Effect of calcium reduction on the properties of half-fat Cheddar-style cheeses with full-salt or half-salt

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Abstract

Standard-calcium (SCa) and reduced-calcium (RCa) half-fat (16%) Cheddar-style cheeses with full-salt (1.9%) or half-salt (0.9%) were made in triplicate, ripened for 270 d, and analysed for composition and changes in lactose metabolism, pH, proteolysis, water-sorption, fracture properties and heat-induced flowability during maturation. The pressing load applied to the moulded cheese was modified to ensure equal moisture in all cheeses despite the differences in salt and calcium levels. The RCa cheeses were characterized by higher primary proteolysis (αs1-casein degradation, pH 4.6-soluble N development), lower secondary proteolysis (concentration of free amino acids), higher water-holding capacity on reducing relative humidity from 85 to 5%, lower fracture stress and strain, and more extensive flow on heating. Overall, the use calcium reduction, when used in conjunction with moisture normalization, proved an effective means of counteracting the adverse effects of fat reduction on texture and cooking properties in half-fat, half-salt cheese.

Key words: Cheddar, moisture normalization, calcium, composition, texture, flowability.
1. Introduction

Health guidelines generally recommend that people in the developed world consume less fat, salt and sugar, as high consumption of these nutrients coincides with increased risk of heart disease, stroke and type 2 diabetes (Mozaffarian, 2016). Consumer research indicates that food labels with ‘reduced-fat’, ‘fat-free’ and ‘reduced-sodium’ are appealing to consumers (Kim, Lopetcharat, Gerard, & Drake, 2012); consequently, many food companies, including cheese manufacturers, are interested in developing reduced-fat and reduced-salt products to increase market share (Pinto et al., 2016).

Fat reduction in cheese is generally associated with a firmer and more rubbery texture, lower opacity, poorer baking/grilling quality (lack of flow, too little free oil, dryness, crusting/puffing), and altered or, sometimes, unacceptable flavour attributes (e.g., umami) (Fenelon & Guinee, 2000; Henneberry, Wilkinson, Kilcawley, Kelly, & Guinee, 2015a; Henneberry et al., 2015b). These effects are attributed to, *inter alia*, alterations in the biochemical changes during maturation (pH, proteolysis, lipolysis), higher concentration of casein, more voluminous and continuous para-casein network, and lower ratios of fat- and moisture-to-protein (Guinee, 2016); it is noteworthy that fat and moisture act as lubricants on fracture surfaces and facilitate displacement within the cheese during deformation (e.g., mastication) and cooking. Reducing salt content is sometimes associated with an increase in cheese moisture, growth of adventitious spoilage bacteria, and production of unwanted flavours e.g., bitterness and sour taste (Ganesan, Brown, Irish, Brothersen, & McMahon, 2014; Murtaza et al., 2014; Rulikowska et al., 2013). The limited literature available on the effects of simultaneous fat and salt reduction in cheese report that reducing both nutrients results in a firmer cheese with an inferior taste and extent of flow when heated (Henneberry et al., 2015a, 2015b; McCarthy, Wilkinson, Kelly, & Guinee, 2015, 2016).
Reducing calcium has been investigated as an approach to improve the texture and functionality of reduced-fat Cheddar and Mozzarella cheese (Henneberry et al., 2015a; Metzger, Barbano, Kindstedt, & Guo, 2001; Sheehan & Guinee, 2004), the hypothesis being that reduction in the degree of calcium-induced cross-linking would mitigate the adverse effect of the higher concentration and volume fraction of the casein network. Calcium reduction can be achieved by lowering the pH at coagulation and/or at whey drainage, either by pre-acidification of the cheesemilk using food grade acids (e.g., lactic acid), CO$_2$ injection, increasing the level of starter culture inoculation, and/or extending the curd-holding time (in the cheese vat) prior to whey drainage (Czulak, Conochie, Sutherland, & Van Leeuwen, 1969; Guinee, Feeney, Auty, & Fox, 2002; Ma and Barbano, 2003; Metzger et al., 2001). Where pre-acidification of cheese milk is used, the moisture content of the resultant reduced-fat cheese tends to increase (Henneberry et al., 2015a; Sheehan & Guinee, 2004; Upreti & Metzger, 2006a), while extending the holding time in the vat is usually coincides with a lower moisture content (Lee, Johnson, & Lucey, 2005; Tunick, Guinee, Van Hekken, & Malin, 2007). The effect of the different methods of calcium reduction on moisture probably relate to their effect on the rate of gel firming during cutting and stirring and, thereby the ability of the curd particles to contract and synerese (Guinee, Pudja & Mulholland, 1994). Hence, lowering calcium tends to result in higher moisture cheese, when undertaken by prolonging time in the cheese vat prior to whey drainage. In reduced-fat, reduced-salt cheese, reducing moisture content is undesirable as it further lowers the moisture-to-protein ratio and exacerbates the texture and cooking defects, while increasing moisture level leads to a further lowering of the salt-in-moisture (S/M) concentration for a given salt level. Hence, for a reduced-fat, reduced-salt cheese, the moisture content would ideally be set at a particular level to optimize the balance of positive (e.g., lowering casein level, higher lubricity) and negative (e.g., reduction in S/M level, low pH, sour taste) effects. Moreover, to ascertain
directly the influence of altering fat and salt on reduced-fat, reduced-salt cheese, it is desirable to minimize the confounding effects of changing moisture content *per se* and its indirect effects on factors such as lactose and pH.

The objective of the current study was to evaluate the influence of calcium reduction on the compositional, biochemical and textural properties of reduced-fat Cheddar cheeses with full (1.9 %) or half (0.9 %) salt, while maintaining equal moisture content in all treatment cheeses.

