Effects of reducing fat and salt on the composition, biochemical, sensory, functional and rheological properties of Mozzarella-style cheese

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ABSTRACT

Studies on the quality and improvement of reduced-fat, reduced-salt Mozzarella-style cheese

The aims of this thesis were to (a) study the properties of Low Moisture Part-Skim (LMPS) Mozzarella; (b) investigate the effects of 40% fat and 30% salt reduction on the properties of LMPS Mozzarella; (c) improve the functional characteristics of reduced-fat, reduced-salt Mozzarella through reducing the degree of calcium-induced casein cross-linking; (d) investigate the impact of fat, salt and calcium reduction on the volatile and sensory properties of LMPS Mozzarella.

Initially, four cheeses investigating the various combinations of fat and salt content; full-fat, full-salt (FFFS); full-fat, reduced-salt (FFRS); reduced-fat, full-salt (RFFS); reduced-fat, reduced-salt (RFRS) were produced. Subsequently, an additional two cheeses investigating the effect of calcium reduction in reduced-fat cheese; reduced-fat, full-salt, low calcium (RFRSLC) and reduced-fat, reduced-salt, low calcium (RFRSLC) were studied. Cheeses were analysed for their composition, biochemical, functional and sensory properties. Results indicated that reducing the fat content of Mozzarella impacted negatively on the functionality and sensory properties of the cheese, while the magnitude of the effect of salt reduction was relatively minor in comparison to the effect of fat, nevertheless, resulted in a reduction in cheese firmness and chewiness. Reducing the calcium content of reduced-fat Mozzarella counteracted the negative impact of fat reduction; however, the resultant cheese was still inferior to full-fat Mozzarella, primarily due to deficits in both the flavour profile and functional properties. This study highlights the properties of highest importance for pizza cheese, the impact of fat, salt and calcium reduction and the impact of heating on the sensory properties.

Publications

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Chapter 1: Literature Review

1. *Pasta-filata* cheeses

*Pasta-filata* cheeses are a group of cheeses originating from the northern Mediterranean region (Italy, Greece, Turkey, the Balkans) characterised by their particular physical attributes that arise from their unique manufacturing method (McMahon and Oberg, 2017). The term *pasta-filata* translates to ‘stretched curd’ and is indicative of the nature of the procedure used in the manufacture of cheeses such as Mozzarella, Provolone and Kashkaval. *Pasta-filata* cheeses are traditionally produced from goat, water-buffalo, cow and sheep milk and are produced in a distinctive manner whereby prior to moulding the cheese is subjected to a stretching step in a hot water or dilute brine solution (McMahon and Oberg, 2017). This process results in the elongation and re-ordering of the protein fibres into a parallel orientation giving these cheeses their characteristic functional properties, e.g., heat-induced-flow and stringiness. During stretching, the curd is exposed to water at ~77 °C until it reaches an internal temperature of ~57 °C; protein bundles are reoriented due to the kneading action causing fat globules to pool together. Exposure of curd to high temperatures results in the inactivation of chymosin and starter cultures (McMahon, 2016). Other cheese constituents such as fat, water, calcium and dissolved substances e.g., salt, orientate themselves around the newly ordered protein matrix.

2. Mozzarella cheese

Mozzarella is a fresh cheese consumed within a short timeframe following manufacture, typically after a few weeks (McMahon, 2016). Therefore, it does not undergo the extended maturation process of other cheeses, such as Cheddar, which possess a strong flavour profile.
resulting from the extensive biochemical reactions, e.g., glycolysis, proteolysis and lipolysis, which occur during extended maturation.

Mozzarella cheese, as defined by the FDA Code of Federal Regulations (CFR, 2018), contains a minimum of 45 % milk fat-in-dry-matter, and a moisture content between 52 and 60 %. Codex Alimentarius (FAO/WHO, 2011) gives further guidelines for the fat-in-dry-matter content of both high and low moisture Mozzarella (Table 2). This cheese is typically consumed as a table cheese or as an ingredient in composite foods such as pizza. However, there are many variants of Mozzarella, each with their own specific end uses; including low-moisture (LM) Mozzarella, part skim (PS) and low-moisture part skim (LMPS) Mozzarella. The compositional parameters for the various types of Mozzarella are summarised in Table 1 & 2.

