A detailed investigation was undertaken to determine the effects of four single starter strains, \textit{Lactococcus lactis} subsp. lactis 303, \textit{Lc. lactis} subsp. cremoris HP, \textit{Lc. lactis} subsp. cremoris AM2, and \textit{Lactobacillus helveticus} DPC4571 on the proteolytic, lipolytic and sensory characteristics of Cheddar cheese. Cheeses produced using the highly autolytic starters 4571 and AM2 positively impacted on flavour development, whereas cheeses produced from the poorly autolytic starters 303 and HP developed off-flavours. Starter selection impacted significantly on the proteolytic and sensory characteristics of the resulting Cheddar cheeses. It appeared that the autolytic and/or lipolytic properties of starter strains also influenced lipolysis, however lipolysis appeared to be limited due to a possible lack of availability or access to suitable milk fat substrates over ripening. The impact of lipolysis on the sensory characteristics of Cheddar cheese was unclear, possibly due to minimal differences in the extent of lipolysis between the cheeses at the end of ripening. As anticipated seasonal milk supply influenced both proteolysis and lipolysis in Cheddar cheese. The contribution of non-starter lactic acid bacteria towards proteolysis and lipolysis over the first 8 months of Cheddar cheese ripening was negligible.

Keywords: Starter lactic acid bacteria, proteolysis, lipolysis, descriptive sensory analysis, Cheddar cheese.
Materials and Methods

Starter cultures for cheesemaking

*Lc. lactis* subsp. *lactis* 303 was obtained from Chr. Hansen’s Ireland Ltd, Little Island, Cork, Ireland and *Lc. lactis* subsp. *cremoris* AM2, *Lc. lactis* subsp. *cremoris* HP, and *Lb. helveticus* DPC4571 were obtained from the culture collection of Moorepark Food Research Centre, Teagasc, Fermoy, Co. Cork. The cultures were maintained in reconstituted skim milk (RSM, 100 g/l; Golden Vale Food Products Ltd, Cork, Ireland) at −80 °C. Prior to cheesemaking lactococcal and *Lb. helveticus* cultures were grown overnight at 23 and 37 °C respectively, in heat treated (95 °C for 30 min) RSM until the pH reached ~4.9.

Cheese manufacture

Antibiotic-free raw milk was obtained from the spring-calving Friesian herd at the Dairy Production Centre, Moorepark during mid lactation (ML) and late lactation (LL), ~180 and ~250 d of lactation, respectively. ML and LL raw milk was standardized to a protein to fat ratio of 0.98 and 0.92 respectively, pasteurized at 72 °C for 15 s and cooled to 31 °C. Initially Cheddar cheeses were manufactured at pilot scale (500 l vats) using 303, AM2 and HP starters and ML milk (303ML, HPML and AM2ML). Subsequently it was decided to include 4571 in the study to determine the impact of this highly proteolytic/autolytic starter on lipolysis in Cheddar cheese. Cheeses were made from LL milk with 303 and 4571 starters (303LL and 4571LL), 303LL cheeses were used as a control cheese as it has been shown that stage of lactation impacts on the biochemical and sensory properties of Cheddar cheese (Hickey et al. 2006). An inoculum level of 1.5 g/100 g for *Lc. lactis* 303, HP and AM2 and 1.8 g/100 g for *Lb. helveticus* 4571 was added to cheese milk. Cheeses were manufactured using conventional cheesemaking methods; curd was cooked at 38.5 °C, pitched at pH 6.15, milled at pH 5.35 and salted at 2.7% (wt/wt). Mean manufacturing times for 303ML, 303LL, HPML, AM2ML and 4571ML cheeses were 225, 220, 190, 285 and 400 min, respectively. The duration of cheese manufacture was longer using 4571 due to its poor acidification properties. As a result of the slow decrease in pH, the whey from 4571LL cheeses was tested for bacteriophage, however none were detected. All cheeses were made in triplicate and sampled at d 14 and 224 ripening for compositional and sensory analysis, respectively. All other analyses were carried out at d 1, 14, 28, 56, 112, 168 and 224.