### 2. Materials and methods

#### 2.1 Cheese manufacture

Previous studies that have shown that in the absence of process intervention moisture content of cheese increases on reducing calcium content, via pre-acidification of cheese milk (Guinee et al., 2002) or reducing salt content (McCarthy et al., 2015). Hence, preliminary trials were undertaken to establish make procedures which gave moisture normalization across cheeses with different salt and calcium levels. Based on these trials, slight changes, as summarized in Table 1, were made to the manufacturing procedures to ensure similar moisture content in all cheeses.

Half-fat (16 % fat) full-salt (1.9 %) and half-salt (0.9 %) Cheddar cheeses with standard- or reduced-calcium content were each prepared from milk which was standardized to a protein-to-fat ratio of 2.65, pasteurized at 72 °C for 15 s, cooled to 31 °C and pumped to the cheese 500L vats. The four different cheeses were coded as follows: half-fat, full-salt, standard-calcium (SCaFS), half-fat, half-salt, standard-calcium (SCaHS), half-fat, full-salt, reduced-calcium (RCaFS), and half-fat, half-salt, reduced-calcium (RCaHS). The control SCaFS cheese was manufactured, as described by McCarthy et al. (2015). Essentially, the milk was inoculated (1.3 %) with a freeze-dried, DVS mesophilic starter culture (R604Y, Chr.
Hansen Ireland Ltd) and ripened at 31 °C for 30 min, inoculated with rennet (Chy-Max® Plus, 320 IMCU mL⁻¹; Chr. Hansen Ireland Ltd, Rohan Industrial Estate, Little Island, Co. Cork, Ireland) at a rate of 0.18 ml L⁻¹. The gel was cut at a firmness of 45 Pa, and the curd whey mixture was cooked to 38 °C at a rate of 0.25 °C min⁻¹. When the pH of the whey expressed from the curd particles reached 6.20, the whey was removed; the curd was recovered in a finishing vat, held at ~ 38 °C until the pH reached 5.55, milled into chips (size ~ 2.8 × 1.25 × 1.25 cm), dry-salted at a level 2.25 % (w/w), mellowed for 20 min while mixing at 5 min intervals, moulded into 20 kg blocks which were pre-pressed for 30 min. Pre-pressing involved an initial pressure of 0.085 kPa, turning the cheeses after 10 min, increasing the pressure to 0.127 kPa, turning after 20 min and increasing the pressure to 0.17 kPa and pressing for a further 10 min; the total pre-pressure loading (pressure x time) was 3.82 kPa min. Following pre-pressing, the cheeses were pressed at a pressure of 2.5 kPa for different times to vary the pressure loading from 326 kPa min for the SCa cheeses to 239 kPa min for the RCa. The pressed cheeses were vacuum wrapped in Cryovac shrink bags (Cryovac Food Packaging Systems, Beech Road, Clondalkin, Dublin 22, Ireland), stored at 4 °C for 30 d, and matured at 8 °C for 8 months.

The manufacture of the reduced-calcium cheeses involved cooling of the pasteurized milk to 29 °C, and pre-acidification of the milk to 5.8 using a 10 % (w/w) lactic acid solution in de-ionised water (Water Technology Ltd, Togher Industrial Estate, Co. Cork, Ireland) while constantly stirring. Otherwise, the differences in manufacture between the SCa and RCa cheeses are summarized in Table 1.

### 2.2 Sampling of cheese

The cheeses were sampled after various times (14, 30, 90, 150, 210 and 270 d) during maturation. 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 hrs.

2.3 Composition analysis of cheese

Cheese samples were grated and analysed in triplicate at 14 d for fat, NaCl, Ca, moisture and protein using standard IDF methods, as described previously by McCarthy et al. (2015).

2.4 Starter and non-starter lactic acid bacteria (NSLAB) counts

Cheeses were analysed in duplicate for counts of starter and NSLAB on Lactose M17 agar (Sigma-Aldrich) and *Lactobacillus* selection agar (LBS) (Sigma-Aldrich), respectively, as described previously by Hou, Hannon, McSweeney, Beresford, and Guinee (2012).

2.5 Lactose and lactate

The lactose and lactic acid concentration was determined according to Rynne, Beresford, Kelly, and Guinee (2007), 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). The lactic acid concentration was calculated as the sum L (+) and D (-) lactic acid.

2.6 Proteolysis

2.6.1 Urea-PAGE

Polyacrylamide gel electrophoresis (PAGE) of the four cheeses was performed at 14, 90, 150 and 270 d using a separating and stacking gel according to the method of Rynne, Beresford, Kelly, and Guinee (2004), using a Protean II xi vertical slab gel unit (Biorad Laboratories Ltd., Watford, Herts. UK). The gels were scanned using Epson Scan software on a dual lens
Epson Perfection V700 (Photo Model J221A) (Epson Deutschland GmbH, Meerbusch, Germany). The areas of the following bands were expressed as a percentage of the total band area: β-casein; αs1-casein; and αs1-casein (f24-199).

2.6.2 Primary proteolysis

The levels of water soluble nitrogen (WSN) and pH 4.6 soluble nitrogen (pH 4.6-SN) were measured as described by Fenelon, O’Connor, and Guinee (2000) after 14, 30, 90, 150, 210 and 270 d.

2.6.3 Secondary proteolysis

The levels of individual free amino acids (FAA) in the pH 4.6-SN extract were determined using high performance cation exchange column with a Jeol JLC-500V AA analyser (Jeol Ltd., Tokyo, Japan), as described by Fenelon and Guinee (2000) at 14, 30, 90, 150, 210 and 270 d.

2.7 Water activity ($a_w$)

Grated cheese (0.5 g sample) was assayed in duplicate at 25 °C on a Novasina LabMaster AW machine (Novatron Scientific Ltd, Horsham, West Sussex, RH12 1AY, UK) at 14, 30, 90, 150, 210 and 270 d, as described previously by McCarthy et al. (2015).

