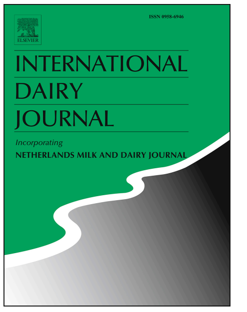
Accepted Manuscript



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Catherine M. McCarthy, Martin G. Wilkinson, Timothy P. Guinee

PII: S0958-6946(17)30156-5

DOI: 10.1016/j.idairyj.2017.07.006

Reference: INDA 4203

To appear in: *International Dairy Journal*

Received Date: 10 March 2017

Revised Date: 18 July 2017

Accepted Date: 19 July 2017

Please cite this article as: McCarthy, C.M., Wilkinson, M.G., Guinee, T.P., Effect of coagulant type and level on the properties of half-salt, half-fat Cheddar cheese made with or without adjunct starter: improving texture and functionality, *International Dairy Journal* (2017), doi: 10.1016/j.idairyj.2017.07.006.

Effect of coagulant type and level on the properties of half-salt, half-fat Cheddar cheese made with or without adjunct starter: improving texture and functionality

Catherine M. McCarthy[a], Martin G. Wilkinson[b], Timothy P. Guinee[a]

[a] *Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland*

[b] *School of Life Sciences, University College Limerick, Co. Limerick, Ireland*

Corresponding author: Tel.: + 353 25 42204; fax: + 353 25 42340.

E-mail address: tim.guinee@teagasc.ie (T. P. Guinee)

ABSTRACT

The potential of increasing proteolysis as a means of enhancing the texture and heat-induced flow of half-fat, half-salt Cheddar cheese made with control culture (CL, *Lc lactis* subsp. *cremoris/lactis*) or adjunct culture (AC, CL + *Lb. helveticus*) was investigated. Proteolysis was altered by substituting bovine chymosin (BC) with camel chymosin (CC), or by a 2.5-fold increase in level of BC. In cheese with CL-culture, increasing BC led to a large increase in pH and more rapid degradation of α_{S1}-casein during maturation, and cheese that was less firm after 180 d. In contrast, substitution of BC with CC in cheeses made with CL-culture had an opposite effect. While chymosin type and level had a similar influence on α_{S1}-casein hydrolysis in the AC-culture cheeses, it did not affect texture or flowability. Grading indicated that cheese made with AC-culture and with a higher level of BC was the most appealing.

1. Introduction

Due to the association of chronic diseases (e.g., cardiovascular disease, hypertension and diabetes) with excessive consumption of saturated fat, salt and sugar, consumers are increasingly interested in products with reduced levels of these nutrients (de-Magistris & Lopéz-Galán, 2016; Ezzati & Riboli, 2013). This, in turn, has led to a renewed focus on the contribution of fat, salt and sugar to the quality of food products, and in the case of cheese a search for new approaches to counteract the negative effects on quality of reducing fat and salt.

Reducing fat and salt in Cheddar cheese below critical levels (e.g., < 20% for fat and < 1.2% for salt) impairs texture and cooking properties (Guinee, Auty, & Fenelon, 2000; McCarthy, Wilkinson, Kelly, & Guinee, 2016). This is manifested in the cheese becoming excessively firm, long and rubbery, by a loss of meltability and flow on heating, and by the flavour becoming sour and more bitter (Drake, Boylston, Spence, & Swanson, 1997; Guinee et al., 2000) These changes are aligned with an increase in volume fraction and density of the casein network, a lower moisture-to-protein ratio, a lower rate of α_{S1}-casein breakdown (Fenelon & Guinee, 2000; McCarthy et al., 2016) and a reduction in the lubrication and moistness otherwise afforded by fat and moisture, respectively (Guinee, 2016). Various approaches have been studied to mitigate these shortcomings: high heat treatment of milk and denaturation of whey proteins in situ to reduce the extent of *para*-casein aggregation (Guinee et al., 1998; Rynne, Beresford, Kelly, & Guinee, 2004); addition of fat mimetics such as microparticulated whey proteins (Schenkel, Samudrala, & Hinrichs, 2013), carbohydrate-based materials such as Stellar™ 100X and Novagel® RCN-15 (McMahon, Alleyne, Fife, & Oberg, 1996), and sucrose polyesters (Rudan, Barbano, & Kindstedt, 1998); addition of non-globular fat (melted butter) to comminuted curd prior to remoulding to achieve a critical level of free oil on the cheese surface during heating (Wadhwani, McManus, & McMahon, 2011); the use of polysaccharide-producing cultures to increase moisture retention (Costa et al., 2010); and reducing the degree of calcium cross-linking (Henneberry, Kelly, Kilcawley, Wilkinson, & Guinee, 2015).

Proteolysis in various cheese types, including Cheddar, Mozzarella, Meshanger and Iranian White, has been accelerated by increasing the quantity of coagulant added to the cheese milk (Dave, McMahon, Oberg, & Broadbent, 2003; de Jong, 1977) and the use of coagulant with a higher ratio of proteolytic-to-milk clotting activity than calf rennet or chymosin, e.g., proteases from *Endothia parasitica* (Yun, Barbano, & Kindstedt, 1993), *Rhizomucor miehei* (Soltani, Boran, & Hayaloglu, 2016) and *Rhizomucor pusillus* (Sheehan, O'Sullivan, & Guinee, 2004). A four-fold increase in the level of added chymosin resulted in a more rapid degradation of α_{S1}- and β-caseins and a decrease in complex modulus (G*; index of firmness) of unheated directly-acidified Mozzarella cheese, and an increase in the flow of the heated cheese, to an extent dependent on the fat content (low-fat, 0.1; reduced-fat, 11.0; or control, 19.5%, w/w) of the cheese (Dave et al., 2003). Nevertheless, the firmness and flow of the reduced- and low-fat cheeses were inferior to those of the control cheese made with the regular level of added chymosin. Hence, the authors concluded that it was not possible to fully compensate for reduction in fat level solely by accelerating cheese proteolysis (Dave et al., 2003). Such a trend is consistent with the exponential increase in firmness and chewiness of hard/semi-hard cheese with protein content, which increases as fat content is reduced (Guinee, 2016). Analogously, Sheehan et al. (2004) found that substitution of chymosin with *Rhizomucor pusillus* protease enhanced primary and secondary proteolysis, but did not significantly affect the rheology or functionality of reduced-fat Mozzarella. The absence of an effect of increased proteolysis on the rheological and melt properties of reduced-fat Mozzarella may be attributable to a number of factors including the relatively high protein-to-fat ratio of Mozzarella (~1.2) compared with other cheeses (~0.8 in Cheddar cheese), the dilution and thermal inactivation of the coagulant at the relatively high temperature (58 to 62 °C) to which the curd is heated during plasticisation, and the overall low level of proteolysis during its relative short storage period.

The residual chymosin activity in Cheddar cheese is three- to four-fold higher than in Mozzarella (Feeney, Fox, & Guinee, 2001). Hence, owing to its lower protein-to-fat ratio, longer maturation time and the higher retention of added coagulant, it is expected that altering the level of proteolysis would elicit a more pronounced effect on the texture and functionality of reduced-fat Cheddar compared with Mozzarella. This premise is supported by the results of studies on the effect of substitution of bovine chymosin, with camel cyhmosin, which is less proteolytic, on reduced-fat Cheddar cheese (Børsting et al., 2012; Govindasamy-Lucey, Lu, Jaeggi, Johnson, & Lucey, 2010). These studies found that the replacement of bovine chymosin with camel chymosin resulted in a higher content of intact α_{S1}-casein, and cheese that was harder, less bitter, and less fluid on heating. However, the use of an adjunct culture (*Lactobacillus delbrueckii*) resulted in a significant reduction in the concentration of bitter-tasting peptides and bitterness in reduced-fat Cheddar cheese made with bovine chymosin after maturation at 9 °C for 56 or 196 d (Børsting et al., 2012). Based on the foregoing, it was hypothesised that increasing the level of added coagulant together with an adjunct culture could be applied advantageously to increase proteolysis and improve the rheological and functional quality of reduced-fat reduced-salt Cheddar cheese, while minimising the risk of bitter flavour in the cheese associated with a higher concentration of chymosin-produced peptides or their derivatives (Børsting et al., 2012; Lemieux and Simard, 1991); the likelihood of bitterness development is increased in reduced-salt cheese owing to the lower extent of starter cell autolysis and associated peptidase activity (Wilkinson, Guinee,

& Fox, 1994). Yet, such an approach has, to our knowledge, not been used to enhance the quality of reduced-fat, reduced-salt Cheddar cheese.