Cheese composition

Grated cheese samples were analysed in duplicate for pH (BS, 1976), fat (International Dairy Federation (IDF), 1986), salt (IDF, 1988), total nitrogen (IDF, 1993) and moisture by drying to a constant weight at 102 °C (IDF, 1982).

Microbiological analysis

Microbiological analysis of the cheese was carried out in duplicate at each sampling point. Starter bacteria were enumerated up to d 56 ripening on LM17 agar after 3 d incubation at 30 °C (Terzaghi & Sandine, 1975). *Lb. helveticus* 4571 was enumerated anaerobically on MRS pH 5.4 agar after 3 d incubation at 37 °C. NSLAB were enumerated anaerobically on LBS agar (Rogosa et al. 1951) following 5 d incubation at 30 °C.

Starter autolysis in cheese

Autolysis of starter cultures in cheese during ripening was monitored by assaying in triplicate for the intracellular enzyme lactate dehydrogenase (LDH), released into the cheese juice at d 1, 14, 28 and 56 of ripening. Cheese juice was extracted from cheeses as described by Wilkinson et al. (1994b). LDH activity was measured by a modification of the method of Wittenberger & Angelo (1970) by measuring the decrease in absorbance at 340 nm (Spectronic Genesys 5 spectrophotometer, Milton Roy Company, Rochester, NY, USA) resulting from the pyruvate-dependent oxidation of NADH in the presence (for lactococcal starter strains) or absence of fructose-1, 6 bis-phosphate (for *Lb. helveticus* 4571 starter strain). Results were expressed as units/ml of cheese juice, where one unit is defined as the amount of LDH that catalyses the oxidation of 1 μM NADH per min.

Assessment of proteolysis in cheese during ripening

Proteolysis was monitored by measuring the percentage of total N soluble at pH 4.6 (pH 4.6-SN) and 5% phosphotungstic acid (PTA-SN) according to the methods of Kuchroo & Fox (1982) and Stadhouders (1960), respectively. Nitrogen was determined by the macro-Kjeldahl method (IDF, 1993). Individual free amino acids (FAA) were determined in 12% trichloroacetic acid filtrates prepared from the pH 4.6-SN fraction according to Wilkinson et al. (1992). Filtrates were analysed using a Jeol JLC-500/V amino acid analyzer fitted with a Jeol Na⁺ high performance cation exchange column (Jeol Ltd., Welwyn Garden City, Herts, UK).
Assessment of lipolysis in cheese during ripening

Individual free fatty acids (FFA) (C4:0 to C18:1) in cheese, were quantified by gas chromatograph flame ionized detection (GC FID) according to Hickey et al. (2006).

Descriptive sensory analysis

At the end of ripening (d 224), cheeses for sensory analyses were removed from storage, cut into 5 x 200 g blocks, vacuum packed and stored at –20 °C. Cheeses were stored frozen until sensory analysis for all samples could be carried out. Descriptive sensory analysis was carried out using the methods described by Hannon et al. (2005) with the following modifications; cheeses were individually assessed for odour and flavour by a panel of 9 assessors, 8 females and 1 male. Descriptive sensory assessment of the 9 test cheeses were carried out in duplicate and took place during 5 sessions, over 3 consecutive days. Data were recorded and scored using Compusense Five V4.0 (Compusense Inc., Ontario, Canada).

Statistical analysis

Statistical analysis of data was determined separately on the experimental cheese trials manufactured using ML (303ML, HPML and AM2ML cheeses) or LL milk (303LL and 4571LL cheeses). A randomized complete block design, which incorporated the treatments (starter type), and 3 blocks (replicate trials) was used for analysis of the response variables relating to cheese composition (Table 1). Analysis of variance (ANOVA) was carried out using the general linear model (GLM) procedure of SAS (2003) where the effect of treatment and replicates were estimated for all response variables. Duncan’s multiple-comparison test was used as a guide for pair comparison of the treatment means. The level of significance was determined at P<0.05.