2.8 Dynamic water vapour sorption analysis

The water sorption of the cheese as a function of relatively humidity (RH) was measured gravimetrically using an SPS11 automatic multisample moisture sorption analyser (Project-Messtechnik, DÜlm, Ulm, Germany), as described by McCarthy et al. (2016). The cheese samples (~ 1 g) were equilibrated at 85 % RH for 12 h and the RH was reduced stepwise
from 85 to 5% RH at intervals of 10%. The time at each % RH was 12 h, which allowed stabilization of sample weight loss. The results are expressed as water loss per 100 g cheese as a function of RH and water-to-protein ratio (WPR) of the desorbed cheeses at 5% RH.

2.9 Fracture properties

Six cubes (25 mm$^3$) were cut from each of the four treatment cheeses 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 analyzer (model TA-HDI, Stable Micro Systems, Godalming, UK) equipped with a 5 mm compression plate and fitted with a 100 kg load cell, as previously described by McCarthy et al. (2016). The following rheological parameters were calculated from the resultant force/time curves: firmness ($\sigma_{\text{max}}$), defined as the force at 70% compression; fracture stress ($\sigma_f$), the force at fracture as determined from the inflection point of the force/time curve; and fracture strain ($\varepsilon_f$), the displacement at fracture expressed as a % of original sample height.

2.10 Meltability of the heated cheese

The flowability was assessed using a modification of the Schreiber and Olson-Price methods (Guinee & O’Callaghan, 2013). The former is indicative of the melting behaviour of cheese when exposed as a topping (e.g., open sandwich) during oven heating, and the latter when the cheese is covered, or largely covered, during cooking (e.g., as a cheese slice in hamburger). For the Schreiber method, a disc of cheese, 4.75 cm in diameter and 4.5 mm in height, was placed on a circular glass dish and heated for 4 min at 280 °C in a convection oven (Binder FD 35, Binder GmbH, Tuttingen, Germany). After cooling to room temperature, the diameter of the cooled cheese was measured at four locations, and the flow during heating was defined as the % increase in mean diameter of the cheese disc. For the Olson-Price
A cheese cylinder (~15 g; 22 mm in diameter, 37.5 mm long) was heated in an enclosed tube at 180 °C for 7.5 min and cooled to room temperature; the length of the cheese was measured and the flow on heating was expressed as % increase in length of the cheese cylinder.

2.11 Statistical analysis

Four treatment cheeses (SCaFS, SCAHS, RCaFS and RCaHS) were made on each of three separate occasions. The effect of treatment on cheese composition at 14 d was determined by applying analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute, 2011). For paired comparison of treatment means, Tukey’s multiple-comparison test was used; the level of significance was determined at \( P < 0.05 \).

A split-plot design was used to determine how cheese characteristics (such as the extent of pH 4.6-SN or flow) measured throughout ripening were affected by calcium, ripening time and their interaction in the FS and HS cheeses, and by the effects of salt, ripening time and their interaction in the SCa and RCa cheeses. Analysis for the split-plot design was carried out using the PROC MIXED procedure of SAS 9.3 (SAS Institute, 2011). Tukey’s multiple-comparison test was used to identify the statistically significant differences \( P < 0.05 \) between different treatment levels. Regression analysis, performed using Microsoft Excel 2010 software, was used to investigate potential relationships between some variables (e.g., flowability and intact casein); the significance of correlations was determined by applying Student’s t-test to the correlation coefficient (r), where n is the actual number of data points, and df is the degrees of freedom (n-2).

3. Results

3.1 Cheese composition
The composition of the different cheeses is shown in Table 2. Reducing calcium had little effect, apart from giving a slight, but significant, increase (1 %, w/w) in fat-in-dry-matter (FDM). The higher FDM in the RCa cheese most likely occurred as a result of the small numerical, but insignificant, differences in the levels of fat, moisture and protein between the RCa and SCa cheeses. Reducing salt content led to a lower content of S/M, but otherwise had no effect on composition.

3.2 Starter and NSLAB counts

Starter lactococci count decreased significantly during maturation, from ~ 1 x 10^9 cfu g⁻¹ at 14 d to 3 to 10 x 10^7 cfu g⁻¹ at 270 d. Simultaneously, the population of NSLAB increased, from ~ 1 to 3 x 10^3 cfu g⁻¹ at 14 d to ~ 3 x 10^8 cfu g⁻¹ at 270 d (data not shown). Calcium reduction did not significantly affect the viability of starter lactococci or NSLAB in the FS or HS cheeses, as reflected by the similar magnitude of the mean counts in the SCa or RCa variants of both the FS and HS cheeses over the 270-day ripening period (Table 3). In contrast, reducing salt significantly increased the mean starter viability in the SCa cheese at times ≥ 90 d and the growth of NSLAB in both the SCa and RCa cheese at times ≥ 30 d.

3.3 Changes in lactose and lactate during ripening

The levels of lactose decreased significantly in all cheeses during ripening, from ~ 0.20 % and 0.15 % in the FS and HS cheeses, respectively, at 14 d to ~ 0 % at 150 d (Fig. 1a, 1b). Simultaneously, the concentration of total lactate increased from ~ 1.48 % at 14 d to 1.6 to 1.7 % at 270 d (Fig. 1c, 1d). Reducing calcium content did not affect the concentrations of lactose or total lactate in the FS or HS cheeses (P > 0.05; Table 3). The ratio of L (+) lactate-to-D (-) lactate (L/D lactate) increased significantly in all cheeses during maturation, from
0.05 at 14 d to 0.65 at 270 d, and was not significantly affected by calcium or salt reduction (data not shown).

Reducing salt content resulted in a lower mean level of residual lactose over the 270 d ripening period in both the SCa and RCa cheeses, and a significant increase in the mean lactate content of the RCa cheeses (but not in the SCa cheeses).