The primary aim of the current study was to investigate the effect of increasing the levels of primary and secondary primary proteolysis, by the combined effects of a 2.5 fold increase in added bovine chymosin and the use of an adjunct culture (*Lactobacillus helveticus*) on the properties of reduced-fat, reduced-salt Cheddar cheese. A secondary objective was to determine the effect of reducing primary proteolysis, by substitution of bovine chymosin with camel chymosin, while increasing secondary proteolysis by the addition of an adjunct culture (Fenelon, Beresford, & Guinee, 2002).

2. Materials and methods

2.1. Coagulant strength

Two coagulants were used in cheese manufacture, namely bovine chymosin, BC (~ 200 IMCU mL^{-1}; Chy-Max® Plus) and camel chymosin CC (~ 200 IMCU mL^{-1}; Chy-Max® M); both were obtained from Chr. Hansen (Chr. Hansen, 10–12 Bøge Alle, DK-2970 Hørsholm, Denmark). Prior to cheese manufacture, the coagulants were tested for rennet-clotting strength at pH 6.55 on milk pasteurised at 72 °C and with protein, fat and lactose contents of 3.51, 3.84 and 4.63% (w/w) respectively. The coagulants, BC or CC, were added to the milk (31 °C) at regular levels of 0.18 mL L^{-1} milk (36 IMCU L^{-1}) and 0.13 mL L^{-1} milk (26 IMCU L^{-1}), respectively. Following a 1.5 min stirring period, a 13 g sub-sample was placed in the cell of a controlled stress rheometer (CSL2 500 Carri-Med;TA Instruments, Inc., New Castle, DE, USA) and the storage modulus, G', was measured as described previously Hou et al. (2017). The rennet coagulation time (RCT) was defined as the time required for G' to attain a threshold value of 0.2 Pa. The coagulant strength in chymosin units (CU), where 1 CU was defined as the coagulant activity required to coagulate 10 mL of milk in 100 s at 31 °C, was calculated, as described by Sheehan et al. (2004).

2.2. Cheese manufacture

Half-fat (16%), half-salt (0.9%) Cheddar cheeses were made in triplicate using either BC or CC as coagulants; for each type of coagulant used, cheese was made with control culture (CL, *Lactococcus lactis* subsp. *lactis* and *cremoris*) or control culture in combination with an adjunct culture (AC, CL + *Lactobacillus helveticus*). For all cheeses, milk was standardised to a protein-to-fat ratio of 2.65, pasteurised at 72 °C for 15 s, cooled to 31 °C and pumped to the cheese 500-L vats. The treatments and the major differences between them are summarised in Table 1.

Vats 1 to 3 were inoculated with the CL culture (F-DVS mesophilic starter; R607Y, Chr. Hansen Ireland Ltd) only, and vats 4 to 6 were inoculated with the AC culture (F-DVS R607Y + F-DVS LH-32, Chr. Hansen Ireland Ltd). Cultures were inoculated at rates recommended by the supplier (i.e., 0.01 and 0.005%, w/w, for the CL- and AC-cultures, respectively) and incubated at 31 °C for 30 min. Following incubation, vats 1, 2, 4 and 5 were inoculated with BC at the regular dosage corresponding to 36 IMCU L^{-1} for vats 1 and 4, or 2.5 times the regular dosage corresponding to 90 IMCU L^{-1} for vats 2 and 5. Vats 3 and 6 were inoculated with CC at the regular dosage rate of 26 IMCU L^{-1}. As seen from Table 1, the milk clotting activity as measured (See section 2.1) and expressed as CU was similar in corresponding vats made with BC (1, 4) or CC (3, 6) at a regular dosage, despite the lower dosage volume of CC (0.13 mL L^{-1}) compared with BC (0.18 mL L^{-1}). Using data from preliminary experiments, the temperature of the milk at renneting was maintained at 31 °C in vats 1, 3, 4 and 6, and adjusted to 28 °C for vats 2 and 5 so as to maintain similar gelation times (38–40 min) across all treatments (Table 1). The required quantity of coagulant for each vat was calculated from its milk clotting strength, diluted 1:10 in de-ionised water, and added to the cheese milk which was then agitated for 1.5 min to ensure uniform distribution. A milk sample (~50 mL) was taken immediately from the cheese vat, placed in an insulated glass container, and taken to an adjacent laboratory where it was assayed for changes in storage modulus, G', over 1 h using low amplitude strain oscillation rheometry as described by Hou et al. (2017). For all cheese vats (treatments), the gel was cut when G' , an index of gel strength, reached 25 Pa. Cheeses were made using a standardised procedure, as described by McCarthy, Wilkinson, Kelly, and Guinee (2015). The pressed cheeses (~22 kg blocks) were vacuumed wrapped, stored at 4 °C for 30 d, and matured at 8 °C for 8 months.

The six different cheeses were denoted as follows (Table 1): CLBC1, CL culture with regular level of bovine chymosin (vat 1); CLBC2.5, CL culture with bovine chymosin at 2.5 times the standard level (vat 2); CLCC, CL culture with camel chymosin at the regular level (vat 3); and the corresponding cheeses made with the AC culture, namely ACBC1 (vat 4), ACBC2.5 (vat 5) and ACCC (vat 6). In the Results and Discussion sections, cheeses made with the CL- and AC-cultures are referred to as CL- and AC-cheeses, respectively.

2.3. Sampling of cheese

For each treatment, a block of cheese was sampled after various times (1, 30, 60, 120, 180 and 270 d) during ripening. At each sampling time, a vertical slice (~1.5 cm thick) was removed from one of the outside faces of the block and discarded, and a slice (~2 kg) which included the freshly-cut surface, was taken for analysis. Samples were analysed within 48 h.

2.4. Composition analysis of cheese

Cheese samples were grated and analysed in triplicate at 14 d using standard IDF methods for fat (ISO, 2004), salt (ISO, 2006), moisture (ISO, 1985), calcium (ISO, 2007) and protein (ISO, 2014).

2.5. Enumeration of viable bacteria

Aseptically taken cheese samples (~ 10 g) were homogenised with ~ 90 mL of sterile trisodium citrate (20 g L^{-1}) in a stomacher (Stomacher, Laboratory-Blender 400) for 8 min at room temperature. The resultant mixture (a 1:10 dilution) was serially diluted. Starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB) were enumerated as described previously by Hou et al. (2017). *Lactobacillus helveticus* were enumerated on MRS agar (pH 5.4) after anaerobic incubation at 45 °C for 3 d (Fenelon et al., 2002). The cheeses were analysed in duplicate at 1, 30, 120 and 180 d for all three trials.

2.6. Lactose and lactate

The lactose and lactic acid concentration was determined in duplicate using a Megazyme Lactose and D-Galactose (Rapid) Assay procedure and a D-/L-Lactic Acid (Rapid) Assay procedure, respectively (Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland) as described by Rynne, Beresford, Kelly, and Guinee (2007). The lactic acid concentration was calculated as the sum L(+) and D(–) lactic acid.

2.7. Proteolysis

2.7.1. Urea-polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) of all cheeses, from two of the three trials, was performed at 30, 120, 180 and 270 d on a Protean II xi vertical slab gel unit (Biorad Laboratories Ltd., Watford, Herts, UK) using a separating and stacking gel according to the method of Rynne et al. (2004). Cheese (i.e., ~14 mg) was dissolved on a protein basis (4.75 mg protein) in 1 mL of sample buffer, incubated at 55 °C for 15 min, and filtered through glass wool to remove fat deposits. Similarly, sodium caseinate powder, which served as a non-hydrolysed casein control, was dissolved in protein solvent to give an equivalent concentration of protein. The operating voltage was 280 V until the samples ran through the stacking gel and then 300 V as the samples ran through the separating gel. The resultant gels were stained (0.25%, w/v, Coomassie Blue G250 dye), de-stained (10%, v/v, acetic acid) and scanned using a dual lens Epson Perfection V700 Photo Model J221A with Epson Scan software (Epson Deutschland GmbH, Meerbusch, Germany). The area of the β-casein, α_{S1}-casein and α_{S1}-casein (f24–199) bands were expressed as a percentage of total band area. The bands were identified according to the notation Mooney, Fox, Healy, and Leaver (2008) and McSweeney, Pochet, Fox, and Healy (1994).

2.7.2. Primary proteolysis

The level pH 4.6-soluble nitrogen (pH 4.6-SN) was measured in triplicate as described by Fenelon and Guinee (2000) after 30, 60, 120, 180 and 270 d.

2.7.3. Secondary proteolysis

The levels of individual free amino acids (FAAs) in the pH 4.6-SN extract were analysed in triplicate using high performance cation exchange column with a Jeol JLC-500V

AA analyser (Jeol Ltd., Tokyo, Japan), as described in by McCarthy, Kelly, Wilkinson, and Guinee (2017) at 30, 120, 180 and 270 d.

