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Effect of galactose metabolising and non-metabolising strains of *Streptococcus thermophilus* as a starter culture adjunct on the properties of Cheddar cheese made with low or high pH at whey drainage

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ABSTRACT

Cheddar cheese was made using control culture (*Lactococcus lactis* subsp. *lactis*), or with control culture plus a galactose-metabolising (Gal^+) or galactose-non-metabolising (Gal^-) *Streptococcus thermophilus* adjunct; for each culture type, the pH at whey drainage was either low (pH 6.15) or high (pH 6.45). *Sc. thermophilus* affected the levels of residual lactose and galactose, and the volatile compound profile and sensory properties of the mature cheese (270 d) to an extent dependent on the drain pH and phenotype (Gal^+ or Gal^-). For all culture systems, reducing drain pH resulted in lower levels of moisture and lactic acid, a higher concentration of free amino acids, and higher firmness. The results indicate that *Sc. thermophilus* may be used to diversify the sensory properties of Cheddar cheese, for example from a fruity buttery odour and creamy flavour to a more acid taste, rancid odour, and a sweaty cheese flavour at high drain pH.

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1. Introduction

Cheddar cheese manufacture has changed much in recent decades with advances in mechanisation and the increase in plant throughput. Specific features of large-scale modern manufacture are the production of different variants (e.g., mild, mature, vintage), the extensive use of direct vat starter (DVS) cultures, and a higher pH at whey drainage, for example, ~6.4–6.5 compared with ~6.1–6.2 in traditional Cheddar cheese made using bulk starter culture. In such factories, even where milk protein standardisation using membrane filtration is not practiced, the various cheesemaking steps, such as starter addition, rennet addition, gel cutting and whey drainage, tend to be performed on the basis of time rather than on some objective parameter such as pH of milk or curd at different stages of manufacture, or gel firmness at cutting. Another feature, at least in Irish Cheddar cheese plants, is the routine use of starter culture adjuncts, including *Streptococcus thermophilus*, which is used primarily for its thermo- and phage- resistance properties, but also apparently to affect flavour. *Sc. thermophilus* is also reported to give faster acid production during Cheddar manufacture (Michel & Martley, 2001), which is likely to be associated with a more effective protein hydrolysis and peptide uptake (Cogan et al., 2007; Law & Haandrikman, 1997), and with the non-utilisation of the galactose moiety of lactose, by most *Sc. thermophilus* strains (Thomas & Crow, 1984; Tinson, Hillier, & Jago, 1982a).

Most strains of *Sc. thermophilus* used in the dairy industry are unable to metabolise galactose (Hutkins, Halambeck, & Morris, 1986; Hutkins, Morris, & McKay, 1985; Robitaille, Moineau, St-Gelais, Vadeboncoeur, & Britten, 2007; Thomas & Crow, 1984; Vaillancourt, Moineau, Frenette, Lessard, & Vadeboncoeur, 2002). De Vin, Rådström, Herman, and De Vuyst (2005) reported that only ~16% of 49 strains of *Sc. thermophilus* evaluated on M17 medium supplemented with galactose were galactose positive. Similarly,

preliminary studies in the authors' laboratory found that only 8% of 51 strains of *Sc. thermophilus* from the Moorepark culture collection metabolised galactose. Thomas and Crow (1984) investigated the galactose-metabolising ability of *Sc. thermophilus* from different sources and found that most were galactose negative (Gal^-) because of failure to induce galactokinase, resulting in the excretion of galactose when grown in lactose-containing broth. When grown under lactose limitation in J8 broth containing 20 mM galactose, partial galactose utilisation occurred and the proportion of galactose used depended on the generation time of cells during incubation.

Hence, the use of *Sc. thermophilus* (which primarily metabolises only the glucose moiety of lactose) as an adjunct culture usually results in the accumulation of galactose during cheese manufacture (Michel & Martley, 2001; Thomas, Turner, & Crow, 1980; Tinson et al., 1982a). Bley, Johnson, and Olson (1985) reported that the use of a 0.5% (w/w) non-galactose-fermenting *Sc. thermophilus* as an adjunct resulted in higher level of residual galactose in one month-old stirred curd Cheddar (compared with the control cheese) and intensified the degree of browning in processed cheese made therefrom. Similarly, Michel and Martley (2001) found that Cheddar cheese made using *Sc. thermophilus*, as an adjunct culture to *Lactococcus lactis* subsp. *cremoris* or *Lactococcus lactis* subsp. *lactis* strains, had a high residual galactose level of ~26.6 mmol kg^{-1} (0.48%, w/w) at 1 d. Moreover, the residual galactose content increased as the scald temperature was increased from 38 °C to 41 °C (data not reported). Tinson, Ratcliffe, Hillier, and Jago (1982b) reported that high levels of residual galactose (33 mmol kg^{-1}, 0.56%, w/w) in 8 wk-old Cheddar cheese made using *Sc. thermophilus* (0.5%, w/w) as an adjunct to *Lc. lactis* subsp. *cremoris* coincided with a higher production of CO_2, leading to the development of slits and fractures in the cheese at 8 and 14 wks. This was most probably caused by the growth of non-starter lactic acid bacteria (NSLAB) that are able to metabolise galactose.

The accumulation of galactose in cheese can lead to problems such as (i) providing a readily fermentable carbohydrate which could influence the development of NSLAB flora and possibly lead to defects, (ii) the presence of a reducing sugar in cheese that can cause excessive Maillard browning on heating, and (iii) early gas production in Cheddar cheese (Mullan, 2000; Ortakci, Broadbent, Oberg, & McMahon, 2015). Moreover, the presence of galactose in whey can affect the rate of growth of lactose crystals during whey processing and increase the propensity of the resultant whey powder to browning during storage (Dattatreya, Lee, & Rankin, 2010; Paterson & Smakman, 2011). While many of the foregoing studies (Bley et al., 1985; Hutkins et al., 1986; Michel & Martley, 2001) studied the effects of *Sc. thermophilus* as an adjunct on composition and sugar metabolism, we are unaware of any that investigated their effects on proteolysis, rheology or sensory properties, despite its apparent impact on flavour development. Moreover, there appear to be few, if any, studies on the comparative effect of galactose positive (Gal^+) and galactose negative (Gal^-) *Sc. thermophilus* as adjunct culture on the latter aspects of cheese quality.

The objective of the current study was to compare the effects of Gal^+ and Gal^- strains of *Sc. thermophilus* as an adjunct culture on the composition, sugar metabolism, pH, proteolysis, volatile compounds, texture, microbiology and sensory properties of Cheddar cheeses prepared made with a high drain pH (6.45), as in modern manufacture, or a low drain pH (6.15), as in more traditional manufacture.

2. Materials and methods

2.1. Preparation of cheese milk

Holstein-Friesian cows' milk (3000 kg) was obtained from a spring-calving herd (Moorepark, Fermoy, Ireland). Milk samples were standardised to a protein to fat ratio of 0.96:1, stored overnight at 8 °C, pasteurised at 72 °C for 15 s, cooled to 31 °C, and pumped to cheese vats (500 L).

2.2. Starter cultures for cheesemaking

Defined strain starter cultures were used in cheesemaking (*Lc. lactis* subsp. *lactis* strains 227 and 303; Chr. Hansen Ireland Ltd., Little Island, Ireland). Both cultures were grown overnight at 24 °C in reconstituted 10% (w/v), antibiotic-free skim milk powder solution (Golden Vale Food Products Ltd., Charleville, Ireland) that had been heat treated at 95 °C for 30 min. When the pH of the inoculated milk reached between pH 4.5 to 5.0, the cultures were cooled and stored at 4 °C until required for cheesemaking (1 d).

Adjunct starter cultures of *Sc. thermophilus* from the Moorepark culture collection were screened on the basis of sugar metabolism, acidification rate and salt sensitivity. One galactose metabolising (DPC 1796) and one galactose non-metabolising (DPC 5095) *Sc. thermophilus* strain were selected for cheesemaking. Both cultures were grown overnight at 37 °C in reconstituted 10% (w/v), antibiotic-free skim milk powder solution (Golden Vale Food Products Ltd.) as described above.

For convenience, the cultures used in cheesemaking were denoted as follows: control culture C, consisting of *Lc. lactis* subsp. *lactis* strain 227 and 303, each inoculated at a level of 0.075% (w/w); Gal^+ culture, consisting of the control culture and a galactose-metabolising *Sc. thermophilus* DPTC 1796 (inoculated at a level of 0.25%, w/w)*;* and Gal^- culture, consisting of the control culture C plus galactose non-metabolising *Sc. thermophilus* DPTC 5095 (inoculated at a level of 0.25%, w/w).