LM Mozzarella contains a minimum of 45 % milk fat-in-dry-matter with a moisture content between 45 and 52 %. PS Mozzarella contains between 30 to 45 % milk fat-in-dry-matter with between 52 to 60 % moisture. LMPS Mozzarella is not typically consumed as a table cheese, but rather is used as an ingredient in foods such as pizza. This cheese typically contains between 30 to 45 % milk fat-in-dry-matter and 45 and 52 % moisture.
Table 2. Compositional parameters of Mozzarella cheese as defined by Codex Alimentarius*

<table>
<thead>
<tr>
<th>Fat in Dry Matter (%, w/w)</th>
<th>High-Moisture Mozzarella Max moisture content (%, w/w)</th>
<th>Low-Moisture Mozzarella Max moisture content (%, w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18≤30</td>
<td>-</td>
<td>66</td>
</tr>
<tr>
<td>30≤40</td>
<td>76</td>
<td>61</td>
</tr>
<tr>
<td>40≤45</td>
<td>71</td>
<td>58</td>
</tr>
<tr>
<td>45≤50</td>
<td>69</td>
<td>55</td>
</tr>
<tr>
<td>50≤60</td>
<td>66</td>
<td>53</td>
</tr>
<tr>
<td>60≤85</td>
<td>62</td>
<td>47</td>
</tr>
</tbody>
</table>


There are no specifications for the salt content of Mozzarella; however, analysis of commercially available LMPS Mozzarella in the USA revealed the average sodium content of the 746 samples analysed was 666mg/100g cheese (Agarwal et al., 2011). Moreover, the EU has published legislation regarding the use of the term ‘reduced in’; defining it as “any claim likely to have the same meaning for the consumer, may only be made where the reduction in content is at least 30 % compared to a similar product, except for micronutrients where a 10 % difference in the reference values as set in Council Directive 90/496/EEC shall be acceptable and for sodium, or the equivalent value for salt, where a 25 % difference shall be acceptable” (EU Regulation (EC) No 1924/2006).

2.1. Production volumes and trends in consumption of Mozzarella

Total production of Mozzarella in the US in 2017 exceeded 1.9 billion kg (Statista, 2018a) and in 2014 sales of pizza were estimated at 38 billion USD (Hynum, 2014). By comparison, production volumes of Cheddar cheese in the US in 2017 reached 1.7 billion kg (Statista, 2018c). The per capita consumption of Mozzarella in the US has increased from 4.11
kg in 2000 to 5.11 kg in 2015 (Statista 2018b), demonstrating the ever-increasing popularity of Mozzarella.

### 2.2. Major uses

Traditional Mozzarella is commonly used as a table cheese; however, other variants such as LM, PS and LMPS Mozzarella are more typically used as ingredients in composite foods, such as pizza and lasagne with the production of pizza cheese now exceeding that of all other pasta-filata cheeses (McMahon and Oberg, 2017). In its 2013 report, the USDA described pizza as a ‘key food’ in the American diet where it was found to be a significant contributor of 14 nutrients, including fat, calcium and sodium (Nickle and Pehrsson, 2013). Pizza is thought to contribute ~ 6.3% of the sodium and 5.9% saturated fat in the average US diet (USDA, 2006) and an overconsumption of sodium and fat is associated with an increased risk of hypertension, stroke and heart disease (He and MacGregor 2009; Mosfegh et al., 2012).

### 2.3. Plasticization

The term ‘pasta-filata’ is of Italian origin and translates to ‘stretched curd’, referring to a unique processing step these cheeses undergo as part of their manufacture. Fig. 2 shows the typical make procedure for pasta-filata cheese. This step traditionally involves kneading and stretching the acidified curd in hot water or dilute brine using wooden tools resulting in reordering of the protein fibres. However, in modern manufacturing, the process takes place in a cooker/stretcher.