2.8. Free fatty acids

The concentrations of individual free fatty acids (FFAs) ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1:0}$, $C_{18:2:0}$ and $C_{18:3:0}$) at 270 d were assayed in triplicate using gas chromatography with flame ionised detection as previously described by McCarthy et al. (2017).

2.9. Rheology

Six cubes (25 mm^3) were cut from each of the six treatment cheeses (~ 4 °C) using a Cheese Blocker (Bos Kaasgereedschap, Bodengraven, Netherlands). The cubes were compressed to 30% original height at a cross head velocity of 1 mm s^{-1} on a TAHDi texture analyser (model TA-HDI, Stable Micro Systems, Godalming, UK) equipped with a 5 mm compression plate and fitted with a 100 kg load cell, using conditions described by Henneberry et al. (2015). The following rheological parameters were calculated from the resultant force/time curves: firmness (σ_{max}) defined as the force at 70% compression; fracture stress (σ_f), the force per unit surface area of sample at fracture as determined from the inflection point of the force/time curve; and fracture strain, (ε_f), the displacement at fracture expressed as a % of original sample height.

2.10. Functionality of the heated cheese

2.10.1. Flowability

The flowability was assessed in quadruplicate using the modified Schreiber method as previously described in McCarthy et al. (2016). The flow during heating was defined as the % increase in mean diameter of the cheese disc.

2.10.2. Work required to stretch the cheese

The work required to stretch the molten cheese (~95 °C) (EW) were measured in triplicate using uniaxial extension on a TAHDi texture analyser at a velocity of 10 mm s^{-1}, as described by McCarthy et al. (2016). The analysis was undertaken in triplicate and the work required to extend the molten cheese to 380 mm (EW) was calculated from the resultant force/time curves.

2.11. Cheese grading

The six treatment cheeses were assessed at 120 and 270 d by a commercial grader from Ornua (Ornua Co-operative Limited Head Office, Grattan House, Mount Street Lower, Dublin 2, Ireland). All cheeses were assigned a random code and were tasted in duplicate. The grading comments were recorded.

2.12. Statistical analysis

Six treatment cheeses (CLBC1, CLBC2.5, CLCC, ACBC1, ACBC2.5 and ACCC) were each manufactured on three separate occasions (trials) over a two-week period. Analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute, 2011), was applied to determine the effect of coagulant on cheese composition at 14

d. Tukey's multiple-comparison test was used for paired comparison of treatment means with the level of significance determined at $P < 0.05$. A repeated measure design was used to determine the separate effects of treatment (coagulant or culture type), ripening time, and the interaction between treatment and ripening time on the cheese properties investigated over maturation. The main plot factor was coagulant or culture type and the sub-plot factor was ripening time. The PROC GLM procedure of SAS (SAS Institute, 2011), which involved 2 factors (coagulant and culture type) as class variables, was used for the data analyses. The significance of correlations was determined by applying Student's t-test to correlation coefficients, where n is the actual number of data points, and df is the degrees of freedom (n-2).

3. Results

3.1. Composition

Analysis of the data of the three replicate trials indicated that coagulant or adjunct culture did not significantly affect composition (Table 2), an outcome consistent with the standardisation of key cheesemaking parameters (e.g., pH at different stages, firmness of gel at cutting).

3.2. Enumeration of viable bacteria

Starter lactococci decreased significantly in all cheeses during maturation from ~ 10^9 cfu g^{-1} at 1 d to ~$10^{7.2}$ cfu g^{-1} at 270 d (data not shown). *Lb. helveticus* populations decreased significantly in the AC-cheeses from $10^{6.6}$ cfu g^{-1} at 1 d to ~10^1 cfu g^{-1} at 180 d, with the decrease being most pronounced in the period 1 to 30 d; as expected, *Lb. helveticus* was not detected in cheeses made with the CL culture. Concomitantly, the population of NSLAB increased in all cheeses over ripening from ~$10^{2.3}$ cfu g^{-1} at 1 d to ~$10^{7.2}$ cfu g^{-1} at 270 d. Neither starter culture nor NSLAB populations were significantly affected by coagulant (Table 3).

3.3. Changes in lactose and lactic acid

Lactose was present at very low levels in all cheeses initially (< 0.06% at 1 d) and was non-detectable after 180 d (data not shown); it was unaffected by coagulant (Table 3) or adjunct culture. The concentration of total lactate increased in all cheeses over ripening from ~1.45% at 1 d to ~1.7% at 270 d. While the mean concentration of total lactate over the 270-day ripening period was not affected by treatment (Table 3), the level in the CLBC2.5 or ACBC2.5 at times ≥ 120 d was significantly higher than that in the corresponding CLBC1, CLCC, ACBC1 and ACCC cheeses.

3.4. pH

There was an interaction between ripening time and coagulant on the pH of CL- and AC- cheeses (Fig. 1, Table 3). The pH of CLBC1 and CLCC remained constant at ~5.25, while that of CLBC2.5 increased significantly during ripening from ~5.2 at 1 d to 5.7 at 270 d (Fig. 1; Table 3). The pH of all the AC-cheeses increased significantly during ripening, from ~5.20 at 1 d to atypically-high pH values at 270 d, i.e., ~5.80 in ACCC or ~6.0 in ACBC1 and ACBC2.5.

3.4. Proteolysis

3.4.1. Urea-PAGE

The gel electrophoretograms for the six cheeses at 30, 120, 180 and 270 d from trial 1 are shown in Fig. 2; similar profiles were obtained for cheeses in trial 2. Both α_{S1}- and β-caseins decreased significantly in all cheeses during maturation (Table 4), to an extent dependent on coagulant and ripening time (Table 5). For both the CL- and AC-cheeses, α_{S1}-casein was hydrolysed to the fractions f24–199, f102–199, and f33-*, and β-casein to the fractions f29–209 (γ_1), f106–209 (γ_2) and f108–209 (γ_3). Simultaneously, the concentrations of intact α_{S1}- and β-caseins (as % of total casein) decreased from ~10–21% and 14–16% at 30 d, to ~ 4–13% and 6–11% at 270 d (Fig. 2; Table 5).

Coagulant had a significant effect on the rate of α_{S1}-casein hydrolysis (Fig. 2; Table 4), which was slowest in CLCC and most rapid in CLBC2.5, where it was almost completely degraded after 180 d. Hence, the concentration of intact α_{S1}-casein was highest in CLCC at times ≥ 120 d and lowest in CLBC2.5 at ≥ 30 d. The level of proteolysis of α_{S1}-casein in BC1 was intermediate between that of BC2.5 and CC for both the CL- and AC-cheeses. Despite its influence on level of hydrolysis, coagulant did not influence the profile α_{S1}-casein-derived peptides.

Coagulant did not affect the mean level of β-casein degradation over the 270-day ripening period or pattern of breakdown products in the CL- and AC-cheeses; a similar trend was observed at all ripening times, apart from 270 d where the proportion of intact β-casein in the CLBC2.5 and ACBC2.5 cheeses was slightly, but significantly, lower than that of the corresponding CLBC1 or CLCC, and ACBC1 or ACCC cheeses.

3.4.2. pH 4.6-soluble N formation

Casein hydrolysis was paralleled by a significant increase in pH 4.6-SN during ripening (Fig. 3a,b), from ~7.5 to 25% TN in the cheeses made with CL-culture and ~10 to 28% TN in the cheeses made with AC-culture.

Coagulant significantly affected pH 4.6-SN in both the CL- and AC-cheeses, for which the mean level in CLBC2.5 and ACBC2.5 was higher than that of the corresponding CLBC1 or CLCC, and ACBC1 or ACCC cheeses, respectively. The mean concentration of pH 4.6-SN in the cheeses made with CC was lower than that of cheeses made with the regular level of BC (BC1) when using the CL-culture, but similar when using the AC-culture (Fig. 3a,b).

The addition of commercial adjunct culture increased the level of primary proteolysis (as measured by the increase in pH 4.6-SN) in all cheeses; the effect was significant only in ACBC1 and ACCC.

3.4.3. Free amino acids

FAAs increased significantly in all cheese during maturation, with the mean concentration being affected by coagulant and adjunct culture (Fig. 3c,d; Table 4).

When using the CL-culture, the level of FAAs in CLBC2.5 was significantly higher than that in CLBC1 or CLCC, with the difference becoming more pronounced with ripening time. After 270 d, the concentration in CLBC2.5 was ~3.2–3.6-fold higher than that in CLBC1 or CLCC. In contrast, coagulant did not significantly affect the level of FAAs in the AC-cheeses for which the mean levels in ACBC1 and ACCC were significantly higher than those in the corresponding CLBC1 and CLCC cheeses. The FAA concentration in ACBC1, ACBC2.5 and ACCC were similar to that in CLBC2.5; hence, the use of adjunct increased the FAA levels in cheeses made using the regular level of BC (BC1) or CC but not in cheese where an increased level of BC (BC2.5) was used. The principal FAAs in all cheeses were glutamate, leucine, phenylalanine, lysine, valine and proline.

