese quality due to its ability to form calcium lactate crystals, which appear as undesirable white specks on the surface of cheese blocks (Kubantseva, Hartel, & Swearingen, 2004; McSweeney & Fox, 2004). The fermentation of residual lactose, which is generally complete within about 1 week in Cheddar cheese, results in a decrease in pH. Shakeel-Ur-Rehman, Waldron, and Fox (2004) reported an inverse relationship between pH and lactose content (at 1 day) of Cheddar cheese in which the lactose level was altered by curd washing or by addition of lactose powder to the cheese milk. However, there is little information on the effect of milk pasteurisation temperature on cheese microflora, pH or lactose metabolism.

The aim of this study was thus to examine the effect of increasing milk pasteurisation temperature on age-related changes in lactose metabolism, pH and the growth of NSLAB in half-fat Cheddar cheese.

2. Materials and methods

2.1. Cheese manufacture

Raw milk (2000 L) was standardised to a total protein-to-fat ratio of 2.35 and pasteurised at different temperatures (72, 77, 82 or 87 °C) for 26 s; the 26 s holding period is typical of that now being used in commercial cheese manufacture (Rynne et al., 2004). Defined strain starter cultures, *Lactococcus lactis* subsp. *cremoris* strain 303 and strain 227 (Chr. Hansen Ireland Ltd., Rohan Industrial Estate, Little Island, Cork), were grown separately overnight at 23 °C in reconstituted (10%, w/v), antibiotic-free skim milk powder (Golden Vale Food Products Ltd., Charleville, Co. Cork) which had been heated at 90 °C for 30 min. Each strain was inoculated at a level of 0.7% (w/w) into the cheesemilk and Cheddar cheeses were made as described by Rynne et al. (2004); cheese-making trials were performed in triplicate.

2.2. Gross compositional analysis of milk and cheese

Pasteurised milks were analysed for fat by the Röse-Gottlieb method (IDF, 1986), total protein by the macro-Kjeldahl method (IDF, 1993), and lactose content using a Milkoscan 605 (A/S N, Foss Electric, Denmark). The cheeses, which were those cheeses previously reported by Rynne et al. (2004), were measured for moisture and protein, at 14 days, using standard IDF methods. The pH at different time intervals throughout ripening was determined on a slurry prepared from 20 g of cheese and 12 g of de-ionised water (Guinee, Auty, & Fenelon, 2000).

2.3. Lactate in cheese

For all trials, the concentrations of D(−)- and L(+)-lactate in the cheeses were measured throughout ripening using a Boehringer Mannheim UV test kit (Cat No. 11 112 821 035, Boehringer Mannhein/R-Biopharm AG, Darmstadt, Germany). The level of lactose in cheeses was measured at 1 and 30 days using a Boehringer Mannheim UV test kit (Cat No. 0176 303).

Before analysis, cheese was finely grated (<1 mm), and approximately 1 g was weighed into a 100 ml volumetric flask and the weight recorded. Deionised water (75 ml) at 60 °C was added to the flask, which was placed in a water bath at 70 °C for 15 min with occasional shaking. Then 5 mL aqueous solution of potassium hexacyanoferrate (3.6 g $K_4[Fe(CN)_6] \times 3H_2O$/100 mL), 5 mL aqueous solution of zinc sulfate (7.2 g $ZnSO_4 \times 7H_2O$/100 mL) and 10 mL of NaOH (0.1 N) were added with shaking after each addition. The mixture was cooled to room temperature, made up to 100 mL with deionised water, and filtered through Whatman 42 filter paper. The concentrations of lactose, and D(−)- and L(+)-lactate in the resultant clarified filtrates were then enzymatically evaluated according to test kit instructions. The total lactate was calculated as the sum of the D(−)- and L(+)-lactate content in the cheeses. Each analysis was performed in duplicate.