3.4 pH

The changes in pH over the 270 d ripening period are shown in Fig. 1e and 1f. The pH of the standard-calcium cheeses (SCaFS, SCaHS) increased significantly by ~ 0.15 to 0.2 pH units, compared to ~ 0.07 in the RCaHS cheese. While the pH of the RCaFS cheese did not change significantly during ripening, it increased initially up to 150 d and, thereafter, decreased slightly but non-significantly. Hence, the mean pH in the SCaFS and SCaHS cheeses over the 270 d ripening period was significantly higher than that of the corresponding RCaFS and RCaHS cheeses, with the difference becoming more pronounced with ripening time (Table 3). The lower pH of the RCa cheeses is consistent with their lower ratio of phosphorous-to-lactate (Table 2), considering that phosphate is a major pH buffering component in cheese (Upreti & Metzger, 2006b).

Reducing the salt content of the SCa cheese did not significantly affect the mean pH over ripening, but resulted in a significantly lower pH in the RCa cheese (Fig. 1e and 1f). The different effect of salt on the pH of the SCa and RCa cheeses may be due to the larger decrease in S/M and phosphorous/lactate ratio on reducing salt in the RCa cheese compared to the SCa cheese.

3.5 Proteolysis

3.5.1 Urea-PAGE
Both $\alpha_s1$- and $\beta$-caseins were increasingly hydrolysed during maturation (Fig. 2a, 2b, 2c), the former into $\alpha_s1$-casein f24-199, f102-199, and f33-*; and the latter, into $\beta$-casein fractions f29-209 ($\gamma_1$), f106-209 ($\gamma_2$) and f108-209 ($\gamma_3$), as identified by Mooney, Fox, Healy, and Leaver (1998). Simultaneously, the concentrations of intact and $\alpha_s1$-and $\beta$-caseins decreased significantly (Table 3).

Reducing calcium content of the FS and HS cheeses accelerated the degradation of $\alpha_s1$-casein, with minimal effect on the hydrolysis of $\beta$-casein. Hence, the concentration of intact $\alpha_s1$-casein in the RCaFS and RCaHS cheeses was significantly lower than that of the corresponding SCaFS and SCaHS at all ripening times (Table 3, Fig. 2a, 2b). The level of $\alpha_s1$-casein f24-199, the primary degradation product of $\alpha_s1$-casein, in the RCaFS and RCaHS cheese was significantly higher than that in the SCaFS and SCaHS cheeses at 14 d, but significantly lower at all other times owing to its degradation into various peptides, including $\alpha_s1$-CN (f102–199), $\alpha_s1$-CN (f121–199), $\alpha_s1$-CN (f33*) and $\alpha_s1$-CN (f99-199) (Mooney et al., 1998). The hydrolysis of $\beta$-casein was minimally affected by reducing calcium content, apart from the occurrence of $\beta$-CN (f1–192), which was found at higher levels in the RCa cheeses at all time points (Fig. 2a, 2c). Compared to calcium, salt content did not significantly influence casein hydrolysis.

### 3.5.2 Soluble N

The levels of pH 4.6-SN, as a % of total N, increased significantly in all cheeses over the 270-day ripening period ($P < 0.05$), from ~ 4 % of total N at 14 d to ~ 15 to 20 % of total N at 270 d (Fig. 3a, 3b).

Consistent with the higher proteolysis of $\alpha_s1$-casein in the RCa cheeses, the mean level of pH 4.6-SN in the RCaFS and RCaHS cheeses over ripening was significantly higher than that the corresponding SCaFS and SCaHS cheeses. Moreover, there was a significant
interaction between calcium content and ripening time with the difference in pH 4.6-SN between the SCaFS and RCaFS cheeses becoming more pronounced as ripening progressed (Table 3). A similar trend was found for WSN, the magnitude of which was on average ~1.25 fold that of pH 4.6-SN for all cheeses (data not shown). In contrast, reducing salt had no significant effect on levels of pH 4.6-SN or WSN in the SCa or RCa cheese.

3.5.3 Free amino acids (FAA)

The concentration of total FAA increased significantly in all cheeses during maturation (Fig. 3c, 3d). In contrast to the trend for pH 4.6-SN, the mean level of FAA in the SCa cheeses (SCaFS, SCaHS) over ripening was significantly higher than that in the RCaFS and RCaHS cheeses. The effect of calcium content was interactive with ripening time, whereby the difference between the SCa and RCa cheeses increased with ripening time, e.g., from ~ 400 mg kg\(^{-1}\) at 14 d to ~ 1500 mg kg\(^{-1}\) at 270 d for the FS cheese (Table 3). The principal FAA in all cheeses were glutamate, leucine, phenylalanine, lysine, cysteic acid and valine. Reducing salt did not significantly affect the levels of FAA in the SCa or RCa cheeses.

3.6 Water activity (\(a_w\))

The \(a_w\) decreased significantly in all cheeses over ripening (data not shown), from ~ 0.955 and 0.961 at 1 d to ~ 0.940 and 0.945 at 270 d, in the FS and HS cheeses respectively. Calcium content did not affect \(a_w\) in either the FS or HS cheeses (Table 4). Owing to the inverse relationship between \(a_w\) and S/M content (Marcos, Alcala, Leon, Fernández-Salgueiro, & Esteban, 1981), the mean \(a_w\) of the HS cheeses (0.95) over ripening was significantly higher than that of the FS cheeses (0.94).

3.7 Dynamic water vapour sorption analysis

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Dynamic water vapour sorption provides a measure of the retention of water by the cheese matrix, and more specifically the water entrapped by the calcium phosphate para-casein network, when the cheese is exposed to drying, for example when lowering the RH or during grilling or baking. Consequently, it provides an index of the water-holding capacity of the cheese, which is an important characteristic affecting the functionality of heated cheese. The water vapour desorption curves on reducing the RH from 85 to 5% are shown in Fig. 4a and 4b for the 270 day-old cheeses from trial 1; similar profiles were obtained at 14, 90 and 150 d (data not shown). The weight loss on desorption decreased significantly in all cheeses during maturation (Fig. 4c, d). This trend concurs with the concurrent increase in protein hydrolysis (Fig. 2 and 3), which involves the splitting of peptide bonds and water uptake, and the reduction in \( a_w \). Consequently, the water-to-protein ratio (WPR) of the desorbed cheese increased significantly (Fig. 4e, f).