3.5. Free fatty acids

The concentrations of total and individual FFAs were measured at 270 d (data not shown). The principal FFAs in all six cheeses in descending order were $C_{16:0}$, $C_{18:1:0}$, $C_{18:0}$, $C_{14:0}$ and $C_{12:0}$. In the cheeses made using CL-culture, CLCC had a significantly higher level of total FFAs compared with CLBC1 and CLBC2.5, e.g., 439 mg kg^{-1} versus 343 and 360 mg kg^{-1}, respectively. In general, CLCC had greater concentrations of $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1:0}$ and $C_{18:2:0}$ than CLBC1 and/or CLBC2.5 (data not shown). A similar trend was found in the AC-cheeses, for which the concentration of total FFA in ACCC at 270 d were significantly higher than that in ACBC1 and ACBC2.5.

3.6. Fracture properties

Fracture stress (σ_f) and firmness (σ_{max}) decreased significantly in all cheeses over maturation (Fig. 4a–d), from ~692 to 330–530 kPa, and 460 to 220–330 (N), respectively.

Coagulant had a significant effect on σ_f and σ_{max} in the cheeses made with the CL culture (Table 4) but not in cheeses made using the AC-culture. In the former, σ_f and σ_{max} were significantly higher in CLCC at times ≥ 120 d. Hence, the mean σ_f and σ_{max} over the 270 d ripening period was higher in CLCC compared with CLBC1 and CLBC2.5. Moreover, the effect of coagulant was interactive with ripening time with the difference between CLCC and CLBC1 or CLBC2.5 increasing as ripening progressed. In contrast, the fracture strain (ε_f) was unaffected by coagulant and the interaction between coagulant and ripening time.

Conversely, coagulant did not significantly affect the σ_f, σ_{max}, or ε_f in the cheeses made using adjunct culture (Table 4, Fig. 4a–d).; nevertheless, ACBC2.5 had a significantly lower ε_f at 270 d compared with ACBC1 or ACCC for which ε_f values were similar i.e., 0.34 versus 0.53 or 0.53, respectively.

3.7. Functionality of the heated cheese

3.7.1. Extent of flow

The flowability of the heated cheeses increased significantly during maturation (Fig. 4e,f). Although the cheese made with CC had the lowest extent of flow when compared with the BC1 or BC2.5 cheese on heating, the effect of coagulant was not significant in the CL- or AC-cheeses (Table 4).

3.7.2. Work required to stretch the cheese

The work required to extend the molten cheese mass decreased for all cheeses during maturation, from ~770 mJ at 30 d to ~300 mJ at 270 d. Despite the fact that EW for cheeses made using CC (CLCC, ACCC) was the highest at most ripening times, the mean values over ripening for the different coagulant treatments did not differ significantly for the CL- or AC-cheeses (Table 4).

3.8. Cheese grading

After 120 d, the grader noted that all cheeses had a curdy texture. The CL-cheeses lacked an acceptable finish and contained bitter notes. CLBC1 lacked a salty taste; CLBC2.5 and CLCC tasted saltier (like a standard Cheddar cheese) and were considered to have a better taste and less bitter finish (compared with CLBC1). The AC-cheeses were characterised as having subtle sweet flavour notes and were considered less savoury than the CL-cheeses. While the ACBC1-cheese was perceived as lacking the typical 'salty' taste of Cheddar towards the end of mastication, the ACCC or ACBC2.5 were found to be characteristically salty. At this stage of ripening, the grader considered the ACCC cheese to be the best tasting (Table 6).

Following evaluation at 270 d, the grader noted that the lack of fat was very obvious in CLBC1 and CLCC cheeses but not in CLBC2.5 cheese. Although the CL-cheeses had sweet notes, the cheeses lacked a good finish which was attributed to the lack of 'saltiness'. Overall, the ACBC2.5 and ACCC cheeses were considered the most acceptable and as being suitable for sale as a 'sweet'-style Cheddar cheese, a variant of Cheddar which is becoming increasingly popular in the Irish and UK markets. Despite both sharing 'sweetish' flavour notes, the taste profiles of the latter cheeses were nonetheless quite different, with the ACBC2.5 cheese perceived as having had a smooth texture and strong sweet flavour notes, and the ACCC cheese as having had a steady texture and a taste that was initially sweet but finished slightly sharp. Although ACBC1 tasted sweet, it was perceived as lacking in 'saltiness' (Table 6).

4. Discussion

The current study investigated the effects of altering coagulant, type and level, as a means of improving the properties of half-salt, half-fat Cheddar-style cheeses made using control culture, CL (consisting of a blend of *Lc. lactis* subsp. lactis, *Lc. lactis* subsp. *cremoris*) or adjunct-containing culture, AC (consisting of the CL-culture with added *Lb. helveticus*). The coagulant treatments, used with both the CL- and AC-cultures, included BC at the regular level (CLBC1 and ACBC1), BC at 2.5 times the regular level (CLBC2.5 and ACBC2.5), and CC at the regular level (CLCC and ACCC). Coagulant had no effect on gross composition, concentrations of lactose and total lactate, or the populations of starter or NSLAB in the CL- or AC-cheeses.

The pH in all cheeses ex-press (~5.2 at 1 d) was similar, as expected because of the equal levels of lactic acid and pH-buffering substances (calcium, phosphate, protein). However, coagulant had a notable effect on the extent of pH change during maturation, whereby the pH increased by ~0.1–0.2 pH units in CLBC1 and CLCC, and ~0.5–0.75 units in CLBC2.5, ACBC1, ACBC2.5 and ACCC. A similar trend was noted for FAAs, i.e., for which the increase during maturation in the CLBC1 and CLCC was notably lower than that in CLB2.5, ACBC1, ACBC2.5 or ACCC. Hence, linear regression analysis indicated a positive correlation between pH and total FAA concentration in both the CL- ($r = 0.97$, $df = 22$) and AC- ($r = 0.89$, $df = 22$) cheeses. The level of pH change during cheese maturation is controlled by the balance of factors that reduce pH (i.e., lactic acid concentration), buffer pH (i.e., buffering capacity which is controlled inter alia by the concentration of calcium phosphate and the protein side-chains of glutamate and aspartate residues), and/or increase pH (production of FAAs) (Salaün, Mietton, & Gaucheron, 2005; Upreti and Metzger, 2006, 2007). The amino groups of FAAs have dissociation constants (pKa > ~ 9.0) well in excess of the initial cheese pH (5.0 to 5.35) and are, thus, likely to become protonated in the cheese environment. Hence, the gradual increase in cheese pH is concomitant with the progressive decrease in hydrogen ion activity as FAA accumulate during maturation; such an effect would be most pronounced in cheeses with higher FAAs, i.e., CLBC2.5, ACBC1, ACBC2.5 and ACCC.

The hydrolysis of α_{S1}-casein was greatly accelerated by increasing the level of BC, as evidenced by the lower content of intact α_{S1}-casein and higher level of pH 4.6-SN in

CLBC2.5 and ACBC2.5, compared with CLBC1 and ACBC1, at all ripening times (Fig. 2, 3a, b, Table 5). The increase in primary proteolysis with dosage level of BC is well documented for cheeses such as Meshanger (de Jong, 1977), Cheddar (Creamer, Iyer, & Lelievre, 1987) and Mozzarella (Dave et al., 2003). In contrast, cheese made with CC (CLCC, ACCC) had a significantly higher content of intact α_{S1}-casein than cheeses made with BC (CLBC1, ACBC1) at most ripening times. A similar finding by Bansal et al. (2009) was attributed to the low level of added CC (~30% reduction in the level of added enzyme milk clotting units compared with BC) and its relatively low unspecific proteolytic activity (on bonds other than Phe_{105}–Met_{106} of κ-casein), which has been found to be only ~20% of that of BC on bovine milk (Kappeler et al., 2006). The lower unspecific proteolytic activity of CC was confirmed by Møller, Rattray, and Ardö (2012), who found that although CC and BC shared similar modes of proteolytic action on dilute solutions (1%) of bovine α_{S1}-casein (at pH 6.5) and β-casein (at pH 6.5 and 5.2), CC was markedly less proteolytic.