Fat losses during the wet stretching step can affect both cheese yield as well as the composition of the resultant cheese. Losses of up to 30% of fat can occur during the process (Rehman et al.,
Cheesemilk can be standardised to counteract these losses by altering the ratios of protein to fat content to ensure that the resultant cheese has the desired fat content. The make procedure of a pasta-filata cheese can be similar to that of Cheddar up until the cheddaring and salting stage. For LMPS Mozzarella curd is cheddared, salted and mellowed similarly to Cheddar cheese; however, following this step, the curd is moved to a cooker/stretching for the stretching or plasticization step. Prior to plasticization, a curd pH of between 5.2-5.3 is typically targeted in order to ensure the optimum casein-associated calcium content during the process (McMahon and Oberg, 2017). However, if cheesemilk is pre-acidified in substitution for, or in addition to, the use of starter cultures, a higher pH (5.6-5.7) at plasticization is acceptable. Typically, curd with a casein-associated calcium content of 25-28 mg/g protein and pH value of ~ 5.1-5.3 at plasticization ensures that the resultant LMPS Mozzarella has acceptable functionality (flow and stretchability on baking) after 1-2 weeks storage at 4-8 °C. Calcium governs the ability of the curd to stretch and plasticize in hot water (McMahon, 2016). Curd that contains too much calcium will fail to form a smooth, pliable consistency during plasticization. Conversely, curd containing too little calcium will become overly soft and fluid-like (McMahon and Oberg, 2017). Large scale stretching typically consists of a two-step process. Prior to stretching, the microstructure of the salted curd consists of a protein matrix containing random pockets of serum and fat globules. The curd is immersed in hot water (65-70 °C) in a single or twin-screw auger where it is heated to ~55 °C. At this point it is kneaded and stretched by the augers until the casein matrix becomes re-ordered resulting in protein fibres becoming elongated, with serum channels and fat globules orientating themselves alongside protein fibres (McMahon, 2016); see section ‘Microstructure characterization’ below for further details. Throughout the stretching process the curd is forced through the stretcher by the augers and cheese exits the cooker/stretching typically between 55-65 °C as a smooth cohesive mass and is moulded into blocks and cooled.
2.4. *Microstructure characterization*

Cheese microstructure consists of a three-dimensional gel matrix composed of interconnecting and overlapping *para*-casein strands which encase fat globules, moisture, dissolved substances, microorganisms and enzymes from various sources (e.g., residual coagulant, proteinases, peptidases and lipases of starter and NSLAB) (Everett and Auty, 2017). The microstructure of Mozzarella cheese differs from that of a typical cheese in that it consists of parallel protein fibres separated by long channels of accumulated fat globules and free serum (McMahon and Oberg, 2011). This is as a result of the plasticization step during manufacture where curd is exposed to high temperatures and mechanical kneading and stretching, resulting in the realignment of protein fibres into parallel columns surrounded by serum channels and fat globules.

The protein matrix is held in place through calcium-induced cross-linking and casein-casein interaction, creating rigidity and structure in the matrix (Guinee, 2016a), while the serum channels and fat globules disrupt the casein-casein interactions (Everett and Auty, 2017). The continuity of the cheese matrix is interrupted by curd junctions, on a macrostructural level, and through hydrolysis of the *para*-casein network on a microstructural level (Guinee, 2003). During ripening, cheese microstructure undergoes changes, the extent of which is determined by numerous factors including levels of residual chymosin, microorganisms and their enzymes and alterations in the equilibrium of minerals between the serum and *para*-casein matrix (Fox et al., 2000).