A split plot design was used to monitor the effect of treatment, ripening time and their interaction on the response variables measured at regular intervals during ripening i.e. LDH activity, pH 4.6 S-N, 5% PTA-SN, concentrations of FAA and FFA. ANOVA for the split plot was carried out using the GLM procedure of SAS (2003). Statistically significant differences (P<0.05) between the different treatments were determined by Fisher’s least significant differences.

Duplicate scores from each assessor were analysed by ANOVA using SPSS V10.0 (SPSS Inc. Chicago, IL 60611, USA) using a significance level of P<0.05. Data were then averaged across replicates, standardized (1/Standard Deviation of the mean score for each attribute) and analysed by means of principal component analysis (PCA) using The Unscrambler V8.0 (Camoz, Oslo, Norway). ANOVA was performed on duplicate scores to determine the principal components (PC) that gave significant differences (P<0.05) between cheese effects. Duplicate scores were then averaged and PCA was performed.

Results

Cheese composition

Compositional analysis at d 14 for 303ML, HPML, AM2ML, 303LL and 4571LL cheeses are shown in Table 1. Some significant (P<0.05) compositional differences were observed in ML cheeses for protein and pH. Moisture and moisture in non-fat substances (MNFS) were significantly different (P<0.05) in 4571LL compared with 303LL cheeses. The composition of all cheeses were within thresholds recommended for good quality commercial Cheddar cheese (Gilles & Lawrence, 1973), apart from fat in dry matter (FDM) which was below 5% in all cheeses except 303LL.
Microbiology of the cheeses

Starter populations at d 1 were 9.4, 8.2, 6.0, 9.1 and 8.0 log cfu/g in 303ML, HPML, AM2ML, 303LL and 4571LL cheeses, respectively and viability declined in all except 303ML and 303LL cheeses up to d 56 (Fig. 1a). Starter populations in AM2ML decreased to 4.6 log cfu/g by d 28 and to 3.4 and 6.6 log cfu/g in 4571LL and HPML cheeses by d 56.

NSLAB populations increased in all cheeses during ripening, reaching 7.5, 6.8, 7.7, 7.4 and 6.3 log cfu/g in 303ML, HPML, AM2ML, 303ML and 4571LL cheeses, respectively at d 224 (Fig. 1b). NSLAB populations were markedly lower in 4571LL cheeses throughout ripening.

Fig. 1. Changes in mean populations of (a) starter and (b) NSLAB in Cheddar cheese made from mid lactation milk with Lactococcus lactis subsp. lactis 303 (○), Lc. lactis subsp. cremoris HP (●), and Lc. lactis subsp. cremoris AM2 (△) and late lactation milk with Lc. lactis subsp. lactis 303 (▲) and Lactobacillus helveticus 4571 (□). Values presented are the means of three replicate trials.

Starter autolysis

Autolysis was monitored up to d 56 ripening (Fig. 2). In cheeses made from ML milk, mean LDH activity in AM2ML cheeses increased steadily throughout ripening and was significantly higher ($P<0.01$) than in HPML and 303ML cheeses. Significant differences were not observed between HPML and 303ML cheeses. In cheeses made from LL milk, mean LDH activity in 4571LL cheeses was significantly higher ($P<0.05$) than 303LL during ripening. Autolysis in 303ML and 303LL cheeses was comparable.

Fig. 2. Lactate dehydrogenate (LDH) activity in cheese juice expressed from Cheddar cheese made from mid lactation milk with Lactococcus lactis subsp. lactis 303 (○), Lc. lactis subsp. cremoris HP (●), and Lc. lactis subsp. cremoris AM2 (△) and late lactation milk with Lc. lactis subsp. lactis 303 (▲) and Lactobacillus helveticus 4571 (□). Values presented are the means of three replicate trials.