Compared with α_{S1}-casein, β-casein underwent a much lower degree of proteolysis during maturation, with the levels at 270 d corresponding to ~45 and 60% of those at 30 d. This resistance of β-casein to hydrolysis by BC in Cheddar cheese has been attributed to a concentration-induced aggregation (at concentrations ≥20 g 100 g^{-1} in aqueous dispersion) which limits the access of the enzyme (Phelan, Guiney, & Fox, 1973). β-Casein hydrolysis was not affected by increasing the level of BC or by the substitution of BC with CC, as seen from the similar concentrations of intact β-casein in all cheeses at most ripening times, apart from 270 d (Fig. 2, Table 5). In corollary, the results of studies investigating the effect of reducing coagulant or BC also suggest little, or no, effect of incrementally reducing the level of added calf rennet from 100 to 20% of normal on β-casein in Cheddar cheese (Creamer et al., 1987). The similar degradation rates of β-casein hydrolysis in cheeses made with BC1 and CC is consistent with results of Børsting et al. (2012) for reduced-fat Cheddar. However, it contrasts with the results of Møller et al. (2012), which showed that the β-casein hydrolysis in reduced-salt Cheddar cheese (0.85%, w/w) made with CC proceeded more slowly than that in cheese made with BC during ripening, but concurs with those of Bansal et al. (2009), who reported no difference in the level of degradation of β-casein in Cheddar cheeses made with BC or CC. The discrepancy with the results of Møller et al. (2012) may relate to inter-study differences in cheese pH, fat content of cheese and/or β-casein concentration (Phelan et al., 1973), which is higher in half-fat Cheddar (current study) than full-fat Cheddar (Møller et al., 2012).

The levels of FAAs in CLBC2.5 were markedly higher than that in CLBC1 or CLCC. Considering that bacterial counts were similar in all cheeses, this result suggests that the higher level in CLBC2.5 is due to the higher level of added chymosin. The potential contribution of coagulant to FAA development in cheese has been demonstrated by early studies on aseptic model cheeses made with or without starter culture or calf rennet (Visser, 1977), and more recently in Cheddar cheeses with different levels of residual chymosin activity, as varied by the addition of different levels of the chymosin inhibitor, pepstatin (O'Mahony, Lucey, & McSweeney, 2005). The concentration of chymosin-derived peptides, which are degraded to peptides of lower molecular weight and FAAs by starter culture peptidases (McSweeney, 2004), is likely to vary according to the level of residual chymosin activity which in turn is affected by the dosage of added coagulant. The significant contribution of adjunct culture to the development of FAAs is exemplified by the significantly higher levels of FAAs in the each of the AC-cheeses compared with the matching CL-cheeses at times ≥ 120 d. The higher, but similar concentrations of FAA in the AC-cheeses, despite their differences in extent of α_{S1}-casein hydrolysis, suggests that the rate of degradation of chymosin-derived peptides by starter culture/adjunct peptidases rather than the concentration of chymosin-derived peptides per se, is the rate-limiting step affecting the development of FAA in regular Cheddar cheese without adjunct culture. The accelerating effect of adjunct *Lactobacillus* on FFA development is consistent with previous studies on full-fat and reduced-fat Cheddar cheeses (Børsting et al., 2012; Fenelon et al., 2002).

Fracture stress (σ_f) and firmness (σ_{max}) correlated positively with intact α_{S1}-casein (r = 0.86, df = 22) and inversely with pH 4.6-SN (r = 0.80, df = 22) in the CL-cheeses. Hence, the CLCC cheese was firmest while the CLBC2.5 was softest. The effects of coagulant on the fracture properties concur with those from previous studies comparing CC with BC in Cheddar (Bansal et al., 2009; Govindasamy-Lucey et al., 2010), reduced-fat Cheddar (Børsting et al., 2012), and the effect of increasing level of added BC in Meshanger (de Jong, 1977) or Mozzarella (Dave et al., 2003). Such effects are consistent with an attenuation of the calcium-phosphate *para*-casein network of cheese commensurate with the hydrolysis of α_{S1}-casein (Guinee, 2016). Creamer, Zoerb, Olson, and Richardson (1982) concluded that the sequence of residues f14-24 of α_{S1}-casein is strongly hydrophobic and contributes to extensive interaction of *para*-casein molecules within the network; hence, its cleavage by chymosin leads to an overall weakening of the cheese matrix, making it more prone to deformation on compression. Nevertheless, O'Mahony et al. (2005) concluded that the softening of Cheddar cheese during early ripening (21 days post manufacture) was essentially independent of the hydrolysis of α_{S1}-casein at Phe_{23}–Phe_{24} and was instead correlated more closely to the partial solubilisation of the colloidal calcium phosphate cross-linking of the casein constituting the *para*-casein network of the curd.

Surprisingly, coagulant did not alter the fracture properties of the AC-cheeses, despite having had a similar effect on α_{S1}-casein degradation in both CL- and AC-cheeses. This prompts the question why cheeses having similar composition and levels of primary proteolysis α_{S1}-casein degradation) behaved so differently during large strain deformation? The difference may reside on how the effects of proteolysis are influenced by pH. The values of σ_f and σ_{max} in cheese increase with pH in the range 5.0 to 6.0 (Visser, 1991; Watkinson et al., 2001), an effect most likely associated with the deposition of serum calcium and phosphate as insoluble calcium phosphate (Guinee et al., 2000) that binds to, and enhances, the cross-linking of the casein molecules. It is probable that the effect of pH, which increased in all AC-cheeses from 5.2 to ~5.8–6.0 during ripening, is dominant, negating the influence of the difference in the concentration of intact α_{S1}-casein between the ACCC, ACBC1 and ACBC2.5 cheeses. Of course, validation of such a hypothesis would require a study of the interactive effects of pH and proteolysis in model cheese systems where calcium content and residual chymosin are maintained constant.

Apart from the above, other effects associated with altering coagulant and adding adjunct culture included changes in the concentration of total FFAs. The addition of the adjunct starter culture and increasing the level of added BC improved grading comments, as confirmed by the 270 day-old ACBC2.5 receiving the most favourable comments. Descriptions assigned to the latter cheese included ‘smooth’ texture and ‘sweet’ flavour notes. The positive effects of adding a *Lb. helveticus* adjunct on the flavour of reduced-fat Cheddar cheese have also been found by others (Børsting et al., 2012; Fenelon et al., 2002) for reduced-fat Cheddar cheese and Møller, Rattray, & Ardö (2013) for reduced-salt Cheddar made with camel chymosin, where it reduced the concentration of bitter peptides at 280 d.

5. Conclusion

The effect of coagulant type (bovine chymosin, BC; camel chymosin, CC) or level (at regular or increased levels for BC, i.e., BC1 or BC2.5) on the texture and functionality of half-fat, half-salt Cheddar-style cheese made using a control culture, CL, or an adjunct-containing starter culture, AC, was investigated. The results showed coagulant type and level affected the levels of intact α_{S1}-casein, pH 4.6-SN, FAAs, pH and fracture properties to an extent depending on the culture type used. Notably, the texture (reduction in fracture stress and firmness) was improved on lowering the content of intact α_{S1}-casein in cheese made using the CL culture by increasing the level of added BC; an opposite effect occurred on replacing BC with CC. These effects were not observed in cheese made with the AC culture, perhaps of their relatively high pH. Nevertheless, cheeses made using the AC culture had higher levels of pH 4.6-SN, lower firmness and fracture stress, and higher heat-induced flowability than the corresponding cheeses made using the CL culture. Moreover, the adjunct culture in combination with a higher dosage of BC resulted in the 270 day-old cheese having a 'sweet flavour' and being generally more 'pleasant'. Hence, the use BC at an elevated level in combination with an adjunct culture (*Lb. helveticus*) provides a means of improving the quality of reduced-fat, reduced-salt Cheddar cheese.

Acknowledgements

This work was funded by the Department of Agriculture, Fisheries and Food, under the Food Institutional Research Measure, Dublin 2, with project reference no. 2012219. The authors kindly acknowledge Mr. Enda Howley (Ornua Co-operative Limited Head Office, Grattan House, Mount Street Lower, Dublin 2, Ireland) for cheese grading.

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Figure legends

Fig. 1. Changes in pH of half-fat, half-salt Cheddar-style cheeses made with control culture, CL (closed symbols) or adjunct culture, AC (open symbols) and using different coagulant treatments: bovine chymosin at the regular level, BC1 (●,○) or at 2.5 fold the regular level, BC2.5 (■,□), or camel chymosin at the regular level, CC (▲,△). Values are the means of three replicate trials; error bars represent standard deviations of the mean.