2.4. Microbiological analysis of cheese

Microbiological analysis was performed on 10 g of cheese taken from a plug that was aseptically removed from the centre of each cheese block. The sample was placed in a sterile bag (Stomacher® lab system classic 400 standard bags, Seward Limited, 98 Great North Road, London) with 90 mL of sterile 2% (w/v) trisodium citrate, and blended for 4 min at room temperature (Stomacher Lab-Blender 400; Seward Medical, London). A serial dilution of the resultant slurry was performed in 9 mL of sterile maximum recovery diluent (Oxoid, Basingstoke, UK) and the bacterial groups were enumerated on the following agars: starter lactococci on LM17 agar (Becton Dickson & Company, Cockeysville, USA) incubated at 30 °C for 3 days (Terzaghi & Sandine, 1975) and non-starter lactic acid bacteria (NSLAB) on LBS agar (Becton Dickson & Company, Cockeysville, USA) incubated aerobically with an overlay for 5 days at 30 °C (Rogosa, Mitchell, & Wiseman, 1951). Microbiological analysis was performed in duplicate at each sampling time.

2.5. Statistical analysis

Three replicate cheesemaking trials were undertaken on different days; in each, cheeses were produced from milks pasteurised at each of four temperatures (72, 77, 82 or 87 °C). A randomised complete block design, which incorporated the four treatments (pasteurisation temperatures) and three blocks (replicate trials), was used for analysis of the response variables relating to composition of the cheesemilk and cheeses. Analysis of variance (ANOVA) was carried out using a SAS procedure (SAS 1995, SAS® User's Guide: Statistics, Version 6.12 Edition. Cary, NC: SAS Institute) where the effects of treatment and replicates were estimated for all response variables. Duncan's

multiple-comparison test was used as a guide for pair comparisons of the treatment means; the level of significance was determined at $P < 0.05$.

A split-plot design was used to monitor the effects of treatment, storage time and their interaction on the response variables measured at regular intervals during storage, namely lactate content, pH and starter and non-starter lactic acid bacteria (NSLAB) counts. Analysis of variance for the split-plot design was carried out using a general linear model (GLM) procedure of SAS (SAS 1995, SAS® User's Guide: Statistics, Version 6.12 Edition. Cary, NC: SAS Institute). Statistically-significant differences ($P < 0.05$) between different treatment levels were determined by Fisher's least significant difference.

3. Results and discussion

3.1. Gross compositions of milks and cheeses

The compositions of milks and cheeses have been described in a previous paper by Rynne et al. (2004). Increasing pasteurisation temperature generally had little effect on the gross composition of milk, as would be expected. In contrast, pasteurisation temperature significantly increased the levels of moisture (from a mean of 45.2%, w/w, at 72 °C to 49.9%, w/w, at 87 °C) and moisture in non-fat substance (MNFS) in the cheeses, and reduced the contents of fat and protein (Rynne et al., 2004).

3.2. Lactose and lactate levels in cheese

3.2.1. Lactose

The level of lactose was very low ($\leqslant 0.14$ g/100 g) in all cheeses at 1 day (Table 1) and did not differ significantly with milk pasteurisation temperature. However, there was a non-significant numerical increase in lactose level with pasteurisation temperature, which might be attributed to the increase in moisture content of the cheese, which is the solvent for lactose. At 30 days there was virtually no lactose in the cheese (Table 1), an occurrence which agrees with previous studies for full-fat Cheddar cheese which show that lactose is rapidly utilised (e.g., ~15 days), especially at low salt-in-moisture levels, i.e., < 4.0%, w/w (O'Connor, 1974; Thomas & Pearce, 1981; Turner & Thomas, 1980); the S/M levels of all cheeses in the current study were < 3.4% (w/w).

3.2.2. Total lactate

The level of total lactate increased significantly ($P < 0.001$) with pasteurisation temperature (Fig. 1(a), Table 2). This effect may be attributed to the higher initial levels of lactose in the cheese curd during the early stages of pressing, before its depletion by the starter culture. It is expected that the initial concentration of the lactose in the moisture phase of cheese curd would be similar to that in the serum phase of the cheese milk, and would increase as the level of moisture in the cheese curd increases.