139

140 *2.3. Cheese manufacture and treatments*

141

142 Six different treatment cheeses were manufactured in each of three replicate trials
143 undertaken over a three-week period from October 20 to November 11, 2011. The cheeses
144 were denoted as: high-drain pH (6.45) made using culture C (HDpHC); high-drain pH with
145 Gal^+ culture (HDpHGal$^+$); high-drain pH with Gal^- culture (HDpHGal$^-$); low-drain pH (6.15)
146 with culture C (LDpHC); low-drain pH with Gal^+ culture (LDpHGal$^+$); low-drain pH with
147 Gal^- culture (LDpHGal$^-$).

148 The manufacture of cheese involved inoculation of cheesemilk with *Lc. lactis* subsp.
149 *lactis* strain 227 and 303, each at a level of 0.75% (w/w). Additionally, *Sc. thermophilus* 179
150 was added to milk for the HDpHGal$^+$ and LDpHGal$^+$ cheeses, and *Sc. thermophilus* 5095 to
151 milk for the LDpHGal$^-$ and HDpHGal$^-$ cheeses; *Sc. thermophilus* 179 and 5095 were each
152 inoculated at a level of 0.25% (w/w). The mean initial count of the *Lc. lactis* subsp. *lactis* was
153 ~ 1×10^7 cfu mL^{-1} in all milk lots, while that of the *Sc. thermophilus* was ~ 6.2×10^6 cfu mL$^-$
154 ^{1} in the LDpHGal$^+$, LDpHGal$^-$, HDpHGal$^+$ and HDpHGal$^-$ milk. Thirty minutes later, rennet
155 (Chymax Plus, Chr. Hansen Ireland Ltd., 200 IMCU mL^{-1}), diluted 1:10 in de-ionised water,
156 was added at a level of 36 IMCU kg^{-1} based on a protein level of 3.3 g 100 g^{-1} milk, and
157 mixed in for 1.5 min to ensure uniform distribution. Immediately, a sample of the rennet-
158 treated cheese milk was taken from the cheese vat, and placed in an insulated glass container.
159 Within 2 min, a 13 g subsample was placed in the cell of a controlled stress rheometer (CSL2
160 500 Carri-Med, TA Instruments, Inc., New Castle, DE, USA) located in an adjacent
161 laboratory, and subjected to a low oscillating strain of 0.025 at a frequency of 1 Hz at 31 °C.
162 The development of elastic shear modulus, G', a measure of gel stiffness, was measured as a
163 function of time; when G' reached 54 Pa, cutting of the gel in the cheese vat was initiated.

Following a 1.5 min cutting programme, the resultant curd particle-whey mixture was allowed to stand quiescently (heal) for 10 min, then stirred continuously, cooked at a rate of 0.2 °C min^{-1} from 31 to 38.5 °C, which is typical of the scald temperature used in commercial practice for Cheddar cheese made with, or without, *Sc. thermophilus* as an adjunct culture. Whey was separated from the curd when the pH of the curd reached 6.45 for the high-drain pH cheeses (HDpHC, $HDpHGal^+$, $HDpHGal^-$) and 6.15 for the low-drain pH cheeses (LDpHC, $LDpHGal^+$, $LDpHGal^-$). The curds were Cheddared, milled at pH 5.35, salted at a level of 2.7% (w/w), mellowed for 20 min, placed in rectangular moulds (23 kg), and pre-pressed at 0.13 kPa for 30 min. The moulded cheeses were then placed in a horizontal press and pressed overnight at 2.5 kPa. A total of two cheeses, each weighing ~20 kg, was obtained for each treatment on each of the three separate cheesemaking occasions (trials).

Cheeses were vacuum-packed and stored at 4 °C for 14 d and at 8 °C thereafter.

2.4. Sampling of cheese

Cheeses (from 20 kg blocks) were sampled at different times (1, 14, 30, 90, 180, 270 d) over the 270 day ripening period, as described by Hou, McSweeney, Beresford, and Guinee (2014b).

2.5. Composition analysis of cheese

Grated cheese samples were analysed at 14 d for moisture, protein, fat, NaCl, moisture, ash, Ca and P using standard IDF methods (Guinee, Harrington, Corcoran,

Mulholland, & Mullins, 2000). The pH was measured after each sampling date on cheese slurry prepared from 20 g of grated cheese and 12 g distilled water (Guinee et al., 2000).

2.6. Microbial counts in cheese

Starter lactococci were enumerated on LM17 agar after incubation at 20 °C for 5 d and the *Sc. thermophilus* adjunct cultures were also enumerated on LM17 agar after incubation at 43 °C for 3 d (ISO/IDF, 2010).

Cheeses were analysed for counts of non-starter lactic acid bacteria on LBS agar, as described previously (Hou, Hannon, McSweeney, Beresford, & Guinee, 2012). Coliform were enumerated by pour-plating on Violet Red Bile Agar (VRBA) incubated at 30 °C for 24 h.

2.7. Lactose and lactate in cheese

Lactose, glucose and galactose were extracted and measured using high performance liquid chromatography (HPLC) as described previously by Hou et al. (2014b); HPLC was performed using a 300 × 7.8 mm Aminex HPX-87C cation exchange carbohydrate column (Bio-Rad Laboratories, Richmond, CA, USA) and detection with a Waters 2414 refractive index detector (Waters, Bray, Ireland). The concentrations of sugars in the cheeses were calculated by comparing the peak area of samples with standard curves. Sugar concentrations were calculated as g 100 g^{-1} cheese.

Similarly, D(–)- and L(+)- lactate were extracted and separated as described previously by Hou et al. (2014b); HPLC was performed using a Phenomenex chirex 3126 cation exchange silica column (Phenomenex, Hurdsfield Ind. Est., Macclesfield, UK) and detection

was with a Waters 2487 dual wavelength absorbance detector (Waters) as described previously by Hou et al. (2014b). The concentration of total lactate was calculated as the sum of D(–)- and L(+)- lactate; each analysis was carried out in duplicate.

2.8. Proteolysis

The level of pH 4.6-soluble nitrogen (pH4.6-SN) was measured as described by Hou, Hannon, McSweeney, Beresford, & Guinee (2014a). The concentration of individual free amino acids (FAAs) in the pH4.6-SN extract were determined using cation-exchange chromatograhy on a Beckman 6300 High Performance Analyser (Beckman Instruments Ltd., High Wycombe, UK), as described by Fenelon, Guinee, Delahunty, Murray, and Crowe (2000).

2.9. Rheology

Six cheeses cubes (25 mm^3 cubes) were cut from each treatment cheese using a Cheese Blocker (Bos Kaasgreedschap, Bodengraven, Netherlands), wrapped in tin foil, and stored at 8 °C overnight prior to analysis. Each cube was compressed by 70% on a texture analyser (model TA-HDI, Stable Micro Systems, Godalming, UK) with a 5 mm compression plate and a 100 kg load cell, as described previously (Hou et al., 2014a). The following rheological parameters were calculated from the resultant force/displacement curves: fracture stress (kPa), the stress at fracture, as indicated by the inflection point of the curve; fracture strain, the fractional displacement at fracture; and firmness, the force required to compress the cheese to 30% of its original height.

2.10. Volatile compounds

The 270 day old cheeses were analysed in triplicate for volatile compounds. For each cheese, a 5 g sample was analysed by solid phase micro-extraction (SPME) coupled to a gas chromatograph (GC)-mass spectrometer (MS). Volatile compounds were separated under the conditions defined by Hannon, Kilcawley, Wilkinson, Delahunty, and Beresford (2007).

2.11. Descriptive sensory analysis

The sensory properties of the 270 day old cheeses from each of the three replicate trials were evaluated using descriptive sensory analysis, as described previously (Hou et al., 2014a). The results are presented as a principal component (PC) plot. Attributes scored for odour included pungent, sweaty/cheesy, rancid, fruity, buttery and caramel; attributes for flavour were pungent, farmyard, creamy, rancid, fruity, buttery, caramel and sweaty/cheesy, while those for taste comprised throat burn, sweet, acid, salt, bitter and astringent.

2.12. Statistical analysis

Three replicate cheesemaking trials were undertaken, each with 6 treatment cheeses, namely LDpHC, LDpHGal^{+}, LDpHGal^{-}, HDpHC, HDpHGal^{+} and HDpHGal^{-}. Analysis of variance (ANOVA) was used to determine if the treatment cheeses differed with respect to response variables, such as compositional factors, at specific time points (e.g., 14 d). ANOVA was undertaken using SAS® version 9.1.2 (SAS Institute, 2004), where the effects of treatment (different drain pH or starter system) and replicates were estimated for all

response variables. Tukey's multiple-comparison test was used for paired comparison of treatment means and the level of significance was determined at $P < 0.05$.