Fig. 2. Urea-polyacrylamide gel electrophoretograms of half-fat, half-salt Cheddar-style cheeses after for 30, 120, 180 or 270 d at 8 °C. The cheeses were made with control starter culture, CL (lanes 1–3) or adjunct culture, AC (lanes 4–6) and using different coagulant treatments: bovine chymosin at the regular level, BC1 (lanes 1, 4) or at 2.5-fold the regular level, BC2.5 (lanes 2, 5); or camel chymosin at the regular level, CC (lanes 3, 6). Sodium caseinate (lane NaCn), loaded at an equivalent weight of protein (4.25 mg per lane) was included as an unhydrolysed casein control. In each panel, the cheeses, defined in Table 2, are: CLBC1, lane 1; CLBC2.5, lane 2; CLCC, lane 3; ACBC1, lane 4; ACBC2.5, lane 5; ACCC, lane 6. Protein bands were identified according to Mooney et al. (1998) and McSweeney et al. (1994): 1, β-casein(f106–209) (γ2); 2, β-casein(f29–209) (γ1); 3, β-casein(f108–209) (γ3); 4, β-casein; 5, β-casein(f1–192); 6, α_{S1}-casein; 7, α_{S1}-casein(f102–199); 8, α_{S1}-casein(f24–199); 9, α_{S1}-casein(f121–199); 10, α_{S1}-casein(f33–*).

Fig. 3. Changes in levels of pH 4.6 soluble-nitrogen (pH 4.6-SN; a,b) and free amino acids (FAA; c,d) of half-fat, half-salt Cheddar-style cheeses made with control culture, CL (closed symbols) or adjunct culture, AC (open symbols) and using different coagulant treatments: bovine chymosin at the regular level, BC1 (●,○) or at 2.5 fold the regular level, BC2.5

(■,□), or camel chymosin at the regular level, CC (▲,△). Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.

Fig. 4. Changes in fracture stress (a,b), firmness (c,d) and extent of flow on heating (e,f) of half-fat, half-salt Cheddar-style cheeses made with control culture, CL (closed symbols) or adjunct culture, AC (open symbols) and using different coagulant treatments: bovine chymosin at the regular level, BC1 (●,○) or at 2.5 fold the regular level, BC2.5 (■,□), or camel chymosin at the regular level, CC (▲,△). Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.

Table 1

Treatments and manufacturing details of experimental half-fat, half-salt Cheddar-style cheese. [a]

Cheesemaking	Control culture (CL)			Adjunct culture (AC)		
	CLBC1	CLBC2.5	CLCC	ACBC1	ACBC2.5	ACCC
Details of cheesemaking steps						
Starter culture	CL	CL	CL	CL	CL	CL
Adjunct culture	-	-	-	AC	AC	AC
Chymosin type/level	BC1	BC2.5	CC	BC1	BC2.5	CC
Chymosin added as:						
mL L^{-1}	0.18	0.45	0.13	0.18	0.45	0.13
IMCU L^{-1}	36	90	26	36	90	26
CU L^{-1} milk	7.4	18.5	7.3	7.4	18.5	7.3
Temperature at set (°C)	31	28	31	31	28	31
pH at set	6.52	6.53	6.52	6.54	6.53	6.53
Gel firmness at cut (Pa)	25	25	25	25	25	25
Time of cheesemaking stages (mins)						
Curd residence (from cut to whey drainage)	168	169	165	182	195	175
Cheddaring (from whey drainage to milling)	113	125	108	105	123	110
Total make time (from starter addition to milling)	354	270	343	380	375	358

[a] Abbreviations are: CL, control starter culture, consisting of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*; AC, adjunct culture consisting of CL plus *Lactobacillus helveticus* as adjunct; IMCU, international milk clotting units, as stated on the label supplied with coagulant; CU, chymosin units, as measured experimentally and defined in the Materials and Methods. Cheese codes are: CLBC1, CLBC2.5 and CLCC refer to the cheeses made using CL culture with bovine chymosin at the regular level (CLBC1) or at 2.5-fold the regular level (CLBC2.5), or with camel chymosin (CLCC) at the regular level; the matching variants made the AC culture are similarly denoted.

Table 2

Effect of coagulant on the composition and pH of 14 day-old half-fat, half-salt Cheddar-style cheeses made using control or adjunct culture. [a]

Compositional factors	Control culture (CL)			Adjunct culture (AC)		
	CLBC1	CLBC2.5	CLCC	ACBC1	ACBC2.5	ACCC
Moisture (g 100 g^{-1})	43.6	43.5	43.5	43.5	43.5	43.7
Protein (g 100 g^{-1})	33.8	33.8	33.7	33.6	33.5	33.5
Fat (g 100 g^{-1})	15.8	15.7	15.5	15.7	15.6	15.7
MNFS (g 100 g^{-1})	51.8	51.6	51.5	51.6	51.6	51.8
FDM (g 100 g^{-1})	28.0	27.7	27.4	27.9	27.7	27.8
NaCl (g 100 g^{-1})	0.94	0.93	0.92	0.96	0.91	0.93
S/M (g 100 g^{-1})	2.2	2.1	2.1	2.2	2.1	2.1
Lactose (g 100 g^{-1})	0.05	0.06	0.05	0.04	0.05	0.04
Total lactate (g 100 g^{-1})	1.5	1.5	1.5	1.5	1.5	1.5
Ca (mg 100 g^{-1})	1108	1091	1104	1113	1116	1116
P (mg 100 g^{-1})	523	546	563	573	574	612
pH	5.20	5.23	5.21	5.20	5.21	5.18

[a] Abbreviations are: MNFS, moisture-in-non-fat-substances; FDM, fat-in-dry-matter; S/M, salt-in-moisture; Ca, calcium; P, phosphorous. Cheese codes are: CLBC1, CLBC2.5 and CLCC refer to the cheeses made using CL culture (*Lactococcus lactis* subsp. *lactis* and *cremoris*) with bovine chymosin at the regular level (CLBC1) or at 2.5-fold the regular level (CLBC2.5), or with camel chymosin (CLCC) at the regular level; the matching variants made the AC culture (CL + *Lactobacillus helveticus*) are similarly denoted. Data are the mean values of three replicate trials; values within a row did not significantly differ ($P < 0.05$) for any of the measured factors.

Table 3

Statistical significances (*P*-values) for effects of coagulant and ripening time on microbiology, lactose metabolism and pH in half-fat, half-salt Cheddar-style cheeses made using control- (CL) or adjunct- (AC) culture. [a]

Factor	Starter	NSLAB	*Lb. helveticus*	Lactose	Total lactate	pH
CL culture						
Main plot						
Coagulant (C)	-	-		-	-	**
Sub-plot						
Ripening time (RT)	***	***		***	***	***
Interaction (C × RT)	-	-		-	-	***
AC culture						
Main plot						
Coagulant (C)	-	-	-	-	-	*
Sub-plot						
Ripening time (RT)	***	***	***	***	***	***
Interaction (C × RT)	-	-	-	-	-	***

[a] Abbreviation: NSLAB, non-starter lactic acid bacteria. Degrees of freedom (df): 2 for coagulant; 3 for ripening time except in the case of pH where there were 5; 6 for interaction of coagulant and ripening time except in the case of pH where there were 10. Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4

Statistical significances (*P*-values) for effects of coagulant and ripening time on primary and secondary proteolysis, and fracture and cooking properties in half-fat, half-salt Cheddar-style cheeses made using control- (CL) or adjunct- (AC) culture. [a]

Factor	α_{S1}-casein	β-casein	pH 4.6-SN	FAAs	Fracture stress	Firmness	Fracture strain	Flow	EW
CL culture									
Main plot									
Coagulant (C)	***	-	*	**	*	*	-	-	-
Sub-plot									
Ripening time (RT)	***	*	***	***	***	***	***	***	***
Interaction (C × RT)	*	-	*	*	-	*	-	-	-
AC culture									
Main plot									
Coagulant (C)	***	-	-	-	-	-	-	-	-
Sub-plot									
Ripening time (RT)	***	*	***	***	***	***	**	***	***
Interaction (C × RT)	**	-	-	-	-	-	-	-	-

[z]Abbreviations are: pH 4.6-SN, pH 4.6 soluble nitrogen; FAA, free amino acids; EW, work required to stretch the heated cheese to 380 mm. Degrees of freedom (df) 2 for coagulant; 4 for ripening time; 8 for interaction of coagulant and ripening time. Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 5

Changes in percentage of intact α_{S1}- and β-casein in half-fat, half-salt Cheddar-style cheeses made using control or adjunct culture. [a]