The moisture loss during desorption in the FS and HS cheeses increased on reducing calcium reduction (Fig. 4b, c). The WPR at 5% RH, an index of the water-holding capacity of the calcium phosphate para-casein network, increased significantly as calcium content was reduced in the FS and HS cheeses and was significantly affected by the interaction between calcium content and ripening time (Table 4; Fig. 4e, f).

Opposite to the effect of calcium, reducing the salt content of the SCa and RCa cheeses led to a significant reduction in the mean WPR over maturation, with the effect being interactive with ripening time in the case of the RCa cheese where the effect of salt became more pronounced during ripening.

### 3.8 Fracture properties

The \( \sigma_f \) and \( \varepsilon_f \) decreased significantly in all cheeses during maturation (Fig. 5a-d; Table 4); a similar trend was noted for firmness (\( \sigma_{\max} \)) (data not shown). Reducing calcium content had a
significant effect, with the mean $\sigma_f$, $\varepsilon_f$, and $\sigma_{\text{max}}$ of the RCaFS and RCaHS cheeses over ripening being significantly lower than that of the respective SCaFS and SCaHS cheeses.

It is also noteworthy that the values of $\sigma_f$ and $\sigma_{\text{max}}$ of SCaHS and RCaHS cheeses were significantly lower than those of the corresponding SCaFS and RCaFS cheeses, despite their similar moisture content. An opposite trend was noted for $\varepsilon_f$, which was higher in SCaHS than in the SCaFS, but lower in RCaHS than in RCaFS.

3.9 Meltability of the heated cheese

The flowability on heating as measured by the Olson-Price (Fig. 5e, f) and Schreiber (data not shown) methods increased significantly during maturation (Table 4). Reducing calcium content led to a significant increase in the mean flowability over ripening in both the FS and HS cheeses. Similarly, reducing salt coincided with a significant increase in the mean flowability in both the SCa and RCa cheeses.

4. Discussion

The current study investigated the effects of reducing calcium content on the properties of full-salt and half-salt Cheddar cheeses. Four different treatment cheeses, namely standard-calcium, full-salt (SCaFS), reduced-calcium, full-salt (RCaFS), standard-calcium, half-salt (SCaHS) and reduced-calcium, half-salt (RCaHS), were manufactured with mean salt contents of 1.9 or 0.9 %, and calcium levels of ~ 1050 or 900 mg g$^{-1}$. In the absence of process intervention, reduction of calcium content (e.g., by pre-acidification of milk prior to renneting) or salt level is usually associated with higher moisture content (Henneberry et al., 2015a; McCarthy et al., 2015; Rulikowska et al., 2013; Upreti & Mezger, 2006a). This makes it difficult to study the direct effects of calcium or salt reduction on cheese properties; moreover, higher moisture content in reduced-salt cheese leads to a lower content of S/M.
content for a given salt level which can influence factors such as starter culture autolysis and proteolysis during maturation (Møller, Rattray, Høier, & Ardö, 2012; Wilkinson, Guinee, & Fox, 1994). Hence, to avoid the confounding effect of higher moisture content associated with reducing salt level, Møller, Rattray, & Ardö (2013) equalized moisture content (~37.5 %) in Cheddar cheeses with salt levels ranging from 0.9 to 2.3 % by altering the scald temperature (33 to 40.5 °C), scalding time (5 to 45 min) and chip size. However, alteration of cooking conditions may affect the growth, viability and autolysis of starter bacteria depending on the strain used (Husson-Kao, Mengaud, Griponm, Laurent, & Chapot-Chartier, 1999; Wilkinson, Guinee, O’Callaghan, & Fox, 1995); this in turn can influence secondary proteolysis in cheese and flavour development (Husson-Kao et al., 1999; Wilkinson et al., 1995). The current study found that manipulation of pressing load (pressure x time) to be an effective means of achieving moisture normalization in cheeses with different levels of salt and calcium, while allowing the scald temperature to be kept constant across treatments.

Reducing calcium content did not significantly affect the mean levels of lactose or lactic acid, or the pH at 1 d in the FS or HS cheeses, even though it had a significant impact on the extent of pH change during maturation. The pH of the SCa cheeses (SCaFS, SCaHS) cheeses increased significantly by ~0.17 pH units while that of the corresponding RCa cheeses remained relatively constant or increased by ~0.07 pH units. Upreti and Metzger (2007) reported a similar trend in full-fat Cheddar denoted as low lactose (with lactose plus lactic levels of ~1.2 to 1.3 % at 1 d and produced from milk without lactose fortification) and low S/M (4.65 to 4.92). The larger increase in pH and higher mean pH of the SCaFS and SCaHS cheeses during ripening may be attributed to their comparatively higher ratio of phosphorous-to-lactic acid, and hence lower phosphate-based buffering capacity (Czulak et al., 1969; Upreti & Metzger, 2007), and higher concentration of free amino acids (as discussed below).
Upreti and Metzger (2006b, 2007) undertook an extensive study on the buffering capacity and pH changes in Cheddar cheese with varying levels of calcium phosphate, lactose and S/M. The authors concluded that the degree of pH change during maturation is controlled by the balance of the factors which promote either a reduction in pH (i.e., fermentation of lactose to lactic acid) or a resistance to pH change (i.e., buffering capacity which is controlled \textit{inter alia} by the concentration of calcium phosphate and the side-chain of protein-bound glutamate. A number of factors may contribute to the increase in pH during maturation, including the solubilisation of calcium phosphate and protonation of the phosphate anion (Upreti, McKay, & Metzger, 2006b; Upreti & Metzger, 2007). Production of FAA and deamination of basic FAA (Salaün, Mietton, & Gaucherón, 2005) are also likely to contribute to the increase in pH during maturation; the amino groups released on proteolysis have dissociation constants (pKa > ~ 9.0) well in excess of the cheese pH (5.0 to 5.35) and are, thus, likely to become protonated in, and thereby reduce the hydrogen ion activity of, the cheese serum phase of the cheese.