Proteolysis

Levels of pH 4.6-SN, 5% PTA-SN and individual FAA increased significantly ($P<0.001$) over ripening in all cheeses (Fig. 3). No significant differences were observed for mean levels of pH 4-6-SN between 303ML, HPML and AM2ML cheeses, however mean levels were significantly
higher ($P < 0.05$) in 4571LL cheeses compared with 303LL cheeses. Mean levels of 5% PTA-SN in AM2ML and HPML cheeses were similar but significantly higher ($P < 0.05$) than in 303ML cheeses. 4571LL cheeses had significantly higher ($P < 0.01$) mean levels of 5% PTA-SN compared with 303LL cheeses. Individual FAA increased significantly ($P < 0.001$) in all cheeses during ripening (results not shown). Mean levels of individual FAA in AM2ML cheeses were significantly higher ($P < 0.05$) than in 303ML and HPML cheeses and FAA levels in 4571LL cheeses were significantly higher ($P < 0.05$) than in 303LL cheeses at the end of ripening (Fig. 4). There were some differences in the ratios of individual FAA between cheeses, however in all cheeses the predominant FAA were glutamic acid and leucine.

**Lipolysis (free fatty acids)**

Levels of individual FFA from C$_4$:0 to C$_{18}$:1 in ML and LL cheeses up to d 224 ripening are presented in Table 2. At d 1 ripening, no significant differences were observed in total FFA (TFFA) between 303ML, HPML and AM2ML cheeses, however TFFA were significantly higher ($P < 0.05$) in 4571LL cheeses compared with 303LL cheeses at this time. During ripening mean levels of TFFA were significantly higher ($P < 0.05$) in AM2ML and HPML cheeses and FAA levels in 4571LL cheeses were significantly higher ($P < 0.05$) than in 303LL cheeses at the end of ripening (Fig. 4). There were some differences in the ratios of individual FAA between cheeses, however in all cheeses the predominant FAA were glutamic acid and leucine.

**Descriptive sensory analysis of the cheese at d 224**

To illustrate sensory differences between the cheeses, the data matrix of attributes were analysed by PCA. One way ANOVA showed that PC 1 and PC 2 accounted for 48% of the experimental variance and discriminated significantly ($P < 0.05$) between cheeses. A biplot of the scores and loadings for the PC 1 and PC 2 is illustrated in Fig. 5a. PC 1 explained 31% of the variation between the sensory characteristics of these cheeses and distinguished AM2ML cheeses from 303ML and HPML cheeses. Sensory analysis made no clear distinction between 303ML and HPML cheeses. Significant sensory differences ($P < 0.05$) were evident between 303ML, HPML and AM2ML cheeses for 6 of the 24 attributes evaluated. The attributes that significantly discriminated between these cheeses, were odour...
Table 2. Individual free fatty acids (FFA) levels expressed as mg/kg cheese, in cheeses made from mid lactation milk with Lactococcus lactis subsp. lactis 303 (303ML), Lc. lactis subsp. cremoris HP (HPML) and Lc. lactis subsp. cremoris AM2 (AM2ML) and late lactation milk with Lc. lactis subsp. lactis 303 (303LL) and Lactobacillus helveticus DPC4571 (4571LL) during ripening and significance for each FFA for the factors: starter type, ripening time and their interaction

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Significance (ML cheeses)

| Starter | NS | NS | NS | * | NS | * | NS | ** | * |
| Ripening Time | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Starter x Time | NS | NS | NS | NS | NS | NS | NS | NS | NS |

Significance (LL cheeses)

| Starter | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Ripening Time | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Starter x Time | NS | NS | NS | NS | NS | NS | NS | NS | NS |

***P<0.001, **P<0.01, *P<0.05; NS, not significant
303LL cheeses had significantly higher \((P<0.05)\) odour ‘pungent’, ‘sulphur’, ‘sweaty’, ‘rancid’ and ‘fruity’ attributes and flavour ‘mouldy’, ‘onion’, ‘fruity’ ‘buttery’ and ‘bitter’ attributes, while 4571 cheeses had significantly higher \((P<0.05)\) ‘cooked animal fat’ and ‘caramel’ odour scores and ‘caramel’, ‘cooked animal fat’ and ‘sweet’ flavour attributes.