The mean level of total lactate increased significantly ($P < 0.001$) with ripening time (Fig. 1(a), Table 2), most notably between days 1 and 30. The increase in lactate content during the first 30 days of ripening is consistent with previous results for full-fat Cheddar cheese (Jordan & Cogan, 1993; Thomas & Crow, 1983; Thomas & Pearce, 1981; Turner & Thomas, 1980) and has been attributed to the metabolism of lactose to lactate by the starter bacteria. However, the significant ($P < 0.01$) increase in lactate concentration between 30 and 270 days (~ 0.20 g/100 g) for all cheeses cannot be entirely attributable to the utilisation of lactose, which was present at only very low levels ($\leqslant 0.02$ g/100 g) at day 30 and which, on metabolism, should, theoretically, give an equal weight content (g/100 g) of lactate. The extra lactate may reflect metabolism of another substrate, e.g., sugars released from the glycomacropeptide of casein and the glycoproteins associated with the fat globule membrane (Beresford & Williams, 2004). Mesophilic lactobacilli have been shown to possess such glycoside hydrolase activity and may be capable of producing lactate from these sugars (Williams & Banks, 1997). It is

Table 1
The effect of milk pasteurisation temperature[A] on the levels of moisture, lactose, total lactate and ratios of L(+)-to D(−)-lactate, protein-to-total lactate and phosphate-to-total lactate in half-fat Cheddar cheese

	Pasteurisation temperature (°C)				SED[B]
	72	77	82	87	
Moisture (g/100 g)	45.2[a]	47.3[ab]	48.8[c]	49.9[c]	1.03
Lactose (g/100 g)					
1 day	0.070[a]	0.087[a]	0.101[a]	0.140[a]	0.04
30 days	0.000[a]	0.004[a]	0.002[a]	0.021[a]	0.01
L(+)-to-D(−)-lactate at 270 days	1.21[a]	1.18[a]	1.11[a]	1.09[a]	0.07
Total lactate (g/100 g) at 30 days	1.66[a]	1.73[a]	1.78[a]	1.90[b]	0.05
Protein-to-total lactate at 30 days	19.7[a]	18.4[b]	17.4[b]	15.9[c]	0.55
Phosphate-to-total lactate at 30 days	399[a]	370[b]	345[b]	310[c]	10.7

[a–c] Values within a row not sharing a common superscript differ significantly, $P < 0.05$; data were analysed from three replicate trials.
[A] The residence time at the pasteurisation temperature was 26 s.
[B] Standard error of difference across the four treatment means.

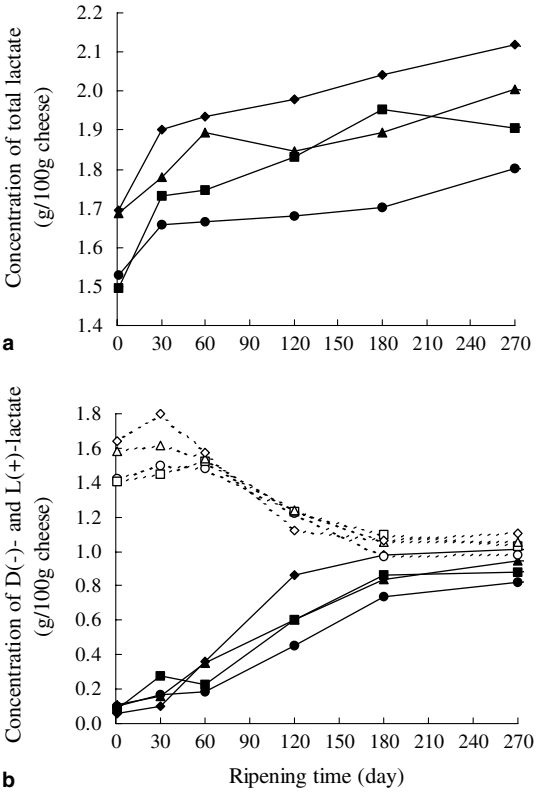

Fig. 1. The effect of milk pasteurisation temperature [72 °C (○, ●), 77 °C (□, ■), 82 °C (△, ▲) or 87 °C (◇, ◆)], with holding for 26 s, on the levels of (a) total lactate (closed symbols) and (b) D(−)-lactate (closed symbols, solid lines) and L(+)-lactate (open symbols, broken lines) in half-fat Cheddar cheese during ripening.