The higher level of primary proteolysis in the RCa cheeses (RCaFS, RCaHS), as reflected by the higher levels of $\alpha_{s1}$-casein degradation and pH 4.6-SN, was most likely associated with higher retention of chymosin in the cheese owing to the lower pH at whey drainage (Bansal, Fox, & McSweeney, 2007; Creamer, Lawrence, & Gilles, 1985; Upreti, Metzger, & Hayes, 2006a). Despite the lack of direct evidence since the level of residual chymosin in the current cheeses was not measured, primary proteolysis is usually associated with hydrolysis by chymosin or plasmin rather than with proteinases or peptidases from starter culture (Milesi, McSweeney, & Hynes, 2008). Another potential factor contributing to the higher primary proteolysis in the RCa cheeses is a greater accessibility of the casein (which is less aggregated owing to the lower density of calcium-mediated crosslinks) to chymosin-induced proteolysis (Fox, 1970). Variation in plasmin activity is unlikely to have...
been a significant factor contributing to the higher primary proteolysis in the RCa cheeses as
the supported by the lack of significant differences between the RCa and SCa cheeses in the
extent of degradation of β-casein (Fig. 2a, c), the preferential casein for plasmin hydrolysis
(Bastian & Brown, 1996). Moreover, studies on plasmin have indicated that plasmin
dissociation from the casein micelle is independent of pH at values > 5 (Grufferty and Fox,
1988) and plasmin activity in experimental cheese did not vary with pH at whey drainage in
the range 6.2 to 6.6 (Farkye & Fox, 1990).

In contrast to the trend for primary proteolysis, the concentration of FAA increased
more rapidly in the SCa cheeses (SCaFS, SCaHS) during maturation, resulting in latter
having significantly higher concentrations than the RCa cheeses at times ≥ 90 d, e.g., ~ 1.4 to
1.5 fold higher at 270 d. FAA are generally considered to accumulate during cheese
maturation as a consequence of the degradation of peptides (e.g., produced by chymosin and
plasmin) by peptidases released during starter cell-die off and autolysis (Wilkinson et al.,
1994). Hence, the relatively high FAA concentration in the SCa cheeses is surprising
considering that starter cell viability decreased at similar rates in the SCaFS and RCaFS
cheeses during maturation. The results suggest that reduction in starter viability in the RCa
cheeses during maturation results, somehow, in a relatively low intra-cellular peptidase
activity (compared to the SCa cheeses), perhaps because of a lower extent of
permeabilization and autolysis, and hence, a lower accessibility of peptides to the
intracellular peptidases (Rice & Bayles, 2008; Sheehan, O’Loughlin, O’Cuinn, FitzGerald, &
Wilkinson, 2005).

Attendant with the changes in proteolysis, the WPR increased in all cheeses with
maturation, with the increase dependent on both calcium and salt levels. The increase in
WPR as calcium content was reduced suggests an enhanced degree of water immobilisation
within the cheese matrix, and concurs with the inverse relationship between casein or para-
casein hydration and calcium content in model systems (protein dispersion) (Creamer, 1985). At the higher calcium content of the SCa cheeses, more numerous calcium-mediated cross-links would be likely to reduce the capacity of the para-casein network to interact with and immobilise water. Hence, Pastorino, Ricks, Hansen, and McMahon (2003) found that injection of Mozzarella cheese with calcium chloride promoted protein interactions, increasing the fusion of the para-casein matrix and decreasing the hydration of the protein network. The reduction in WPR on reducing salt level in the SCa and RCa cheeses is indicative of a concomitant reduction in para-casein hydration. This finding is consistent with the reduction in the level of expressible serum, and the increase in serum-soluble protein, in Mozzarella cheese as the S/M concentration was increased from 0.25 to 3.5 % (w/w) (Guo, Gilmore & Kindstedt, 1997). Similarly, the reduction in WPR is consistent with the decrease in cheese protein solubilisation in model brine solutions as the salt concentration was reduced from 6 to 0% (Everett, Guinee, & Johnson, 2014).

Reducing calcium level resulted in significant reductions in $\delta_f$, $\varepsilon_f$ and $\delta_{\text{max}}$ of the unheated cheese, and an increase in heat-induced flowability. This trend is consistent with the increase in casein hydration and reduction in intact casein (non-hydrolysed casein that is insoluble at pH 4.6), which was found by linear regression to be positively correlated with $\delta_f$, $\varepsilon_f$ and $\delta_{\text{max}}$ and negatively with flowability. Casein is the principal structural component of the cheese matrix that controls the level of deformation (displacement) incurred during compression or on heating (Guinee, 2016). Hence, hydrolysis of the casein comprising the calcium phosphate para-casein network attenuates the structure and its ability to mitigate stress encountered during compression or heat-induced-displacement.

5. Conclusion
The effect of reducing calcium content on the composition, lactose utilization and texture properties of full-salt and half-salt variants of half-fat Cheddar cheeses was investigated. Despite differences in the contents of calcium phosphate and salt, moisture normalization was achieved across all cheese treatments by adjusting pressing load (pressure x time) applied to the moulded cheese. Reducing calcium content from ~ 1060 to 890 mg 100 g$^{-1}$ resulted in a lower phosphate-to-lactic acid ratio, lower pH, more rapid $\alpha_s$-casein degradation, and lower levels of free amino acids. These changes coincided with the reduced-calcium cheeses having lower fracture stress, fracture strain and firmness and higher flowability when melted. Similar trends were found for the effects of reducing calcium in both full-salt and half-salt cheese variants. Hence, reducing calcium would appear to be particularly amenable as an approach to improve the texture and cooking properties of reduced-fat reduced-salt cheeses, especially when used in conjunction with a moisture normalization manufacturing protocol to avoid the negative effect of higher moisture level in cheese with reduced salt content.