**Discussion**

In agreement with previous studies, 303 remained viable during the early stages of ripening with low levels of autolysis, HP decreased in viability but was not accompanied by extensive autolysis, while both AM2 and 4571 decreased in viability accompanied by substantial cell autolysis (Wilkinson et al. 1994a; O’Donovan et al. 1996; Hannon et al. 2003; Sheehan et al. 2006). While minor differences in composition were found between 303ML, HPML and AM2ML cheeses it is unlikely they impacted on cheese ripening. The lower moisture and MNFS in 4571LL cheeses in comparison with 303LL cheeses was probably due to the extended manufacturing time required for 4571LL cheeses, as increasing stir and Cheddaring time increases syneresis (Dejmek & Walstra, 2004).

In agreement with previous studies (O’Donovan et al. 1996; Hannon et al. 2003; Kenny et al. 2006), cheeses produced using the highly autolytic strains, 4571 and AM2 accelerated secondary proteolysis over cheeses produced by 303 and HP. However, significantly higher levels of both 5% PTA-SN and FAA were found in 4571LL cheeses, confirming that intracellular peptidase activity of 4571 is higher than for AM2 (Kenny et al. 2003; Sheehan et al. 2006). Cheeses produced with 303 had the lowest levels of secondary proteolysis due to its high viability and limited autolysis during ripening.
Cheeses produced with 4571 had the highest levels of FFA over ripening and may indicate the link between autolysis and lipolysis as previously suggested by Collins et al. (2003b). Differences in FFA levels in cheeses made using the highly autolytic strain AM2 or the poorly autolytic strains 303 and HP, were only apparent after d 112, suggesting that the lipolytic enzyme complement of individual starters, along with accessibility and availability of suitable milkfat substrates may play a role in determining the extent of starter-strain related lipolysis in Cheddar cheese. At d 1 ripening, significantly higher levels of FFA were found in 4571LL cheeses compared with 303LL cheeses, suggesting that 4571 may be generating FFA in the cheese vat possibly from a combination of its autolytic/lipolytic properties and/or the extended manufacturing time in the vat. In 4571LL cheeses, a slow down in the production of FFA was noted after d 112, suggesting that intracellular esterases of starter bacteria may lack access to suitable milkfat substrates over ripening. LAB esterases appear to have a preference for mono- and di-acylglycerides over tri-acylglycerides (Stadhouders & Veringa, 1973; Holland et al. 2005) and a lack of production/availability of these substrates may be partially responsible for the small differences in FFA content noted over ripening between highly autolytic strains such as AM2 and poorly autolytic strains such as 303 and HP. The decline in FFA generation after d 112 ripening in 4571 cheeses may indicate catabolism of FFA. The influence of a seasonal milk supply on lipolysis was also highlighted, as identified by Hickey et al. (2006). A seasonal milk supply may exert a greater influence on the extent of lipolysis than starter type as levels of FFA were significantly greater in 303LL than 303ML, HPML and AM2ML cheeses. Starter strain type appeared to have a greater impact on lipolysis than compositional differences such as moisture and MNFS, as 303LL cheeses despite having higher moisture and MNFS still had lower levels of lipolysis than 4571LL cheeses.

Starter strain related effects on levels of proteolysis significantly influenced the sensory attributes of the resulting Cheddar cheeses. Differences in the level of FFA between the cheeses at the end of ripening were very low and therefore the impact of lipolysis on sensory character of the cheese is unclear. In agreement with Wilkinson et al. (1994a) and Morgan et al. (1997) Cheddar cheese produced using the poorly autolytic strains 303 and HP were associated with bitterness. In contrast, 4571LL and AM2ML cheeses were associated with ‘sweet’ and ‘caramel’ flavours and odours. Higher levels of glycine, serine, threonine, proline, lysine and/or alanine in 4571LL cheeses possibly contributed to the sweet flavour attributes of this cheese (Fox & McSweeney, 1995). Similarly high levels of glycine, serine, threonine and possibly lysine contributed to the sweet flavour of AM2 cheeses. At the end of ripening 303ML/LL and HPML cheeses were associated with negative lipolytic flavour attributes ‘soapy’ and ‘rancid’, despite having lower levels of lipolysis than 4571LL cheeses, which were not associated with these off-flavours. These results highlight the complexity of Cheddar cheese flavour, and may indicate that higher concentrations of FAA may counteract negative flavours associated with FFA at certain levels.