Age-related protein hydration results in the absorption of serum from serum channels into the protein fibres, causing the protein matrix to swell and encase fat, resulting in coalescence and clumping of fat globules (McMahon and Oberg, 2017). Fat globules occupy the space around the protein fibres and help to limit the degree of aggregation of the *para*-casein micelles and disrupting the continuity of the protein matrix, while the globules themselves exist in
varying degrees of size and coalescence throughout the matrix (McMahon and Oberg, 2017). The resultant structure exhibits a striated appearance with elongated protein fibres running in parallel orientation with occluded fat globules and serum channels dispersed throughout the matrix. Upon reducing the fat content, the volume fraction of the para-casein network is increased (Guinee, 2015), serum channels become smaller and the structure of the casein network becomes more rigid. Detailed study of cheese microstructure can be undertaken through utilisation of various microscopic techniques, including, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), cryo-SEM, and transmission electron microscopy (TEM). Age-related changes in cheese microstructure have been shown to influence various properties of the cheese, e.g., texture, flavour, heat-induced functionality and rheological properties (Guinee et al., 2002; Brickley et al., 2007). An increased volume fraction of the casein network is associated with cheese that is firmer and has a poor heat-induced-flow; however, as cheese ripens proteolysis occurs, resulting in the breakdown of the casein network resulting in an improved heat-induced flow and lower firmness (McCarthy et al., 2016; Guinee, 2016b).

2.5. Functional requirements of Mozzarella cheese

LMPS Mozzarella is known for its elastic texture and its ability to melt and flow evenly upon cooking. A list of the key functional properties of unheated and heated Mozzarella is given in Table 3 below.

In the unheated state, pizza cheese must be firm enough to allow shredding without clumping or balling. Excessively soft Mozzarella can result in the formation of balls upon shredding, leading to damage of machinery and interruption to processing (Guinee, 2016b). The ability to stretch and form strings indicates the stringiness of the cheese; however, a high resistance to stretching indicates a tough, elastic cheese, while a low level of resistance to
stretching indicates cheese of a more fluid nature. Oiling off describes the release of fat from the cheese matrix upon heating that spreads across the cheese surface, offering a degree of protection to the cheese surface during cooking, preventing dehydration and scorching of the cheese surface (Rudan et al., 1999). However, excessive oiling-off during cooking can result in a cheese that has pools of surface fat and is undesirable in appearance to the consumer.

Table 3. Functional properties of Mozzarella cheese.*

<table>
<thead>
<tr>
<th>Functional Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unheated Cheese</strong></td>
<td></td>
</tr>
<tr>
<td>Shreddability</td>
<td>Ability to cut cleanly into shreds of uniform dimensions (typically cylindrically shaped; 2.5cm long) that are resistant to fracture (forming curd dust), sticking, matting or clumping when stored in bins or retail packs</td>
</tr>
<tr>
<td>Sliceability</td>
<td>Ability to be cut cleanly into thin slices without fracturing</td>
</tr>
<tr>
<td>Grate-ability</td>
<td>Ability to fracture easily into small hard particles, that flow freely and resist matting during shearing, crushing, fluidization or piling</td>
</tr>
<tr>
<td>Spreadability</td>
<td>Ability to spread easily when sheared</td>
</tr>
<tr>
<td>Crumbliness</td>
<td>Ability of cheese to fracture (break) easily into small irregularly shaped pieces, for example when rubbed between fingers</td>
</tr>
<tr>
<td><strong>Heated Cheese</strong></td>
<td></td>
</tr>
<tr>
<td>Meltability</td>
<td>Ability to soften on heating</td>
</tr>
<tr>
<td>Flowability</td>
<td>Ability of cheese (shredded, grated, or sliced) to spread or flow on heating</td>
</tr>
<tr>
<td>Stretchability or stringiness</td>
<td>Ability of cheese to form strings and/or sheets when extended</td>
</tr>
<tr>
<td>Oiling-off</td>
<td>Ability of cheese to express a moderate level of free oil on heating, to impart gloss and succulence to the molten cheese mass</td>
</tr>
<tr>
<td>Fluidity</td>
<td>Ability of cheese to attain desired fluidity on heating, and not to congeal too rapidly on cooling</td>
</tr>
<tr>
<td>Blistering</td>
<td>Ability of cheese to display a moderate to low level of blistering in some applications</td>
</tr>
</tbody>
</table>

*Taken from Guinee (2016b).
3. **Age-Related Changes in Mozzarella Cheese**

3.1. *Composition*

Composition has been found to have a significant impact on the physical properties of cheese; the fat, moisture, salt, protein and ratio of insoluble to soluble calcium content all affect the degree of hydration, lubrication and density of the protein matrix. The effect of fat reduction on the composition, biochemistry and functional properties of Mozzarella is covered in Section 4.1. The effect of salt reduction is covered in more detail in Sections 5.1 and 5.2.