The data for changes in individual response variables (such as sugars and micro counts) in the high and low drain pH cheeses over the duration of ripening were analysed using a split-plot design to determine the effects of treatment, ripening time, and their interaction. Analysis of variance for the split-plot design was carried out using a general linear model (GLM) procedure of SAS (SAS Institute, 2004), and significance at $P < 0.05$ determined using Fisher's least significant difference test.

The data for volatile compounds and descriptive sensory analysis, measured at 270 d only, were analysed using PCA by Unscrambler V 6.1 (CAMO AS, N-7041 Trondheim, Norway). The results are presented as a principal component (PC) plot.

3. Results

3.1. Cheese manufacturing time

The use of *Sc. thermophilus* adjunct cultures and alteration of pH at whey drainage had varying effects on the times for the different stages of manufacture, namely the curd residence time in cheese vat (time from gel cutting to whey drainage), Cheddaring time (time from whey drainage to curd milling), and total make time (time from starter culture addition to curd milling).

The curd residence time in the cheese vat for the HDpH cheeses (53–54 min) was significantly lower than that for the LDpH cheeses (105–135 min). However, the Cheddaring time for the HDpH cheeses (~125–150 min) was generally longer than that of the LDpH

286 cheeses (71–87 min) (Table 1). Hence, the overall make time for corresponding LDpH and
287 HDpH cheeses did not significantly differ.

288 Starter culture had a significant effect on the total make time of the HDpH chesses,
289 with that for the HDpHGal$^+$ cheese (260–288 min) being significantly shorter (by ~30 min)
290 than that of the corresponding control HDpHC or HDpHGal$^-$ cheeses.

291

292 *3.2. Composition at 14 d*

293

294 The gross composition of the cheese was affected by pH but not by starter culture
295 type (Table 1). The moisture content of the LDpH cheeses was below the maximum level
296 (39%, w/w) specified for Cheddar cheese (HMSO, 1996), while that of the HDpH cheeses
297 was higher. Despite the difference in moisture content between the LDpH and HDpH cheeses
298 (1.6–2.3%, depending on starter culture system used), the higher moisture content of the
299 HDpH cheeses was significant only in the case of HDpHGal$^-$ cheese. A similar trend was
300 noted for content of moisture-in-fat substances (MNFS).

301

302 *3.3. Changes in sugars during ripening*

303

304 *3.3.1. Lactose and galactose*

305 The effects of drain pH and starter culture system on the changes in lactose and
306 galactose over the course of ripening are shown in Fig. 1a–d and Table 2. The mean level of
307 residual lactose in the LDpH cheeses over the 270 d ripening period was significantly
308 affected by starter culture system, ripening time and their interaction (Fig. 1a). The mean
309 lactose content in the HDpH cheeses was, similarly, influenced by ripening time, but not by
310 starter culture (Fig. 1b).

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Lactose content decreased during maturation (Fig. 1a, b), and was, essentially, fully metabolised in all cheeses by 90 d, apart from the LDpHC cheese that had a significantly higher content than that of the corresponding LDpHGal$^+$ and LDpHGal$^-$ cheeses at this time. The mean lactose level over the 270 d ripening period in the LDpHC cheese was significantly higher than that in the corresponding LDpHGal$^+$ and LDpHGal$^-$ cheeses. The results indicate that residual lactose content in Cheddar cheese (< 180 days old) can be reduced by the use of *Sc. thermophilus* (Gal$^+$ or Gal$^-$) as a culture adjunct when the pH at whey drainage is low, or by increasing the pH at whey drainage when the cheese is made using the control starter culture.

The galactose content at 1 d varied from ~0.2–0.025%, remained relatively constant between d 1 and 14, and thereafter decreased to ≤ 0.05% in all cheeses at 180 d (Fig. 1c,d). Starter culture had a significant effect on the mean galactose level over the 270 d ripening period in the HDpH cheeses, with the mean concentration in the HDpHGal$^+$ cheese being higher than that of the HDPHC or HDpHGal$^-$ cheeses (Table 2). While a similar overall pattern was observed in the LDpH cheeses, the effect of starter culture was not significant, probably because of the relatively large inter-trial variation in galactose content. Overall, the results indicate that the use of the Gal$^+$ *Sc. thermophilus* led to higher residual galactose content in young Cheddar cheese (≤ 30 d), especially where the pH at whey drainage was high, as frequently is the case in large modern cheese manufacturing facilities using DVS cultures.

The mean levels of reducing sugars (lactose plus galactose) over ripening were unaffected by the addition of *Sc. thermophilus* (Table 2), indicating that the associated increase in galactose was offset by the concomitant decrease in lactose content; similarly, the drain pH did not significantly affect the level of reducing sugars for any of the cultures used.

3.3.2. Total lactate

The metabolism of lactose and galactose resulted in a significant increase in lactate content during the first 30 d, from ~1 to 1.3% in the LDpH cheeses and 1.2–1.45% in the HDpH cheeses; thereafter lactate levels remained relatively constant. The mean concentration over the 270 d maturation period was significantly higher in the HDpH cheeses than that in the corresponding LDpH cheeses for each culture type ($P < 0.05$). This trend is consistent with the higher level of moisture (which is the solvent for lactose) in the HDpH cheeses.

The mean level of total lactate in the LDpH or HDpH cheeses over the 270 d ripening period was unaffected by the starter culture type (Table 2; Fig. 1e,f).

3.4. pH changes during ripening

The mean pH of the LDpH and HDpH cheeses over the 270 d ripening period was not significantly affected by the starter culture, time or their interaction (Table 2). In contrast, the pH at whey drainage had a significant effect, with the pH of the LDpH cheeses being slightly (~0.07 pH units), but significantly ($P < 0.05$), higher than that of the corresponding HDpH cheeses at all times. The slightly higher pH of the LDpH cheeses concurs with their lower contents of moisture and lactic acid.

3.5. Microbial counts of starter and non-starter lactic acid bacteria (NSLAB) in cheese

*3.5.1. Starter bacteria (*Lactococcus*)*

The mean count of starter lactococci decreased significantly ($P < 0.05$) in all cheeses during ripening, from ~1×10^{10} cfu g^{-1} at 1 d to ~3.2×10^{7} cfu g^{-1} at 270 d (Table 3; Fig.

2a,b). The inclusion of Gal^+ or Gal^- *Sc. thermophilus* strains in the starter culture did not influence the mean of count over the ripening period.

The lactococci count in the LDpHC cheese at 270 d was slightly, but significantly, higher than that of the corresponding HDpHC cheese ($P < 0.05$). As the counts in both cheeses (LDpHC, HDpHC) were similar at 1 d, the higher count in the LDpHC cheese at 270 d suggests a lower degree of starter cell autolysis, which could be associated with its lower mean level of lactic acid over the 270 d ripening period (Nájera-Domínguez & Gutiérrez-Méndez, 2013).

*3.5.2. Adjunct bacteria (*Sc. thermophilus*)*

The mean count of *Sc. thermophilus* over the 270 d ripening period was significantly affected by starter system and ripening time in both the LDpH and HDpH cheeses (Table 3; Fig. 2c,d).

Sc. thermophilus grew (from ~1×10^6 cfu g^{-1} in the milk following inoculation) during cheese manufacture and pressing to reach counts of ~1×10^9 cfu g^{-1} in the Gal^+ and Gal^- cheeses at 1 d (Fig. 2c,d). The population in the Gal^+ and Gal^- cheese decreased significantly during ripening to ~1×10^5 cfu g^{-1} at 270 d. While the mean count of *Sc. thermophilus* over the 270 ripening period were similar in the LDpHGal$^+$ and LDpHGal$^-$ cheeses, that in the HDpHGal$^-$ cheese was slightly, but significantly, lower than that in the HDpHGal$^+$ cheese.

The mean count of *Sc. thermophilus* in the control cheeses (LDpHC, HDpHC) was significantly lower than that of the corresponding Gal^+ and Gal^- cheeses, which had similar counts at 1 d (1×10^9 cfu g^{-1}). Nevertheless, *Sc. thermophilus* was present in the control HDpHC and LDpHC cheeses at ~10^3 cfu g^{-1} cheese on 1 d, grew to ~1×10^4 cfu g^{-1} between 1 and 5 d, and remained essentially constant at this level through the remainder of ripening.

