



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 characterised by higher primary proteolysis (α_{S1} -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, calcium reduction, when used in conjunction with moisture normalisation, proved an effective means of counteracting the adverse effects of fat reduction on texture and cooking properties in half-fat, half-salt cheese.

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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 et al., 2015a; Henneberry, Wilkinson, Kilcawley, Kelly, & Guinee, 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, Brotherson, & 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,b; 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., 2015b; 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 cheese milk using food grade acids (e.g., lactic acid), CO₂ 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 & 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., 2015b; Sheehan & Guinee, 2004; Upreti & Metzger, 2006a), while extending the holding time in the vat usually coincides with a lower moisture content (Lee, Johnson, & Lucey, 2005; Tunick, Guinee, Van Hekken, & Malin, 2007).

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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 optimise 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 minimise 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 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 that gave moisture normalisation across cheeses with different salt and calcium levels. Based on these trials, slight changes, as summarised 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 that 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 500 L cheese vats. The four different cheeses were coded as follows: SCaFS, full-salt, standard-calcium; SCaHS, half-salt, standard-calcium; RCaFS, full-salt, reduced-calcium; RCaHS, half-salt, reduced-calcium. 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, Rohan Industrial Estate, Little Island, Co. Cork, Ireland) and ripened at 31 °C for 30 min, inoculated with rennet (Chy-Max[®] Plus, ~200 IMCU mL⁻¹; Chr. Hansen 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 of 2.25% (w/w), mellowed for 20 min while mixing at 5 min intervals, moulded into 20 kg blocks that 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 × 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 vacuumed 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 RCa cheeses involved cooling of the pasteurised 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 summarised 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 h.

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 of L (+) and D (–) lactic acid.

2.6. Proteolysis

2.6.1. Urea–polyacrylamide gel electrophoresis

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; α_{S1} -casein; and α_{S1} -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 (FAAs) 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

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