The casein network hydrates over time, i.e., it absorbs free serum from the serum channels, resulting in swelling of the protein fibers. This hydration of the casein network increases with ripening time and is associated with a reduction in cheese hardness, increased flowability and stretchability on cooking in part due to its lubricating effect (Guinee et al., 2002). Increased levels of protein hydration result in greater casein-water interactions and reductions in casein-casein interactions. This imparts improved cooking properties, as casein aggregates flow more easily (Joshi, 2003). Calcium content, or more accurately, casein bound calcium-phosphate, affects protein hydration. Guinee et al. (2002) found that protein hydration increased as the level of casein-bound calcium decreased. Calcium lends structure and rigidity to the protein matrix in the form of calcium-induced casein cross-linking (Carr et al., 2002; McCarthy et al., 2017d). Casein bound calcium phosphate solubilises over ripening, reducing casein-casein interactions (Everett and Auty, 2017), this is demonstrated by the age-related increase in soluble calcium in the cheese serum (Guo and Kindstedt, 1997). Reduction in casein bound calcium has been reported to increase the level of proteolysis in cheese over ripening (Feeney et al., 2002). The degree of hydration of the casein network and calcium solubility all develop over the course of ripening and affect the properties of the cheese.
3.2. Changes in Proteolysis and Lipolysis

The biochemical processes of proteolysis and lipolysis develop over ripening and play an important role in the development of cheese texture and flavour.

Proteolysis describes the biochemical pathway by which intact casein is hydrolysed to peptides of varying molecular mass and to free amino acids. This process is considered to be a significant reaction during ripening as it influences the development of both flavour and physical properties, such as texture and cooking properties (McSweeney and Sousa, 2000; McSweeney, 2017; Ardö et al., 2017). The extent of proteolysis varies between cheese varieties and has been extensively studied and reviewed (Feeney et al., 2002; Dave et al., 2003; Guinee and O’Kennedy, 2009; Hinz et al., 2012; Soodam et al., 2015; Ardö et al., 2017; McCarthy et al., 2017a,b,c; McSweeney et al., 2017).

During cheese ripening, starter culture numbers decline and non-starter lactic acid bacteria (NSLAB) begin to dominate the bacterial population. NSLAB supplement the proteolytic activity of starter cultures, producing peptides and free amino acids (FAAs) throughout ripening as the starter cultures die off (McSweeney, 2017). However, due to the relatively short ripening time of Mozzarella (~75 days), this activity is limited and is of less relevance compared with varieties that undergo a more prolonged ripening time, e.g., Cheddar (McSweeney, 2017). The typical pathway for the breakdown of caseins during proteolysis is shown in Fig. 1.

![Fig. 1: Proteolytic agents in cheese during ripening (Sousa et al., 2001).](image)
The biochemical process of proteolysis is comprised of a primary and secondary stage, modulated by proteolytic enzymes that originate from a number of sources, e.g., native milk enzymes, coagulant, starter cultures, non-starter lactic acid bacteria (NSLAB) or adjuncts. Starter cultures e.g., lactic acid bacteria (LAB) employed in cheesemaking, also contribute proteolytic enzymes that hydrolyse casein over ripening. LAB themselves, along with intracellular enzymes released upon their lysis, contribute to the breakdown of large peptides to small peptides and free amino acids.