noteworthy that the increase in total lactate content between 30 and 270 days was paralleled by an increase of $\sim 10^6$ cfu/g in non-starter lactic acid bacteria (NSLAB) populations in all cheeses over the same period (see Fig. 3). However, a similar increase in total lactate (~ 0.3 g/100 g) following lactose utilisation in full-fat Cheddar with a low salt-in-moisture level (4.1%, w/w) did not coincide with an increase in NSLAB count in the study of Turner and Thomas (1980), suggesting that factors other than NSLAB may contribute to lactate production in Cheddar cheese.

3.2.3. D(−)- and L(+)-lactate

The mean concentrations of D(−)-lactate over the 270 day ripening period increased significantly ($P < 0.05$) with pasteurisation temperature, which is consistent with the concomitant increases in levels of moisture and total lactate (Fig. 1(b); Table 2). In contrast, pasteurisation temperature of the milk did not significantly affect the level of L(+)-lactate in the cheeses, even though, after 1 and 30 days of ripening, the mean levels of this constituent in the cheeses from milk pasteurised at 87 °C were significantly higher than those from milks pasteurised at 72 or 77 °C (Fig. 1(b)).

The level of D(−)-lactate increased significantly ($P < 0.001$) with ripening time up to 180 days and thereafter levelled off; this trend generally coincided with that noted for growth of NSLAB, as discussed below. The age-related changes in the concentration of L(+)-lactate showed an inverse trend to that noted for D(−)-lactate, decreasing significantly ($P < 0.001$) to 180 days and thereafter remaining constant (Fig. 1(b)). These results are consistent with those previously reported for full-fat Cheddar cheese (Jordan & Cogan, 1993; Martley & Crow, 1993; Turner & Thomas, 1980), and indicate the racemisation of L(+)-lactate to D(−)-lactate by NSLAB (McSweeney & Fox, 2004), which reached populations of 10^6 to 10^8 cfu/g at times $\geqslant$180 day. It is unlikely that D(−)-lactate was formed from residual lactose which was essentially all utilised by 30 days, when both the NSLAB counts (Fig. 3) and the concentrations of D(−)-lactate were very low (Fig. 1(b)). In agreement with the results of Turner and Thomas (1980) for full-fat Cheddar cheese with salt-in-moisture levels of $\sim 4\%$ (w/w), L(+)-lactate was the major end-product, being ~ 0.1–0.15 g/100 g higher than D(−)-lactate when the concentrations of the isomers had stabilised, at ripening times $\geqslant$180 day.

3.3. Age-related changes in pH

Increasing the pasteurisation temperature from 72 to either 82 or 87 °C resulted in a significant ($P < 0.01$ or $P < 0.001$, respectively) decrease in cheese pH over the 270 day ripening period (Fig. 2, Table 2). This trend con-

Table 2
Degrees of freedom (df), mean squares (MS) and statistical significances (*P*-values) for changes in total lactate, D(−)-lactate, L(+)-lactate and pH in half-fat Cheddar cheeses made from milks pasteurised at different temperatures[a]

Factors	Total lactate			D(−)-lactate			L(+)-lactate			pH		
	df	MS	*P*	df	MS	*P*	df	MS	*P*	df	MS	*P*
Main plot												
Past. temp.	3	0.239	0.000	3	0.072	0.041	3	0.053	0.069	3	0.118	0.002
Error	6	0.006		6	0.014		6	0.013		6	0.006	
Sub-plot												
Time		0.180	<0.0001		1.517	<0.0001		0.768	<0.0001		0.154	<0.0001
Interaction (past. temp. × time)	15	0.007	0.941	15	0.022	0.139	15	0.018	0.378	18	0.004	0.255
Error	40	0.015		40	0.014		40	0.016		48	0.003	

[a] The residence time at each pasteurisation temperature (72, 77, 82 and 87 °C) was 26 s; data were analysed from three replicate trials.