NSLAB levels developed in cheeses over ripening were comparable to those reported in other studies (Collins et al. 2003b; Hannon et al. 2003, 2005). It appears that 4571 inhibits NSLAB growth possibly by the generation of antimicrobial peptides or by competitive inhibition as most NSLAB are also lactobacilli. The lower MNFS in 4571LL cheeses did not appear to influence NSLAB levels, as Hannon et al. (2003) reported that NSLAB populations only reached 10^3 cfu/g by d 224 in Cheddar cheese made using 4571 with a MNFS of 55%. Crow et al. (1993) suggest that NSLAB are unlikely to influence biochemical activity in cheese below 10^6 cfu/g, however, even at 10^7 cfu/g NSLAB were unlikely to contribute towards lipolysis as a suitable fat substrate may not be available or accessible at maximum NSLAB numbers. NSLAB strains typically found in Irish Cheddar cheese (Lb. casei, Lb. plantarum and Lb. curvatus) are viable, but non-autolytic (Jordan & Cogan, 1993; Fitzsimons et al. 1999; Kiernan et al. 2000), therefore intracellular esterases would not be able to access a suitable substrate. In addition no single NSLAB strain appears to dominate during maturation of Irish Cheddar (Fitzsimons et al. 2001) and no individual strain is likely to reach sufficient numbers to significantly contribute to flavour development. Overall, this report suggests that mesophilic lactobacilli (NSLAB) normally occurring in Cheddar cheese have a limited role in cheese flavour development at least up to d 224 and in particular have little or no impact on lipolysis during ripening. Our conclusion regarding the relative role of lipolysis in Cheddar cheese by starter LAB and NSLAB concurs with that of Stadhouders & Veringa (1973), who suggested that in Gouda cheese lipolysis was primarily due to the lipolytic activity of starter LAB.

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References


Fitzsimons NA, Cogan TM, Condon S & Beresford T 1999 Phenotypic and genotypic characterization of non-starter lactic acid bacteria in mature Cheddar cheese. *Applied and Environmental Microbiology* 65 3418–3426

Fitzsimons NA, Cogan TM, Condon S & Beresford T 2001 Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. *Journal of Applied Microbiology* 90 600–608


Kiernan RC, Beresford TP, O’Cuinn G & Jordan KN 2000 Autolysis of lactobacilli during Cheddar cheese ripening. *Irish Journal of Agricultural and Food Research* 39 95–106

Kuchroo CN & Fox PF 1982 Soluble nitrogen in cheese: Comparison of extraction procedures. *Milchwissenschaft* 37 331–335

McSweeney PLH, Fox PF, Lucey JA, Jordan KN & Cogan TM 1993 Contribution of the indigenous microflora to the maturation of Cheddar cheese. *International Dairy Journal* 3 613–634


Sheehan A, O’Cuinn G, Fitzgerald RJ & Wilkinson MG 2006 Proteolytic enzyme activities in Cheddar cheese juice made using lactococcal starters of differing autolytic properties. *Journal of Applied Microbiology* 100 893–901


Terzaghi BE & Sandine WE 1975 Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology* 29 807–813


Wilkinson MG, Guinee TP, O’Callaghan DM & Fox PF 1994a Autolysis and proteolysis in different strains of starter bacteria during Cheddar cheese ripening. *Journal of Dairy Research* 61 249–262

Wilkinson MG, Guinee TP & Fox PF 1994b Factors which may influence the determination of autolysis of starter bacteria during Cheddar cheese ripening. *International Dairy Journal* 4 141–160