Casein	Control culture (CL)			Adjunct culture (AC)		
	CLBC1	CLBC2.5	CLCC	ACBC1	ACBC2.5	ACCC
30 day-old cheese						
Intact β-casein	13.9^{a}	15.9^{a}	14.9^{a}	14.3^{a}	14.7^{a}	15.6^{a}
Intact α_{S1}-casein	14.0^{a}	10.0^{b}	18.8^{a}	15.5^{b}	11.4^{b}	21.2^{a}
α_{S1}-casein (f24-199)	11.7^{a}	13.3^{a}	6.6^{b}	11.2^{b}	14.9^{a}	4.1^{c}
120 day-old cheese						
Intact β-casein	14.8^{a}	14.4^{a}	13.8^{a}	13.5^{a}	13.3^{a}	13.7^{a}
Intact α_{S1}-casein	9.3^{b}	7.0^{b}	15.0^{a}	8.3^{b}	7.6^{b}	18.9^{a}
α_{S1}-casein (f24-199)	14.1^{a}	11.8^{a}	10.6^{a}	12.7^{a}	11.0^{a}	12.4^{a}
180 day-old cheese						
Intact β-casein	9.1^{a}	9.3^{a}	10.1^{a}	12.4^{a}	11.0^{a}	10.9^{a}
Intact α_{S1}-casein	7.2^{b}	4.4^{c}	11.5^{a}	8.2^{b}	7.1^{b}	15.0^{a}
α_{S1}-casein (f24-199)	9.4^{a}	7.1^{b}	9.8^{a}	11.8^{a}	10.9^{a}	12.7^{a}
270 day-old cheese						
Intact β-casein	8.2^{a}	6.3^{b}	8.8^{a}	8.8^{a}	7.5^{b}	10.9^{a}
Intact α_{S1}-casein	6.4^{b}	3.5^{c}	11.2^{a}	7.8^{b}	6.8^{b}	12.9^{a}
α_{S1}-casein (f24-199)	8.6^{a}	6.7^{b}	9.6^{a}	11.8^{a}	10.2^{b}	11.4^{a}

[a] Cheese codes are: CLBC1, CLBC2.5 and CLCC refer to the cheeses made using CL culture with bovine chymosin at the regular level (CLBC1) or at 2.5-fold the regular level (CLBC2.5), or with camel chymosin (CLCC) at the regular level; the matching variants made the AC culture are similarly denoted. Data are the mean values of three replicate trials; values within a row relating to CL-cheeses or to AC-cheeses and not sharing a common lower-case superscript differ significantly ($P < 0.05$).

Table 6

Grading assessment of 120 and 270 day-old half-fat, half-salt Cheddar-style cheeses made using control or adjunct culture. [a]

Cheese code	Grading comments	
	120-day old cheese	270-day old cheese
CLBC1	Good texture, hint of bitterness, low-salt	Steady texture, slightly dry, tastes like a young cheese
CLCB2.5	Good cheese, smooth texture, poor finish	Smooth texture, good body, subtle sweet notes
CLCC	Slight curdy texture, good flavour, salty finish	Dry mouth-feel, clean flavour, low-fat
ACBC1	Good cheese, sweet flavour notes, low-salt	Steady texture, sweet flavour notes, low-fat
ACBC2.5	Smooth texture, sweet flavour notes, rounded flavour	Very good cheese, smooth texture, sweet flavour notes
ACCC	Curdy texture, plain cheese, not Cheddar-like	Steady texture, slightly dry mouth-feel, pleasant sweet flavour with sharp finish

[a] Cheese codes are: CLBC1, CLBC2.5 and CLCC refer to the cheeses made using CL culture with bovine chymosin at the regular level (CLBC1) or at 2.5-fold the regular level (CLBC2.5), or with camel chymosin (CLCC) at the regular level; the matching variants made the AC culture are similarly denoted.

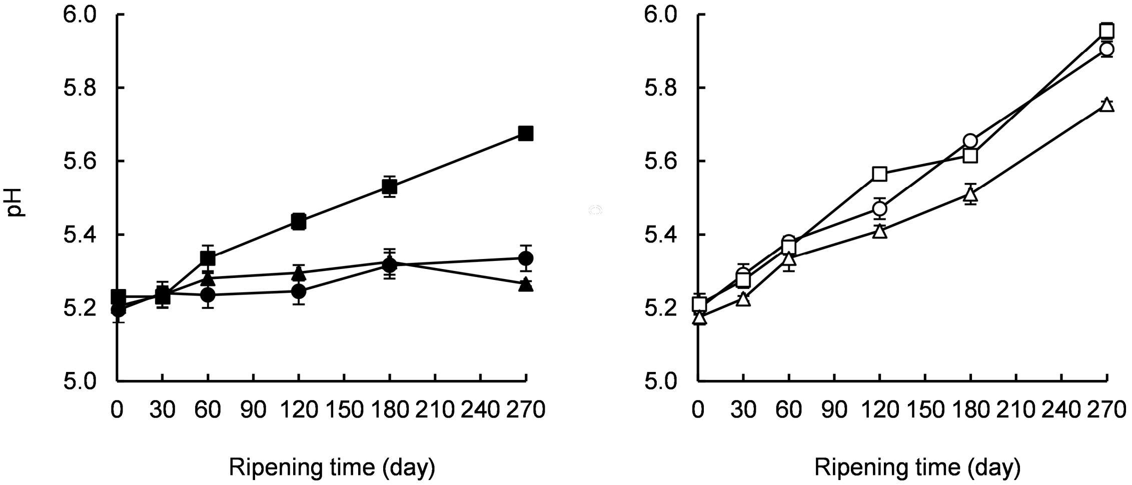
6.0
5.8
5.6
5.4
5.2
5.0
pH
0 30 60 90 120 150 180 210 240 270
Ripening time (day)
6.0
5.8
5.6
5.4
5.2
5.0
0 30 60 90 120 150 180 210 240 270
Ripening time (day)

Fig. 1

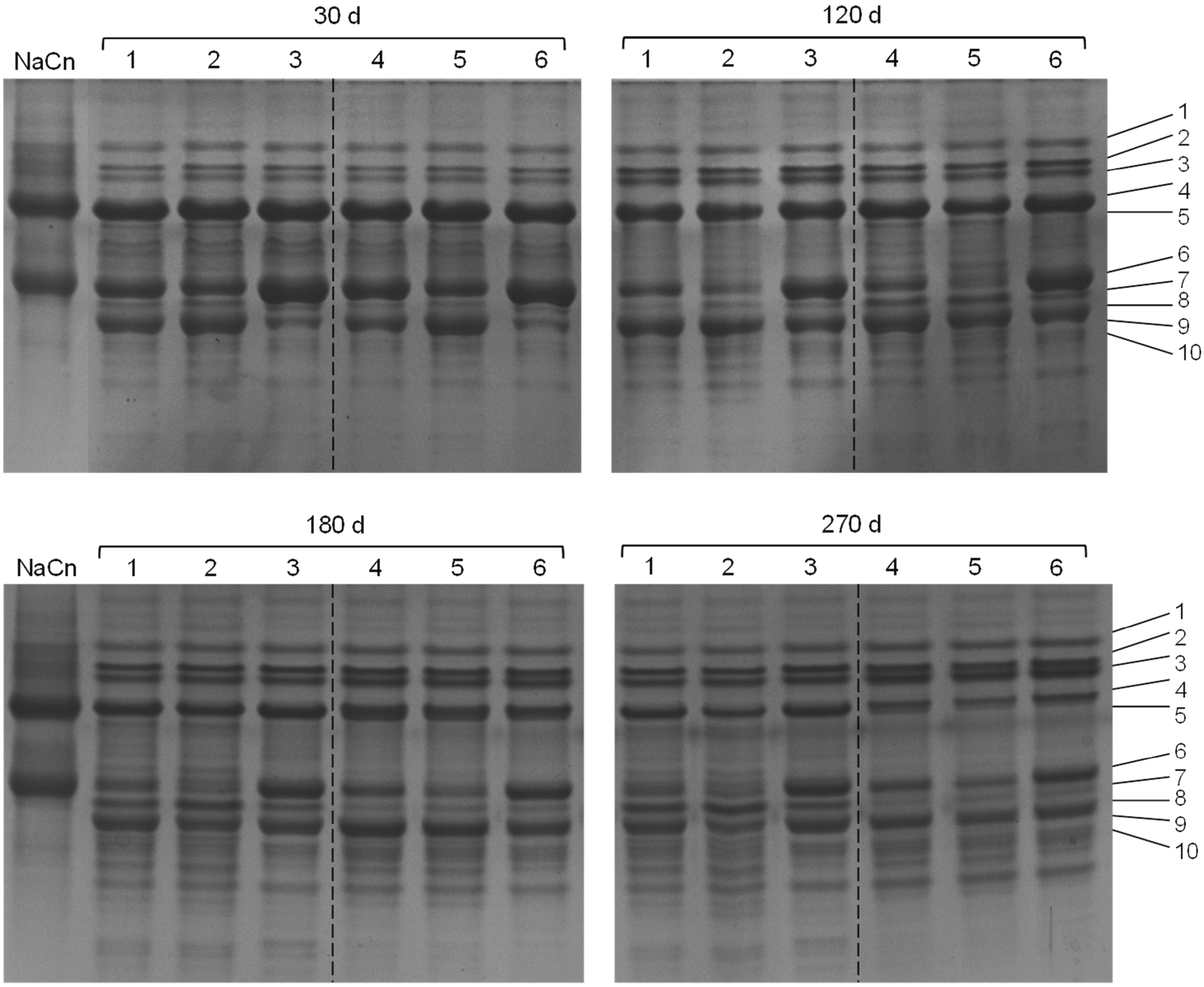
30 d
NaCn
1
2
3
4
5
6
120 d
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
180 d
NaCn
1
2
3
4
5
6
270 d
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10