Acknowledgements

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References


**Figure legends**

**Figure 1.** Changes in concentrations of lactose (a, b), total lactate (c, d) and pH (e, f) in half-fat Cheddar-style cheeses with different calcium and salt levels during ripening: standard-calcium, full-salt (SCaFS, ⬤); reduced-calcium, full-salt (RCaFS, ○); standard-calcium, half-salt (SCaHS, □); and reduced-calcium, half-salt (RCaHS, □). Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.
Figure 2. (a) Urea-polyacrylamide gel electrophoretogram of half-fat Cheddar-style cheeses varying in calcium and salt content after 14, 90, 150 or 270 d maturation: standard-calcium, full-salt (SCaFS, ●); reduced-calcium, full-salt (RCaFS, ○); standard-calcium, half-salt (SCaHS, ■); and reduced-calcium, half-salt (RCaHS, □). The cheeses were loaded with a fixed weight of protein (4.25 mg protein per lane); sodium caseinate (NaCn) was also loaded at 4.25 mg protein per lane as an unhydrolyzed casein control. Protein bands were identified according to Mooney et al. (1998): 1, β-CN (f106-209) (γ2); 2, β-CN (f29-209) (γ1); 3, β-CN f108-209 (γ3); 4, β-CN; 5, β-CN (f1-192); 6, αs1-CN; 7, αs1-CN (f102-199); 8, αs1-CN (f24-199); 9, αs1-CN (f121-199); 10, αs1-CN (f33-*), and (b, c) Corresponding changes in concentrations of αs1- and β-caseins, measured by densitometry measurements on the gel electrophoretogram. Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.

Figure 3. Changes in levels of pH 4.6 soluble-nitrogen (pH4.6-SN; a, b) and free amino acids (FAA; c, d) in half-fat Cheddar-style cheeses varying in calcium and salt content during maturation: standard-calcium, full-salt (SCaFS, ●); reduced-calcium, full-salt (RCaFS, ○); standard-calcium, half-salt (SCaHS, ■); and reduced-calcium, half-salt (RCaHS, □). Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.

Figure 4. Changes in water sorption characteristics of half-fat Cheddar-style cheeses with different calcium and salt levels on lowering the relative humidity (RH) from 85 to 5%; (a, b) moisture in 270 day-old cheeses during desorption, expressed as g 100 g⁻¹ (solid line) or g g⁻¹ protein (broken line); (c, d) total weight lost during desorption as a function of ripening time;
and (e, f) water-to-protein ratio (WPR) following desorption as a function of ripening time.

Cheeses: standard-calcium, full-salt (SCaFS, ●); reduced-calcium, full-salt (RCaFS, ○); standard-calcium, half-salt (SCaHS, ■); and reduced-calcium, half-salt (RCaHS, □).

Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.

Figure 5. Changes in fracture stress ($\delta_f$: a, b), fracture strain ($\varepsilon_f$: c, d), and flow (e, f) on heating half-fat Cheddar-style cheeses with different calcium and salt levels during ripening:

standard-calcium, full-salt (SCaFS, ●); reduced-calcium, full-salt (RCaFS, ○); standard-calcium, half-salt (SCaHS, ■); and reduced-calcium, half-salt (RCaHS, □). Presented values are the means of three replicate trials; error bars represent standard deviations of the mean.
Table 1

Treatments and details of manufacturing process of experimental half-fat Cheddar-style cheese. \(^a\)

<table>
<thead>
<tr>
<th>Parameters</th>
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<td>pH at set</td>
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</tr>
<tr>
<td>Whey drainage</td>
<td>6.20(^a)</td>
</tr>
<tr>
<td>Milling</td>
<td>5.55(^a)</td>
</tr>
<tr>
<td>pH at set</td>
<td>RCaFS</td>
</tr>
<tr>
<td>Whey drainage</td>
<td>5.50(^b)</td>
</tr>
<tr>
<td>Milling</td>
<td>5.35(^b)</td>
</tr>
<tr>
<td>pH at set</td>
<td>SCaHS</td>
</tr>
<tr>
<td>Whey drainage</td>
<td>6.20(^a)</td>
</tr>
<tr>
<td>Milling</td>
<td>5.35(^b)</td>
</tr>
<tr>
<td>pH at set</td>
<td>RCaHS</td>
</tr>
<tr>
<td>Whey drainage</td>
<td>5.50(^b)</td>
</tr>
<tr>
<td>Milling</td>
<td>5.35(^b)</td>
</tr>
</tbody>
</table>

Acidification procedure

| pH adjustment before culture addition            | -           |
| Ripening culture type                           | R604Y       |
| Ripening time (min)                             | 30\(^a\)    |

Details of cheesemaking steps

| Protein-to-fat ratio of the milk                | 2.60\(^a\)  |
| Temperature of milk on adding acid (°C)         | -           |
| Set temperature (°C)                            | 31\(^a\)    |
| Set firmness at cut (Pa)                        | 45\(^a\)    |
| Stirring mode                                   | continuous  |
| Temperature at scald (°C)                       | 38\(^a\)    |
| pH at milling                                   | 5.55\(^a\)  |
| Salt added (% w/w)                              | 2.25\(^a\)  |
| Mellow time (min)                               | 20\(^a\)    |
| Pre-pressing load (kPa min)                     | 3.81\(^a\)  |
| Pressing load (kPa min)                         | 326\(^a\)   |

Time of cheesemaking stages (min)

| Curd residence (from cut to whey drainage)      | 163\(^a\)   |
| Cheddaring (from whey drainage to milling)      | 74\(^b\)    |
| Total make time (from starter addition to milling) | 290\(^a\) |

\(^a\) Cheese codes: SCaFS and SCaHS refer to the full-salt (FS) and half-salt (HS) variants of standard-calcium (SCa) cheese; the corresponding variants for reduced-calcium (RCa) are similarly denoted (see Materials and methods). Starter culture type R604Y consisted of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. Values within a row not sharing a common lower-case superscript letter differ significantly (\(P < 0.05\)).
Table 2

Effect of calcium reduction on the compositional parameters and pH of 14 day-old half-fat Cheddar-style cheeses. 