The low *Sc. thermophilus* count in the control cheeses probably reflects cross-contamination during cheese manufacture, even though care was taken to avoid this.

3.5.3. Non-starter lactic acid bacteria (NSLAB)

NSLAB were present in all cheeses at $\leq 3.2 \times 10^2$ cfu g^{-1} at 1 d and grew during ripening, reaching counts of ~ 3.2×10^6 cfu g^{-1} – 10^7 cfu g^{-1} at 180 d (Table 3; Fig. 2e,f). The mean population in the LDpH cheeses over the 270 d ripening period was significantly affected by starter culture system, with the mean count in the LDpHGal$^+$ cheese being significantly higher than that in the LDpHGal$^-$ cheese, and numerically, though not significantly, higher than that in the LDpHC. Post-hoc analysis showed that the counts in the LDpHGal$^+$ were significantly higher than that in the LDpHGal$^-$ cheese at 1, 14 and 30 d, but similar at all other times.

3.6. Proteolysis

The mean level of pH4.6-SN, which is indicative of hydrolysis of the insoluble intact calcium phosphate *para*-casein into water soluble peptides by residual chymosin, increased significantly in all cheeses during ripening from ~5% of total nitrogen at 1 d to ~26–29% at 270 d (data not shown). The mean level over the 270 d ripening was significantly affected by starter culture system in the HDpH cheeses (Table 4), with the mean level in the HDpHC cheese being significantly lower than that in the HDpHGal$^+$ or HDpHGal$^-$ cheeses for which it was similar; no such difference was found between the LDpH cheeses. However, these differences were quite small (0.6–1.3 %) and are unlikely to have had a notable effect on the physical or sensory properties of the cheese. The pH at whey drainage did not affect the content of pH4.6-SN.

The concentration of FAAs increased significantly during ripening (Fig. 3), with glutamic acid, leucine, phenylalanine and valine being the major FAAs present in all cheeses (data not shown). The mean concentration of FAAs in the LDpH or HDpH cheeses over the 270 d ripening period was not affected by the starter culture (Table 4). In contrast, pH at whey drainage had a significant effect, with the LDpH cheeses having significantly higher mean levels of FAAs than the corresponding HDpH cheeses over the 270 d ripening period. The 270 day old LDpH cheeses had significantly higher levels of total FAAs, glutamic acid, valine, leucine, phenylalanine, proline and lysine than the corresponding HDpH cheeses. The differences in FAA concentration between the cheeses may reflect inter-cheese differences in peptidase activities as affected by pH, NSLAB species (Gobbetti et al., 1999), and degrees of autolysis and permeability of starter and non-starter bacteria (Doolan &Wilkinson, 2009).

3.7. Rheological properties

The mean values of firmness, fracture stress and fracture strain of all cheeses decreased significantly during ripening (Table 5). The decreases are consistent with the increase in primary proteolysis of calcium phosphate *para*-casein network (data not shown), which is the main structural component of the cheese matrix controlling the level of stress in response to applied deformation, e.g., during compression (Guinee, 2016). Starter culture had no effect on rheological properties of either the LDpH or HDpH cheeses (Table 5), a trend compatible with the very small differences in pH4.6-SN between the LDpH cheeses or HDpH cheeses. In contrast, the pH at drainage had a significant effect on firmness, with that of the LDpH cheeses, which had lower moisture content (Table 1), being significantly higher than that of the HDpH cheeses (data not shown).

3.8. Volatile compounds at 270 d

Thirty six different volatile compounds were identified in the 270 day old cheese. These comprised 10 alcohols, 8 ketones, 3 esters, 3 aldehydes, 6 acids, 2 sulphur compounds, 2 alkanes, 1 alkene (octene) and 1 terpene (limonene) were identified in all of the cheeses.

PCA was undertaken to establish if the different cheeses could be separated by the types and concentrations of volatile compounds; a biplot of the volatile compounds is presented in Fig. 4. Principal components PC1 and PC2 accounted for 47% and 25% of explained variance between the cheeses, respectively. Three cheeses, i.e., the control (LDpHC and HDpHC) and HDpHGal$^+$ cheeses, scored positively on PC1, and three (LDpHGal$^+$, LDpHGal$^-$ and HDpHGal$^-$) scored negatively. In contrast, all cheeses, apart from LDpHC and LDpHGal$^+$, scored positively on PC2. Two groupings of cheeses were identifiable based on their proximity on both PC1 and PC2, namely the cheeses made using *Sc. thermophilus* at high drain pH (HDpHGal$^+$ and HDpHGal$^-$) or at low drain pH (LDpHGal$^+$ and LDpHGal$^-$). The HDpHGal$^+$ and HDpHGal$^-$ cheeses were associated principally with ketones (butanone, acetoin), acetic acid, acetoin, and methyl sulphone. The LDpHGal$^+$ and HDpHGal$^-$ cheeses were aligned with a large range of volatile compounds including fatty acids (butanoic, pentanoic, heptanoic and octanoic acid), alcohols (butanol, 3 methyl-1-butanol, 2 heptanol, 1 hexanol), ethyl esters of fatty acids (butanoic, octanoic, hexanoic), ketones (2 nonanone and 2 heptanone), aldehydes (benzeneacetaldehyde, nonanal) and limonene.

The control cheeses (LDpHC and HDpHC) differed from the adjunct-containing cheeses and from each other with respect to volatile compounds. The LDpHC cheese was characterised by the presence of an array of volatiles including alcohols (ethanol, 1- and 2-pentanol, 3-methyl-2-buten-1-ol and 3-methyl-3-buten-1-ol), ketones (acetone, 2-butanone),

460 hydrocarbons (octane, pentane, heptane), acids (butanoic, hexanoic) and carbon disulphide;
461 few volatile compounds were identified in the HDpHC cheese.
462
463 *3.9. Descriptive sensory analysis at 270 d*
464
465 The PCA biplot for the different odour and flavour attributes of the 270 d-old cheeses
466 is shown in Fig. 5. The first two PCs discriminated significantly between the cheeses and
467 accounted for a cumulative explained variance of 77%. Two distinct groupings were evident
468 based on proximity on PC1 and PC2, namely the HDpHGal$^+$ and HDpHGal$^-$ cheeses that had
469 a sweaty, rancid flavour, a rancid and sweaty odour, and acid taste, and the LDpHC and
470 LDpHGal$^+$ cheeses that had buttery flavour, caramel odour and sweet taste. In contrast, the
471 HDpHC and LDpHGal$^-$ cheeses were separated from the above groupings and from each
472 other with respect to sensory characteristics. The former had a fruity, creamy flavour, and a
473 fruity buttery odour, while the LDpHGal$^-$ cheese had a pungent and farmyard flavour,
474 pungent odour, pungent throat and astringent sensations, and bitter taste (Fig. 5).
475
476 **4. Discussion**
477
478 The current study investigated the effects of adding either a galactose metabolising
479 (Gal$^+$) or galactose non-metabolising (Gal$^-$) strain of *Sc. thermophilus* on the properties of
480 Cheddar cheese made using either a low (pH 6.15) or high (pH 6.45) pH at whey drainage.
481 The study is of significance because of the growing use of *Sc. thermophilus* as an adjunct
482 culture and the increase in pH at whey drainage, accompanying the transition from bulk
483 mesophilic starter culture (*Lc. lactis* subsp. *cremoris* or *lactis*) to direct-vat starter mesophilic
484 culture in large modern Cheddar cheese factories. *Sc. thermophilus* was inoculated at a level

485 of 1×10^6 cfu mL^{-1} milk, resulting in counts of 1×10^9 cfu g^{-1} cheese at 1 d, decreasing
486 gradually to ~1×10^5 cfu g^{-1} at 270 d. The use of the *Sc. thermophilus* adjuncts had
487 significant effects on levels of residual sugars (lactose plus galactose), volatile compounds
488 and sensory properties of the 270 day old cheese to a degree dependent on its galactose-
489 metabolising ability and pH, and altering the pH at whey drainage directly affected cheese
490 composition (contents of moisture, MNFS), lactic acid level, pH and firmness irrespective of
491 the culture systems used.