Fig. 2

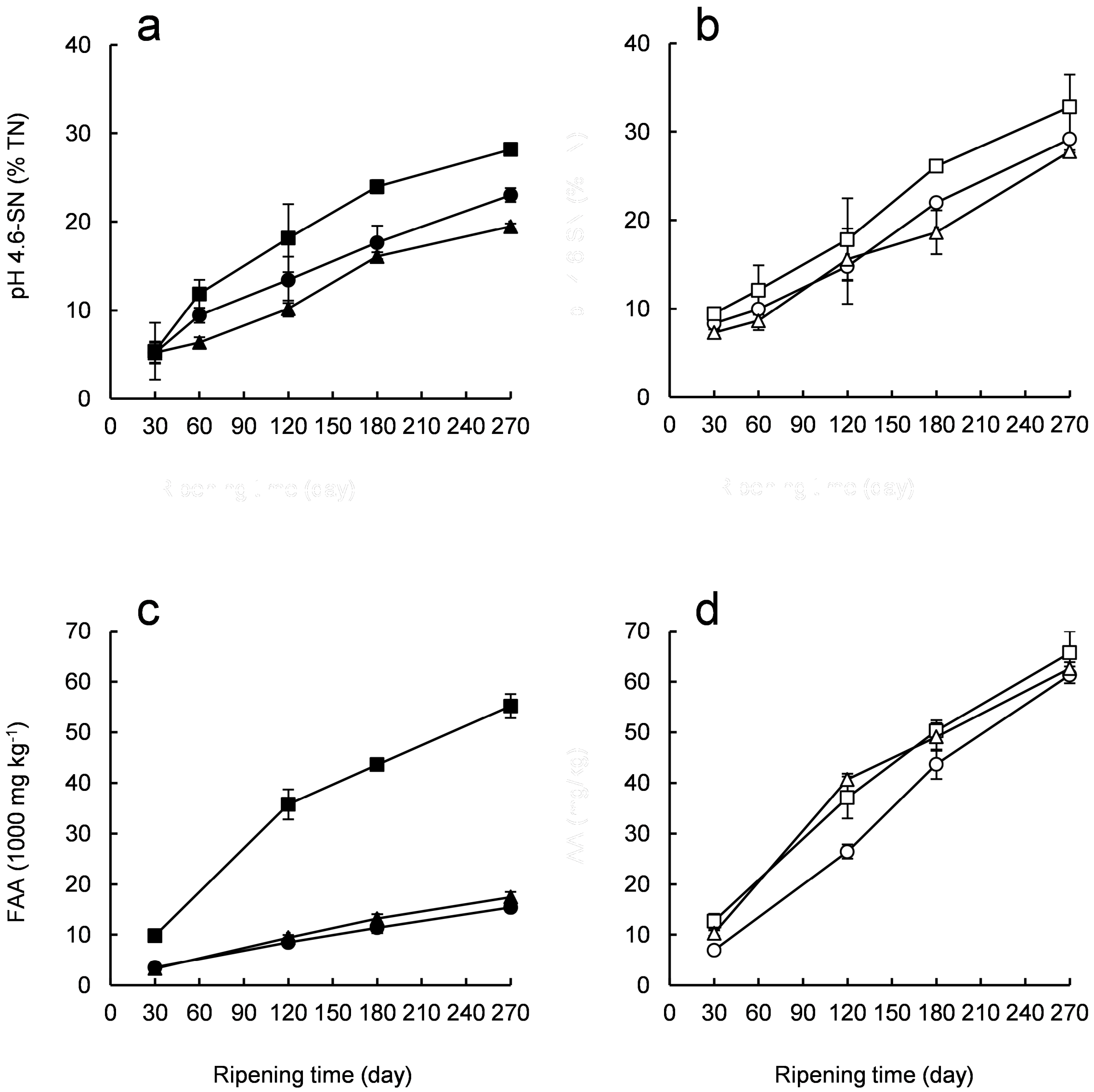
a
pH 4.6-SN (% TN)
40
30
20
10
0
0 30 60 90 120 150 180 210 240 270
b
40
30
20
10
0
0 30 60 90 120 150 180 210 240 270
c
FAA (1000 mg kg-1)
70
60
50
40
30
20
10
0
0 30 60 90 120 150 180 210 240 270
Ripening time (day)
d
70
60
50
40
30
20
10
0
0 30 60 90 120 150 180 210 240 270
Ripening time (day)

Fig. 3

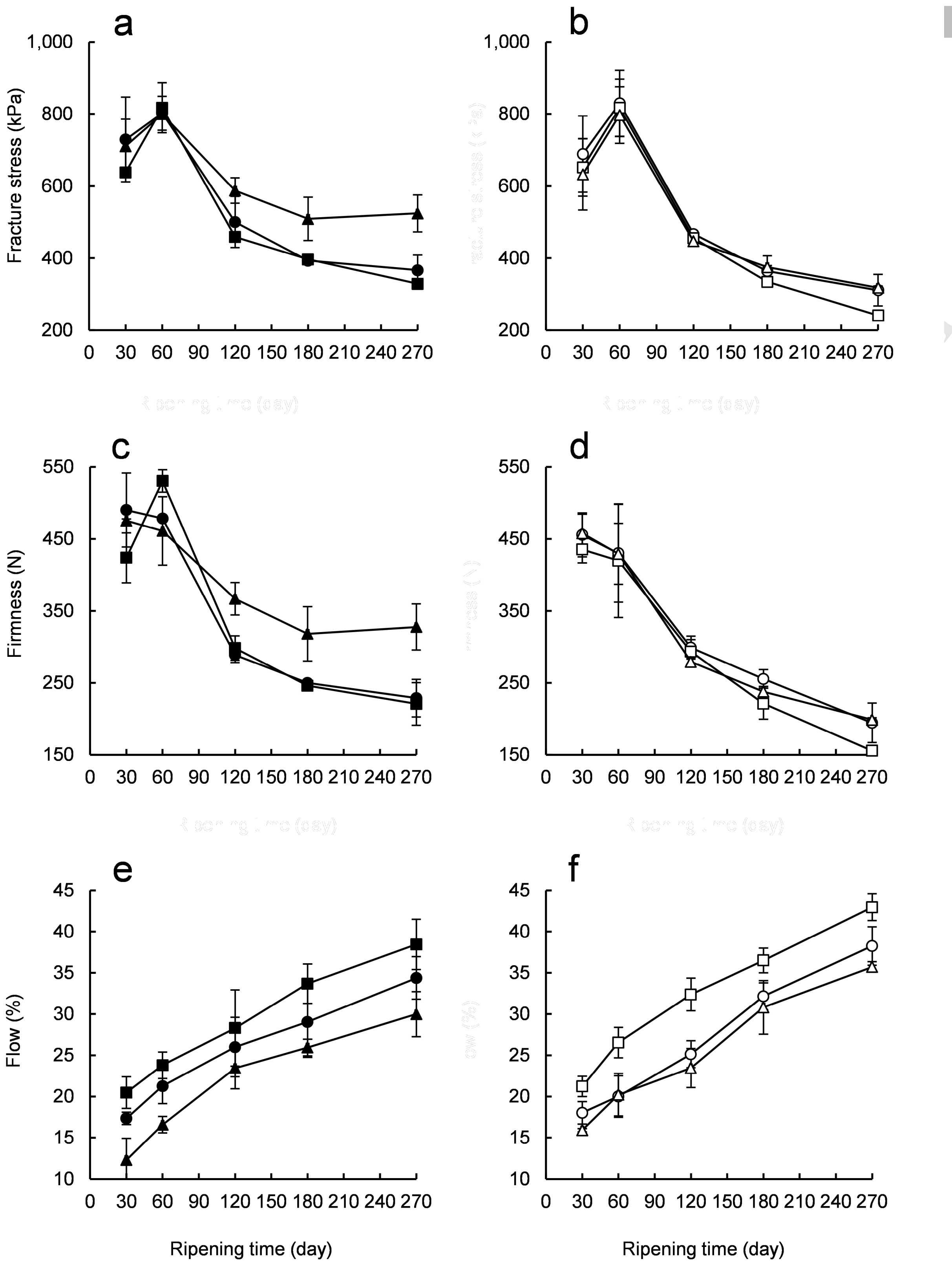
a
b
c
d
e
f
Fracture stress (kPa)
Firmness (N)
Flow (%)
1,000
800
600
400
200
550
450
350
250
150
45
40
35
30
25
20
15
10
0
30
60
90
120
150
180
210
240
270
Ripening time (day)

Fig. 4