<table>
<thead>
<tr>
<th>Compositional factors</th>
<th>Cheese code</th>
<th></th>
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<td></td>
<td>SCaFS</td>
<td>RCaFS</td>
<td>SCaHS</td>
<td>RCaHS</td>
</tr>
<tr>
<td>Moisture (%) (w/w)</td>
<td>44.7&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>44.8&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>44.7&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>44.9&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%) (w/w)</td>
<td>33.9&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>33.8&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>34.4&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>33.7&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%) (w/w)</td>
<td>15.3&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>15.6&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>15.3&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>15.6&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaCl (%) (w/w)</td>
<td>1.83&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>1.84&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>0.94&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;aB&lt;/sup&gt;</td>
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<tr>
<td>MNFS (%) (w/w)</td>
<td>52.8&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>53.1&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>52.6&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>54.1&lt;sup&gt;aA&lt;/sup&gt;</td>
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<td>FDM (%) (w/w)</td>
<td>27.6&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>28.3&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>27.6&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>28.7&lt;sup&gt;aA&lt;/sup&gt;</td>
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<td>S/M (%) (w/w)</td>
<td>4.1&lt;sup&gt;aA&lt;/sup&gt;</td>
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<td>2.1&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;aB&lt;/sup&gt;</td>
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<td>Calcium (mg 100g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1053&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>878&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>1068&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>907&lt;sup&gt;bA&lt;/sup&gt;</td>
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<td>Calcium/protein (mg g&lt;sup&gt;-1&lt;/sup&gt; protein)</td>
<td>31.1&lt;sup&gt;aA&lt;/sup&gt;</td>
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<td>Phosphorus (mg 100g&lt;sup&gt;-1&lt;/sup&gt;)</td>
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<td>Phosphorus/protein (mg g&lt;sup&gt;-1&lt;/sup&gt; protein)</td>
<td>21.4&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>16.2&lt;sup&gt;bA&lt;/sup&gt;</td>
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<td>5.20&lt;sup&gt;aA&lt;/sup&gt;</td>
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<td>5.09&lt;sup&gt;aB&lt;/sup&gt;</td>
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Cheese codes: SCaFS and SCaHS refer to the full-salt (FS) and half-salt (HS) variants of standard-calcium (SCa) cheese; the corresponding variants for reduced-calcium (RCa) are similarly denoted (see Materials and methods). Abbreviations: MNFS, moisture-in-non-fat substances; FDM, fat-in-dry-matter; S/M, salt-in-moisture. Data are the mean values of three replicate trials; values within a row relating to the FS or HS cheeses, not sharing a common lower-case superscript letter differ significantly ($P < 0.05$), values within a row relating to the SCa or RCa cheeses, not sharing a common upper-case superscripted letter differ significantly ($P < 0.05$).
Table 3

Statistical significances (P-values) for changes in microbiology, lactose metabolism, pH, primary and secondary proteolysis in full-salt and half-salt half-fat Cheddar-style cheeses with different calcium levels. "

<table>
<thead>
<tr>
<th>Factor</th>
<th>Starter</th>
<th>NSLAB</th>
<th>Lactose</th>
<th>Total lactate</th>
<th>pH</th>
<th>α_\text{S1}-casein (% TC)</th>
<th>β-casein (% TC)</th>
<th>pH 4.6-SN (% TN)</th>
<th>FAA</th>
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<td>-</td>
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</tbody>
</table>

| Half-salt cheese              |         |       |         |               |    |                            |                 |                 |     |
| Main plot                     |         |       |         |               |    |                            |                 |                 |     |
| Calcium content (CC)          | -       | -     | -       | **            | ***| ***                        | -               | *               | *   |
| Sub-plot                      |         |       |         |               |    |                            |                 |                 |     |
| Ripening time (RT)            | ***     | ***   | ***     | ***           | ***| ***                        | ***             | ***             | *** |
| Interaction (CC × RT)         | -       | -     | -       | ***           |    | -                          | -               | ***             | *** |

Differences in contents of calcium and salt are given Table 1. Abbreviations: NSLAB, non-starter lactic acid bacteria; TC, total casein; pH 4.6-SN, pH 4.6-soluble N expressed as % total N; FAA, free amino acids. There were 1 degrees of freedom (df) for calcium content; 5 df for ripening time except in the case of starter and NSLAB where there were 4 df; and 5 df for interaction of calcium and ripening time except in the case of starter and NSLAB where they were 4 df. The significance for changes in the studied parameters are presented as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Table 4

Statistical significances (P-values) for changes in $a_w$, WPR, fracture properties and flow in full-salt and half-salt half-fat Cheddar-style cheeses with different calcium levels. a

<table>
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<th>Factor</th>
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<th>WPR (5 % RH)</th>
<th>Fracture stress</th>
<th>Firmness</th>
<th>Fracture strain</th>
<th>Flow-Sch</th>
<th>Flow-PO</th>
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<tr>
<td>Calcium content (CC)</td>
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<td>Ripening time (RT)</td>
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<tr>
<td>Interaction (CC × RT)</td>
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<td>Sub-plot</td>
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<td>Ripening time (RT)</td>
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<tr>
<td>Interaction (CC × RT)</td>
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</table>

a Differences in contents of calcium and salt are given Table 1. Abbreviations: $a_w$, water activity; WPR, water-to-protein ratio at 5 % relative humidity; Flow-Sch, flowability determined using the Schreiber method; Flow-PO, flowability determined using the Price-Olson method. There were 1 degrees of freedom (df) for calcium content; 5 df for ripening time, except in the case of WPR where there were 4 df; and 5 df for interaction of calcium and ripening time, except in the case of WPR where there were 4 df. The significance for changes in the studied parameters are presented as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. 
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.