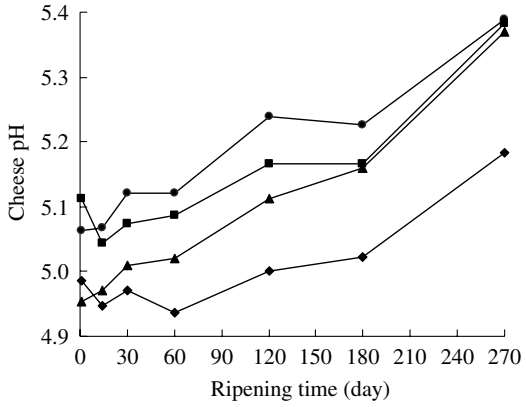

Fig. 2. The effect of milk pasteurisation temperature (72 °C ●; 77 °C ■; 82 °C ▲; or 87 °C ◆), with holding for 26 s, on the pH of half-fat Cheddar cheese during ripening.

curs with that reported by Guinee et al. (1998), who suggested that the decrease in pH may coincide with concomitant increases in the level of moisture and thus lactate (Shakeel-Ur-Rehman et al., 2004), and a reduction in levels of protein and phosphate and, thereby, buffering capacity (Lucey & Fox, 1993). Indeed, the decrease in the ratios of protein-to-total lactate, and of phosphate-to-total lactate as the pasteurisation temperature was elevated from 72 to 82 or 87 °C (Table 1) are expected to promote concomitant reductions in the ratio of buffering capacity-to-lactate and pH values (Guinee et al., 1998) as the pasteurisation temperature is increased. Moreover, the increase in set-to-cut time with pasteurisation temperature in the current study, as previously reported by Rynne et al. (2004), may have also contributed to the lower pH in the cheeses made from the high temperature-pasteurised milk. A lower pH at cutting leads to more phosphate being lost from the curd during cheese manufacture (Rynne et al., 2004), which in turn would result in a lower buffering capacity (Czulak, Conochie, Sutherland, & van Leeuwen, 1969).

The pH of all cheeses increased significantly over the 270 day ripening period, from a mean value of 5.03 for all cheeses at 1 day to 5.33 at 270 day (Fig. 2, Table 2). This trend is in agreement with that reported in previous studies for half-fat Cheddar cheese by Fenelon and Guinee (2000) who attributed the increase in pH to the production of ammonia, via deamination of free amino acids and/or oxidative deamination of amines. Similar increases have been reported in non-Swiss type, bacterially-internal-ripened hard cheeses, e.g., Gouda cheese made from late lactation milk (Lawrence, Creamer, & Gilles, 1987), and semi-hard cheeses produced from UF retentate (Guinee et al., 1995). In the latter study, this was attributed to a relatively high protein-to-lactate ratio and high concentrations of amino N in the cheese. In a recent study, Shakeel-Ur-Rehman et al. (2004) reported that the pH of Cheddar cheeses made from milks with altered lactose levels was inversely correlated with the lactose level in the cheese at day 1. Similarly in the current study, increasing the milk pasteurisation temperature resulted in cheeses with higher lactate levels and lower pH values.

3.4. Levels of starter and non-starter lactic acid bacteria (NSLAB)

The changes in the mean populations of starter and non-starter lactic acid bacteria (NSLAB) in cheese over 120 and 270 days of ripening, respectively, are shown in Fig. 3. Starter counts at times >120 days were not measured as NSLAB may also grow on the medium (LM17) used to enumerate the starter bacteria (Cogan, 2003; Crow, Martley, Coolbear, & Roundhill, 1995) and, therefore, augment the apparent count of the latter especially when they are ⩽1 log cycle below that of the starter (e.g., at 180 day in the current study) (Fig. 3).