492 While lowering the pH at whey drainage resulted in lower contents of moisture (1.6–
493 2.3%) and MNFS (~1.4–2.0%) in all cheeses, the effect was significant only in cheeses made
494 with the Gal^- *Sc. thermophilus* strain. The lower moisture content of the LDpH cheeses was
495 consistent with the longer residence time of the curd particle-whey mixture in the cheese vat
496 prior to whey drainage (Everard et al., 2011). Whey expulsion in the cheese vat is more
497 intense than that which occurs after whey drainage (e.g., during Cheddaring, salting and
498 moulding) because the higher surface area of the curd particles in the cheese vat (compared
499 with slabs of fused curd particles during Cheddaring in finishing vats or on Cheddaring belts)
500 and the higher temperature (~3 °C) compared with that during curd Cheddaring (Dejmek &
501 Walstra, 2004). The numerically higher moisture content of the HDpH cheeses, though non-
502 significant in the case of the HDpHC and $HDpHGal^+$ cheeses, could have practical
503 implications, in terms of compliance to compositional specification, quality and yield.
504 However, normalisation of moisture content in cheese produced at different pH could easily
505 be achieved through process intervention, whereby factors such as firmness of gel at cutting,
506 curd particle size and rate of cooking are altered (Guinee & O'Callaghan, 2010).

507 Owing to their lower moisture content, the LDpH cheeses had a lower mean level of
508 lactic acid, higher pH and higher firmness than the corresponding HDpH cheeses. This trend
509 concurs with the findings of other studies (Chevanan, Muthukumarappan, Upreti, & Metzger,

2006; McCarthy, Wilkinson, Kelly, & Guinee, 2015, 2016; Rynne et al., 2004; Upreti, Bühlmann, & Metzger, 2006). In contrast to the current results, Lee, Johnson, and Lucey (2005) found that a reduction in the pH at whey drainage from 6.17 to 5.82 led to a reduction in pH of Cheddar cheese (from ~5.0 to 4.8), while Tunick, Guinee, van Hekken, Beresford, and Malin (2007) reported no change in in the pH of half-fat Cheddar cheese when reducing the drain pH from 6.3 to 5.85. The inter-study discrepancy on the effect of drain pH on cheese pH probably relates to differences in manufacturing conditions, such as the pH at set and the range of pH investigated, that influence the concentration of calcium phosphate which buffers the pH of cheese upwards (Lucey & Fox, 1993). Reducing drain pH by lowering the set pH (e.g., by pre-acidification of the cheese milk) is conducive to a reduction in the ratio of calcium phosphate to casein, a lower buffering capacity and a lower cheese pH (Lee et al., 2005). Conversely, lowering drain pH by extending the curd residence time of the curd/whey mixture in the cheese vat, as in the current study, is conducive to lower moisture content, a slightly higher pH, and has little, or no, effect on the calcium-to-casein ratio (Table 1).

Lactose was present in all cheeses at 1 d, with levels in the control LDpHC and HDpHC cheeses (~0.25–0.3%) being higher than that (< 0.15%) in the corresponding cheeses ($LDpHGal^+$, $LDpHGal^-$, $HDpHGal^+$, $HDpHGal^-$) made using the Gal^+ or Gal^- strains of *Sc. thermophilus*. Moreover, the use of the *Sc. thermophilus* adjuncts led to faster depletion of lactose, especially in the LDpH cheeses, in which lactose was fully metabolised at 90 d in the $LDpHGal^+$ and $LDpHGal^-$ cheeses compared with 180 d in the LDpHC cheese. High residual lactose content in Cheddar cheeses has been previously reported by others (Hou et al., 2012; Shakeel-Ur-Rehman, Waldron, & Fox, 2004; Upreti & Metzger, 2006). The current study indicated that increasing the pH at whey drainage in the control Cheddar cheese (made with mesophilic culture) and the use of Gal^+ culture are effective means of reducing the residual

lactose content in Cheddar cheese. In contrast to the trend noted for lactose, cheeses made using *Sc. thermophilus* ($LDpHGal^+$, $LDpHGal^-$, $HDpHGal^+$ and $HDpHGal^-$) had relatively high levels of residual galactose (~0.075–0.2% or 4.2–11.34 mM at 1 d) compared with control LDpHC or HDpHC cheeses (~0.025% or 1.3 mM at 1 d), especially at times ≤ 30 d. While the combined concentration of reducing sugars (lactose and galactose) were unaffected by *Sc. thermophilus*, the *Sc. thermophilus*-containing cheese may have a greater propensity to browning on cooking because of the higher concentration of galactose, which has lower molecular mass than lactose and, hence, higher number of reducing groups per unit weight of reducing sugars.

The addition of *Sc. thermophilus* had a notable effect on both the type volatile compounds and sensory properties of the 270 day old cheeses, with the effect dependent on culture phenotype and pH. At high drain pH, the addition of *Sc. thermophilus* increased the range of volatile compounds associated with the cheeses ($HDpHGal^+$, $HDpHGal^-$), including butanoic acid, butanone, acetoin, dimethyl sulphone, and acetic acid; by comparison, the control HDpHC cheese had few relatively few volatile compounds, apart from acetic acid and acetoin. The change in profile of volatile compounds coincided with a marked transition in sensory properties, from a buttery/fruity odour and creamy/fruity flavour in the HDpHC cheese to a sweaty, cheesy and rancid odour and sweaty, cheesy, rancid flavour. Such a trend is consistent with the strong sweaty, cheesy aroma of butanoic acid, and the sour aroma of acetic acid (Kilcawley, 2016; Singh, Drake, & Cadwallader, 2003). Similarly, the addition of *Sc. thermophilus* to the low drain pH cheeses resulted in a major shift in the profile of volatile compounds, from a predominance of short-chain alcohols (ethanol, methyl-butene-ols, pentanol), hydrocarbons and ketones in the control LDpHC cheese to short chain fatty acids (butanoic, pentanoic, heptanoic and octanoic acid), ethyl esters of fatty acids, alcohols, ketones and aldehydes (benzene acetaldehyde, nonanal), in the $LDpHGal^+$ and $LDpHGal^-$

cheeses; nevertheless, the latter cheeses varied with the short chain fatty acids being more closely aligned with the LDpHGal$^-$ cheese and aldehydes and ketones with the HDpHGal$^+$ cheeses. Unlike the trend observed in the HDpH cheeses, the effect of *Sc. thermophilus* on the sensory properties on the LDpH cheeses was dependent on its galactose metabolising ability. Based on their closeness on both PC1 and PC2, the LDpHGal$^+$ and LDpHC cheese were distinguished as a group and characterised as having a caramel odour, sweet taste and buttery flavour, with the intensity of these attributes being higher in the former than the latter. In contrast, the LDpHGal$^-$ had a pungent odour, with a bitter, throat burn, astringent farmyard flavour. Hence, despite the LDpHC and LDpHGal$^+$ cheeses belonging to the same PCA grouping for volatile compounds, they belonged to a different grouping for the corresponding descriptive sensory analyses. This confirms that sensory perception of cheese at any time is complex, being determined by volatile compounds, taste compounds, texture and their interaction (Szczesniak, 2002).

5. Conclusion

The use of *Sc. thermophilus* as an adjunct culture (to *Lc. lactis* subsp. *lactis*) affected the levels of residual lactose and galactose, the profile of volatile compounds and sensory properties of Cheddar cheese to an extent dependent on the drain pH and *Sc. thermophilus* phenotype (Gal$^+$ or Gal$^-$). At high drainage pH (6.45), the use of both Gal$^-$ or Gal$^+$ strains of *Sc. thermophilus* gave Cheddar cheese that had a sweaty, rancid flavour, a rancid and sweaty odour, and acid taste at 270 d, compared with control cheese (without adjunct) that had a fruity, creamy flavour, and a fruity butter odour. Conversely, at low drain pH (6.15), the control cheese and cheese made using Gal$^+$ strain of *Sc. thermophilus* were closer in sensory properties (buttery flavour, caramel odour and sweet taste) than the cheese made using the

Gal$^-$ strain of *Sc. thermophilus*, which had a pungent and farmyard flavour, pungent odour, pungent throat and astringent sensations, and bitter taste. For both the control culture and adjunct-containing cultures, reducing the pH at whey drainage from 6.45 to 6.15 resulted in cheese that had lower levels of moisture and FAA, and was firmer. The results suggest that *Sc. thermophilus* as a starter culture adjunct may be used as a means of creating Cheddar cheese variants with distinctive flavour profiles; but when using Gal$^-$ variant *Sc. thermophilus*, the pH at whey drainage should be increased to avoid the accumulation of high levels of residual galactose during ripening.

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Figure legends

Fig. 1. Changes in the level of residual lactose (a and b), galactose (c and d) and total lactate (e and f) during ripening in low drain pH (broken line, open symbol) and high drain pH (solid line, closed symbol) Cheddar cheeses made with control starter culture (LDpHC, △; HDpHC, ▲), control starter culture with galactose metabolising *Sc. thermophilus* culture adjunct ($LDpHGal^+$,○; $HDpHGal^+$, ●) or control starter culture with galactose non-metabolising *Sc. thermophilus* as culture adjunct ($LDpHGal^-$,□; $HDpHGal^-$, ■). Values are the means of three replicate trials; error bars represent standard deviations of the mean.

Fig. 2. Changes in the counts of starter *Lactococcus* (a and b), *Sc. thermophilus* (c and d) and non-starter lactic acid bacteria (e and f) during ripening in low drain pH (broken line, open symbol) and high drain pH (solid line, closed symbol) Cheddar cheeses made with control starter culture (LDpHC,△; HDpHC, ▲), control starter culture with galactose metabolising *Sc. thermophilus* culture adjunct ($LDpHGal^+$,○; $HDpHGal^+$, ●) or control starter culture with galactose non-metabolising *Sc. thermophilus* as culture adjunct ($LDpHGal^-$,□; $HDpHGal^-$, ■). Values are the means of three replicate trials; error bars represent standard deviations of the mean.

Fig. 3. Changes in the concentrations of total free amino acids (FAA) during ripening in low drain pH (a, broken line, open symbol) and high drain pH (b, solid line, closed symbol) Cheddar cheeses made with control starter culture (LDpHC, △; HDpHC, ▲), control starter culture with galactose metabolising *Sc. thermophilus* culture adjunct ($LDpHGal^+$, ○; $HDpHGal^+$, ●) or control starter culture with galactose non-metabolising *Sc. thermophilus* as

25 culture adjunct (LDpHGal$^-$, □; HDpHGal$^-$, ■). Values are the means of three replicate trials;
26 error bars represent standard deviations of the mean.
27
28 **Fig. 4.** PCA showing the first two principal components of volatile compounds in 270 day
29 old low drain pH and high drain pH Cheddar cheeses made with control starter culture
30 (LDpHC; HDpHC), control starter culture with galactose metabolising *Sc. thermophilus*
31 culture adjunct (LDpHGal$^+$; HDpHGal$^+$) or control starter culture with galactose non-
32 metabolising *Sc. thermophilus* as culture adjunct (LDpHGal$^-$; HDpHGal$^-$). Values are the
33 means of three replicate trials.
34
35 **Fig. 5.** PCA showing the first two principal components of descriptive sensory odour and
36 flavour attribute in 270 day-old low drain pH and high drain pH Cheddar cheeses made with
37 control starter culture (LDpHC; HDpHC), control starter culture with galactose metabolising
38 *Sc. thermophilus* culture adjunct (LDpHGal$^+$; HDpHGal$^+$) or control starter culture with
39 galactose non-metabolising *Sc. thermophilus* as culture adjunct (LDpHGal$^-$; HDpHGal$^-$).
40 Values are the means of three replicate trials.
41
42

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Table 1

Effect of different starter culture and pH at whey drainage on the composition of 14 day old Cheddar cheeses and the times required for different stages of manufacture. [a]

Parameter	Low drain pH cheese			High drain pH cheese		
	LDpHC	LDpHGal$^+$	LDpHGal$^-$	HDpHC	HDpHGal$^+$	HDpHGal$^-$
Cheese composition						
Moisture (%, w/w)	38.5^{a,A}	38.7^{a,A}	38.7^{a,B}	40.1^{a,A}	40.9^{a,A}	41.0^{a,A}
Fat (%, w/w)	30.7^{a,A}	30.5^{a,A}	30.7^{a,A}	29.5^{a,A}	29.1^{a,A}	29.1^{a,B}
Protein (%, w/w)	25.4^{a,A}	25.1^{a,A}	25.3^{a,A}	24.8^{a,A}	24.4^{a,A}	24.3^{a,B}
Salt (%, w/w)	1.74^{a,A}	1.93^{a,A}	1.84^{a,A}	1.73^{a,A}	1.66^{a,A}	1.64^{a,A}
Ca (mg 100 g^{-1})	755^{a,A}	757^{a,A}	742^{a,A}	771^{a,A}	769^{a,A}	746^{a,A}
Calcium to protein (mg g^{-1})	29.7^{a,A}	30.2^{a,A}	29.3^{a,A}	31.1^{a,A}	31.5^{a,A}	30.7^{a,A}
P (mg 100 g^{-1})	486^{a,A}	480^{a,A}	476^{a,A}	487^{a,A}	471^{a,A}	472^{a,A}
S/M (%, w/w)	4.54^{a,A}	5.01^{a,A}	4.74^{a,A}	4.30^{a,A}	4.08^{a,A}	3.99^{a,A}
MNFS (%, w/w)	55.5^{a,A}	55.6^{a,A}	55.8^{a,B}	56.9^{a,A}	57.6^{a,A}	57.8^{a,A}
FDM (%, w/w)	49.9^{a,A}	49.7^{a,A}	50.0^{a,A}	49.2^{a,A}	49.2^{a,A}	49.3^{a,A}
pH	5.25^{a,A}	5.34^{a,A}	5.29^{a,A}	5.25^{a,A}	5.24^{a,A}	5.19^{a,B}
Time for different stages of cheese manufacture (min)						
Curd residence time	133^{a,A}	104^{b,A}	122^{ab,A}	54^{a,B}	54^{a,B}	53^{a,B}
Cheddaring time	71^{a,B}	75^{a,A}	87^{a,B}	150^{a,A}	124^{b,A}	151^{a,A}
Total make time	281^{a,A}	252^{a,A}	279^{a,A}	280^{a,A}	253^{b,A}	284^{a,A}

[a] Cheeses were low drain pH (LDpH) or high drain pH (HDpH) cheeses made with control culture (LDpHC, HDpHC), galactose-metabolising *Sc. thermophilus* culture (LDpHGal$^+$, HDpHGal$^+$), or galactose-metabolising *Sc. thermophilus* culture (LDpHGal$^-$, HDpHGal$^-$). Values within a row relating to LDpH cheeses (LDpHC, LDpHGal$^+$, LDpHGal$^-$) or HDpH cheeses (HDpHC, HDpHGal$^+$, HDpHGal$^-$) and not sharing a common lower-case superscript differ significantly ($P < 0.05$) for effect of starter culture; values within a row relating to cheeses made with control culture (LDpHC, HDpHC), galactose-metabolising *Sc. thermophilus* culture(LDpHGal$^+$, HDpHGal$^+$) or galactose-non-metabolising *Sc. thermophilus* culture (LDpHGal$^-$, HDpHGal$^-$), and not sharing a common upper-case superscript letter differ significantly ($P < 0.05$) for effect of drain pH. Abbreviations are: S/M, salt in moisture; MNFS, moisture in non-fat substances; FDM, fat in dry matter.

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Table 2

Statistical significances (*P* values) for effects of starter culture and ripening time on concentrations of lactose, galactose, total lactate and pH in Cheddar cheeses made using low- or high-drain pH. [a]

Factor	Lactose		Galactose		Lactose + galactose		Total lactate		pH	
	df	*P*	df	*P*	df	*P*	df	*P*	df	*P*
Low drain pH cheese										
Main plot										
Starter system	2	0.005	2	0.158	2	0.869	2	0.227	2	0.583
Sub-plot										
Ripening time	5	<0.001	5	<0.001	5	<0.001	5	<0.001	5	0.250
Interaction (starter system × ripening time)	10	0.0002	10	0.110	10	0.210	10	0.696	10	0.475
High drain pH cheese										
Main plot										
Starter system	2	0.073	2	0.036	2	0.394	2	0.371	2	0.207
Sub-plot										
Ripening time	5	<0.001	5	<0.001	5	<0.001	5	<0.001	5	0.554
Interaction (starter system × ripening time)	10	0.004	10	0.048	10	0.136	10	0.206	10	0.820

[a] Cheeses were made with different starter cultures (C, Gal^+ or Gal^-, as described in Materials and methods) at low or high drain pH. Analysis of variance was carried out using the general linear model (GLM) procedure of SAS, where the effects of treatment, ripening time and their interaction were estimated; there were '2' degrees of freedom (df) for starter system and '5' for ripening time.