The starter count at 1 day was $10^{8.5}$–10^{9} cfu/g for all cheeses, which was typical of that reported elsewhere for half-fat Cheddar (Fenelon et al., 1999; Fenelon, O'Connor, & Guinee, 2000; Fenelon, Beresford, & Guinee, 2002) and full-fat Cheddar (Fenelon et al., 2000; Folkertsma, Fox, & McSweeney, 1996; Hannon et al., 2003; O'Donovan, Wilkinson, Guinee, & Fox, 1996; Wilkinson, Guinee, O'Callaghan, & Fox, 1995) cheeses. The mean starter count across the four treatments increased slightly, but significantly ($P < 0.05$), during the first 30 days of ripening (from $10^{8.9}$ cfu/g at day 1 to $10^{9.2}$ cfu/g cheese at day 30) and thereafter decreased significantly ($P < 0.001$) to day 120 (Fig. 3; Table 3). However, the mean starter population across all treatments decreased significantly on ripening for 120 days (Table 3). The slight increase in starter numbers during the first 30 days of ripening is consistent with the decrease in lactose and increase in lactate contents during the same time period; provided a S/M content of < 6%, starter bacteria have the ability to utilise residual lactose in

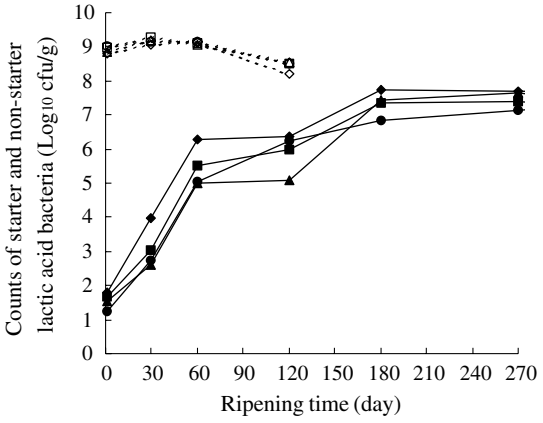

Fig. 3. The effect of milk pasteurisation temperature [72 °C (○, ●), 77 °C (□, ■), 82 °C (△, ▲), or 87 °C (◇, ◆)], with holding for 26 s, on the mean counts of starter (open symbols, broken lines) and non-starter lactic acid bacteria (closed symbols, solid lines) in half-fat Cheddar cheese during ripening.

Table 3
Degrees of freedom (df), mean squares (MS) and statistical significances (*P*-values) for microbiological counts of half-fat Cheddar cheese from milks pasteurised at different temperatures[a]

Factors	Starter culture counts			NSLAB[b] counts		
	df	MS	*P*	df	MS	*P*
Main plot						
Past. temp.	3	0.060	0.067	3	1.938	0.060
Error	6	0.032		6	0.446	
Sub-plot						
Time	5	5.456	<0.0001	5	54.648	<0.0001
Interaction (past. temp. × time)	15	0.046	0.704	15	0.219	0.9802
Error	36	0.061		31	0.608	

[a] The residence time at each pasteurisation temperature (72, 77, 82 and 87 °C) was 26 s; data were analysed from three replicate trials.
[b] Non-starter lactic acid bacteria.

cheese as an energy source and in doing so convert it to lactate (Lawrence et al., 2004; Thomas & Pearce, 1981; Turner & Thomas, 1980). The subsequent decrease in starter numbers has been reported previously (Fenelon et al., 1999, 2000, 2002) and has been attributed to a number of factors, such as depletion of their primary energy source (lactose), autolysis and inhibition by salt (Beresford, Fitzsimons, Brennan, & Cogan, 2001; Farkye, 2000).

The mean starter levels over the 120 day ripening period were not significantly affected by pasteurisation temperature or by the interaction between pasteurisation temperature and stage of ripening (Table 3). This trend suggests that the temperature-dependent increase in denaturation of whey proteins (from ~3 to ~34% of total whey protein on increasing heating temperature of milk from 72 to 87 °C) (Rynne et al., 2004), and their complexation with casein, had little impact on the growth of starter bacteria during cheese manufacture or their viability during maturation.