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Table 3

Statistical significances (*P* values) for effects of starter culture and ripening time on counts of *Lactococcus*, *Sc. thermophilus* and non-starter lactic acid bacteria (NSLAB) in Cheddar cheeses made using low or high drain pH. [a]

Factor	*Lactococcus*		*Sc. thermophilus*		NSLAB	
	df	*P*	df	*P*	df	*P*
Low drain pH cheese						
Main plot						
Starter system	2	0.883	2	0.028	2	0.002
Sub-plot						
Ripening time	5	<0.001	5	<0.001	5	<0.001
Interaction (starter system × ripening time)	10	0.759	10	<0.001	10	0.337
High drain pH cheese						
Main plot						
Starter system	2	0.120	2	<0.001	2	0.284
Sub-plot						
Ripening time	5	<0.001	5	<0.001	5	<0.001
Interaction (starter system × ripening time)	10	0.337	10	<0.001	10	0.145

[a] Cheeses were made with different starter cultures (C, Gal^+ or Gal^-, as described in Materials and methods) at high or low drain pH. Analysis of variance was carried using the general linear model (GLM) procedure of SAS, where the effects of treatment, ripening time and their interaction were estimated; there were '2' degrees of freedom (df) for starter system and '5' for ripening time.

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Table 4

Statistical significances (*P* values) for effects of starter culture and ripening time on levels of pH 4.6-soluble N (pH4.6-SN) and free amino acids (FAAs) in Cheddar cheeses made using low or high drain pH. [a]

Factor	pH4.6-SN		FAAs	
	df	*P*	df	*P*
Low drain pH cheese				
Main plot				
Starter system	2	0.883	2	0.499
Sub-plot				
Ripening time	4	<0.001	4	<0.001
Interaction (starter system × ripening time)	8	0.96	8	0.754
High drain pH cheese				
Main plot				
Starter system	2	<0.001	2	0.081
Sub-plot				
Ripening time	4	<0.001	4	<0.001
Interaction (starter system × ripening time)	8	0.978	8	0.122

[a] Cheeses were made with different starter cultures (C, Gal^+ or Gal^-, as described in Materials and methods) at high or low drain pH. Analysis of variance was carried using the general linear model (GLM) procedure of SAS, where the effects of treatment, ripening time and their interaction were estimated; there were '2' for degrees of freedom (df) for starter system and '5' for ripening time.

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Table 5

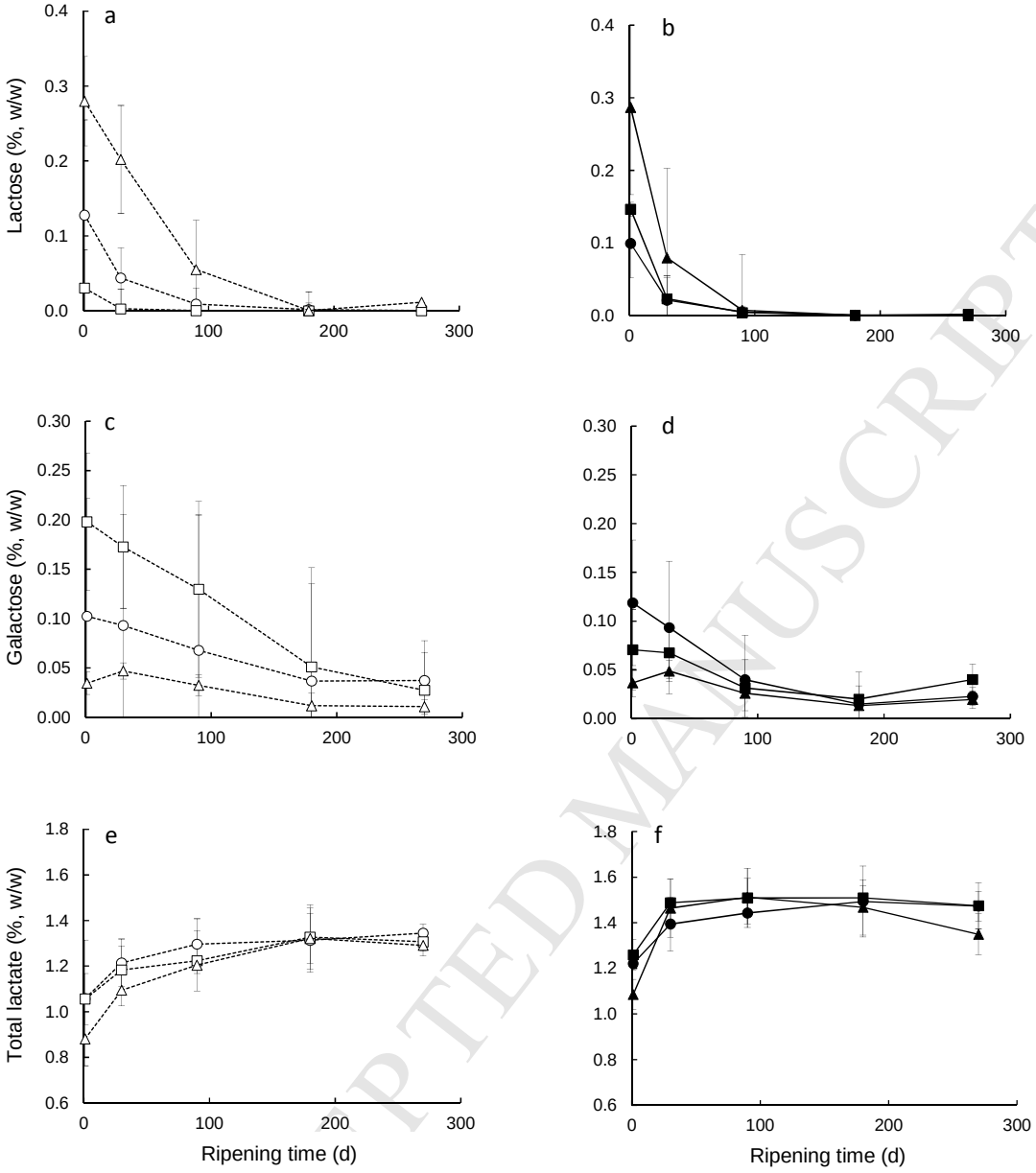
Statistical significances (*P* values) for effects of starter culture and ripening time on the firmness, facture stress and fracture strain of Cheddar cheese made using low or high drain pH. [a]

Factor	Firmness		Facture stress		Fracture strain	
	df	*P*	df	*P*	df	*P*
Low drain pH cheese						
Main plot						
Starter system	2	0.240	2	0.346	2	0.340
Sub-plot						
Ripening time	4	<0.001	4	<0.001	4	<0.001
Interaction (starter system × ripening time)	8	0.949	8	0.453	8	0.294
High drain pH cheese						
Main plot						
Starter system	2	0.066	2	0.163	2	0.773
Sub-plot						
Ripening time	4	<0.001	4	<0.001	4	<0.001
Interaction (starter system × ripening time)	8	0.923	8	0.483	8	0.949

[a] Cheeses were made with different starter cultures (C, Gal^+ or Gal^-, as described in Materials and Methods) at high or low drain pH. Analysis of variance was carried using the general linear model (GLM) procedure of SAS, where the effects of treatment, ripening time and their interaction were estimated; there were '2' for degrees of freedom (df) for starter system and '5' for ripening time.

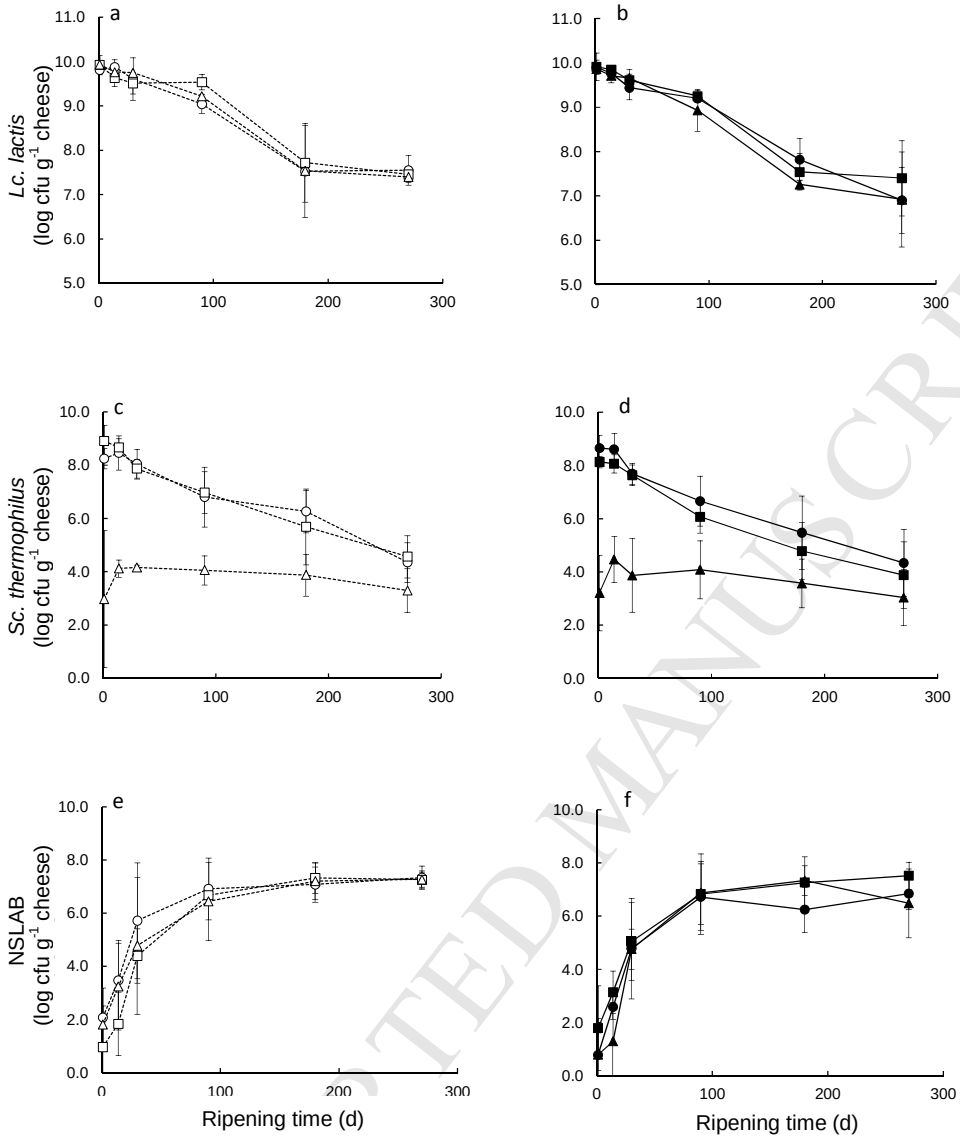
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Figure 1



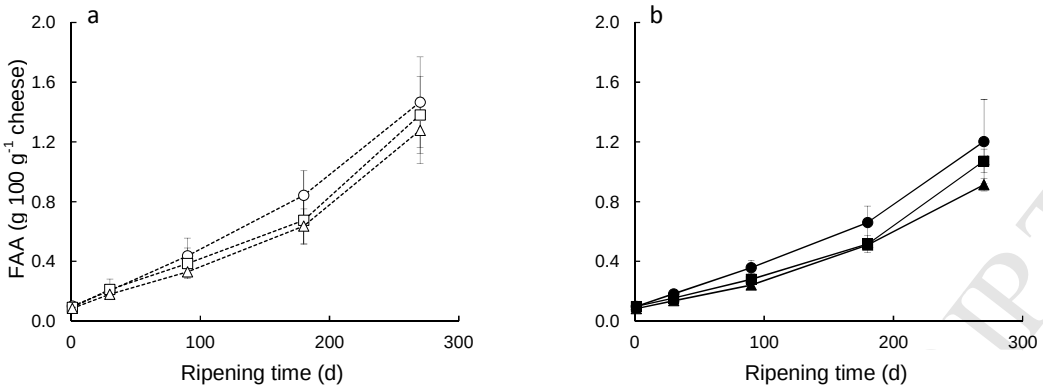
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Figure 2.



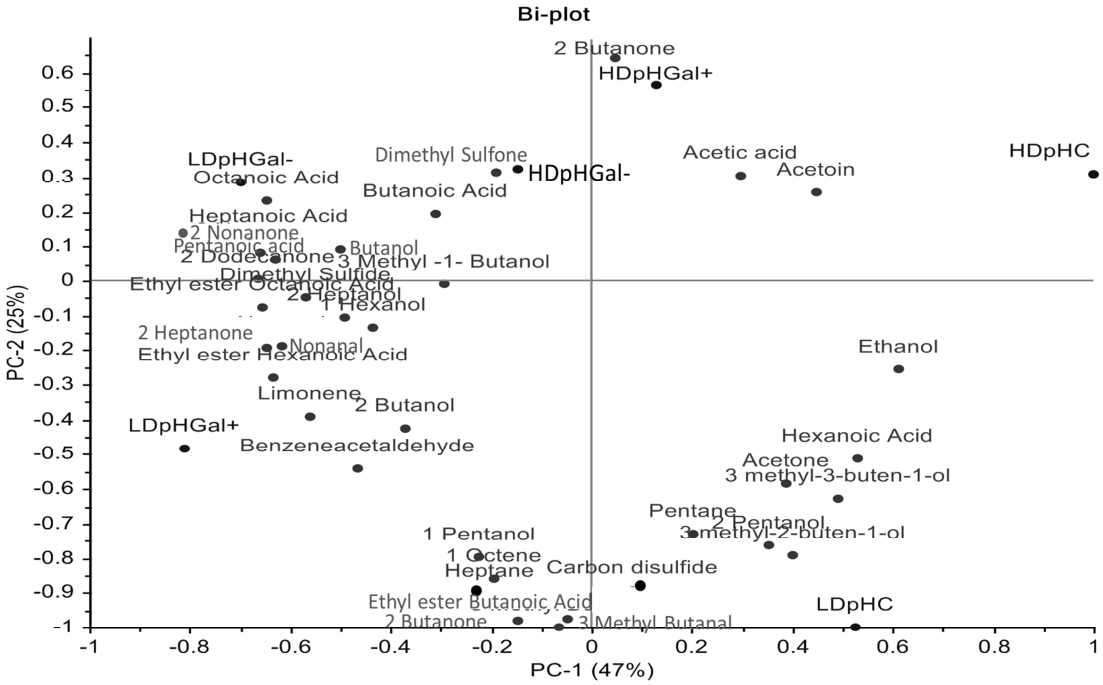
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Figure 3.



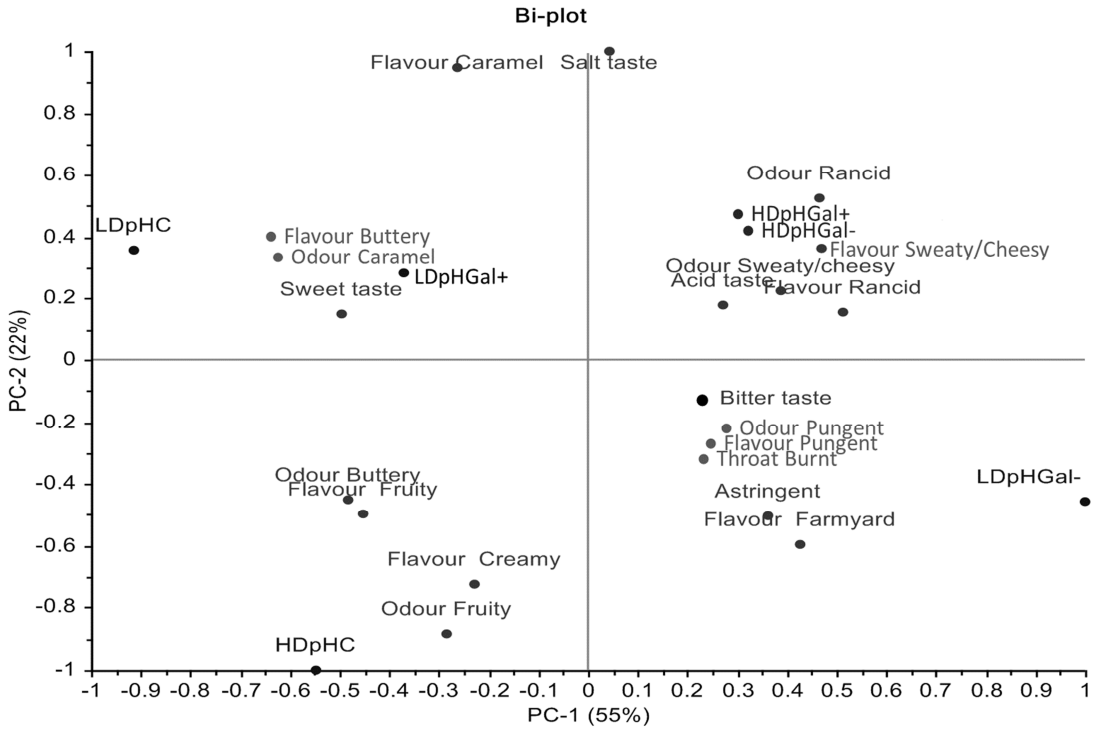
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Figure 4.



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Figure 5.



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