The mean NSLAB counts were not significantly affected by pasteurisation temperature or the interaction between pasteurisation temperature and ripening time, but increased significantly during ripening (Fig. 3; Table 3), especially during the first 60 days, to counts (~$10^{7.5}$ cfu/g), typical of those reported elsewhere for reduced-fat and full-fat Cheddar cheeses (Fenelon et al., 1999, 2000, 2002; Haque, Kucukoner, & Aryana, 1997); NSLAB counts increased significantly ($P < 0.001$) during the first 180 days of ripening and then levelled off towards the end of ripening (Fig. 3). Similar trends for NSLAB have been reported previously for reduced-fat (Fenelon et al., 1999, 2002) and full-fat Cheddar cheese (Jordan & Cogan, 1993; Lane, Fox, Walsh, Folkertsma, & McSweeney, 1997; O'Donovan et al., 1996). However, the rate of growth of NSLAB in the current cheeses appeared to be slower than that in full-fat Cheddar, as reflected by the longer times before NSLAB counts began to level off at numbers (e.g., 10^8 cfu/g) typical of those found in aged full-fat Cheddar, e.g., ~180 day in the current study, compared to ~56–96 days for full-fat Cheddar cheeses ripened at 6–8 °C (Jordan & Cogan, 1993; Lane et al., 1997; O'Donovan et al., 1996). This trend, which was also observed by Fenelon et al. (2000) when comparing Cheddar cheeses of different fat contents, may possibly be due to the lower fat content and, hence, fat globule membrane material, in half-fat Cheddar cheese. Non-starter mesophilic lactobacilli have the ability to utilise sugars derived from the fat globule membrane glycoproteins as an energy source where the lactose level is very low, as in the cheese environment (Beresford & Williams, 2004).

4. Conclusions

Increasing the milk pasteurisation temperature did not significantly affect the mean populations of starter or non-starter lactic acid bacteria (NSLAB) in reduced-fat Cheddar throughout ripening. Lactose was present in all cheeses at 1 day of ripening with the mean levels increasing numerically with pasteurisation temperature. The level of lactate, which occurred in two isomeric forms, D(−)- and L(+)-lactate, increased rapidly during the first 30 days and thereafter more slowly. The level of L(+)-lactate decreased during ripening, while that of D(−)-lactate increased, resulting in a ratio of L(+)-to-D(−)-lactate which decreased to a mean of ~1.2 to 1.1 at 270 day on increasing pasteurisation temperature. The increase in D(−)-lactate coincided with the growth of NSLAB, which increased from $10^{1.5}$ at 1 day to $10^{7.5}$ cfu/g at 270 day. The increases in D(−)-lactate could have a negative impact on cheese quality because of its precipitation in the form of calcium lactate crystals (Kubantseva et al., 2004), which affect cheese appearance and consumer acceptance (Chou, Edwards, Luedecke, Bates, & Clark, 2003; Severn, Johnson, & Olson, 1986); in this context, it is noteworthy that both the total calcium content and the calcium-to-moisture ratio of half-fat Cheddar cheese are significantly higher than those of its full-fat equivalent (Fenelon & Guinee, 1999; Guinee et al., 2000). However, the susceptibility to formation of these crystals in reduced-fat cheese from milk pasteurised at high pasteurisation temperatures could be minimised by reducing the lactose and hence lactate, in the moisture phase of the cheese, e.g., by curd washing. Alternatively, suppression of the growth of NSLAB by the use of starter cultures which produce bacteriocins that

inhibit NSLAB, e.g., Lacticin 3147 (Fenelon et al., 1999), would reduce the risk of calcium lactate crystals forming in reduced-fat Cheddar from high-temperature pasteurised milks.

The increase in total lactate levels resulting from increasing the milk pasteurisation temperature was accompanied by a significant reduction in cheese pH and an increase in the rate of hydrolysis of α_{s1}-casein to α_{s1}-CN(f24-199) (Rynne et al., 2004), and a marked weakening of the *para*-casein matrix, which are conducive to reductions in fracture stress and firmness (Creamer & Olson, 1982; de Jong, 1976, 1977; Guinee et al., 2000; Rynne et al., 2004) and improved texture of reduced-fat Cheddar cheese. Moreover, the lower pH in the cheeses made from milk pasteurised at high temperatures may favour a higher solubility of calcium lactate (Kubantseva et al., 2004) and a lower incidence of calcium lactate crystallisation in reduced-fat Cheddar cheese.

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