Primary proteolysis involves breakdown of intact casein to large- and medium- molecular weight water soluble peptides and is predominantly mediated by residual chymosin and plasmin. These peptides are subsequently hydrolysed during secondary proteolysis by chymosin along with enzymes released from both starter and non-starter bacteria to low molecular weight peptides and free amino acids (FAA) which form volatile flavour compounds (McSweeney and Sousa, 2000; McSweeney, 2017). Chymosin (rennet) acts on the Phe105-Met106 bond of kappa-casein (Horne and Lucey, 2017) releasing the caseino-macro peptide (k-casein f106-199) from the micelle surface. The hydrolysis of the caseino-macropetide, which is lost with the whey, reduces the steric hindrance between the remaining para-casein micelles and predisposes them to rennet-induced gelation. During cheese manufacture, the gel is dehydrated to a cheese curd with the target moisture content (e.g., 48-52%, w/w, for LM Mozzarella, Tables 1 and 2), by subjecting the curd to various steps as described in Fig. 2. Following manufacture, αs1-casein is the primary target of chymosin. Hydrolysis of the Phe23-Phe24 peptide bond of αs1-casein occurs from residual chymosin activity in the cheese and results in a marked weakening of the para-casein matrix which decreases the fracture stress and firmness of the cheese (Creamer and Olson, 1982; Fenelon et al., 2000). Approximately
6% of chymosin added during manufacture is retained in the resultant cheese (Banks and Horne, 2003).

![Process flow for pasta-filata cheese.](image)

Proteolysis plays a key role in the development of typical functional properties associated with Mozzarella (Mistry, 2001; McMahon and Oberg, 2017). Although Mozzarella is subjected to high temperatures during plasticization, which inactivate chymosin to a large extent (Feeney et al., 2001), the development of bitterness is still of concern. McSweeney (2007) stated that the action of both plasmin and chymosin on casein can result in the production of hydrophobic peptides associated with bitterness.
Lipolysis in cheese refers to hydrolysis of milk fat, predominantly in the form of triglycerides, glycerol, mono- and di-glycerides and free fatty acids (Thierry et al., 2017). During lipolysis, compounds especially free fatty acids (FFAs) are released which contribute both directly and indirectly to the development of cheese flavour. FFAs themselves contribute directly to cheese flavour, while volatile compounds released on their further bio-transformation, e.g., esters, thioesters and methyl ketones impart flavour and aroma to the cheese (McSweeney, 2017). Low concentrations of FFAs in Mozzarella can positively contribute to cheese flavour, once they are balanced with appropriate levels of proteolysis (Collins et al., 2003). Lipases and esterases mediate lipolysis and originate from a number of potential sources; milk, rennet, starter cultures, NSLAB bacteria and added lipase preparations (Thierry et al., 2017). However, due to the high temperatures reached by Mozzarella curd during manufacture (~55 °C) and short ripening time at 4 – 8 °C, there is limited lipolytic activity in Mozzarella (Santillo et al., 2011); nevertheless, fat content and its hydrolysis contributes to the characteristic mild, milky flavour associated with Mozzarella (Johnson et al., 2009).

3.3. Physical characteristics of unheated Mozzarella cheese

3.3.1. Rheology and Texture

Rheology is the study of how matter deforms when subjected to stress or strain and can be used to determine how different cheeses behave under similar conditions (e.g., temperature), or how a cheese can transition from a viscoelastic solid to a viscoelastic liquid on heating. Rheology can also give an insight into the cooking behaviour of cheese, and in the case of ingredient cheeses such as LMPS Mozzarella, this is of particular relevance for different cooking applications, e.g., a protected cooking environment (in a sandwich) or an exposed environment (the surface of a pizza). Cheese can be described as being viscoelastic in nature,
i.e., sharing both elastic (Hookean) and liquid (Newtonian) characteristics to an extent depending on different factors including cheese variety, composition, testing conditions, temperature and ripening time (Guinee et al., 2003). Cheese composition affects viscoelastic properties in a number of ways; fat offers lubrication, particularly at fracture points, at temperatures at or above 40°C (Guinee, 2002; Guinee and Kilcawley, 2004), while moisture content impacts the hydration of the protein matrix, in turn affecting the fracture properties. Cheese rheology is temperature dependent and the viscoelastic properties of cheese vary considerably depending on test conditions.

Texture is an important attribute for Mozzarella and can be measured through either instrumental and/or sensory analysis. Instrumental tests such as Texture Profile Analysis (TPA) are less expensive than sensory panels. Instrumental measurements have been validated by correlating measured parameters (e.g., firmness, chewiness, springiness, adhesiveness and cohesiveness) with sensory attributes (Chen et al., 1979; Drake et al., 1999; Breuil and Meullenet, 2001). The response of the cheese matrix to deformation, such as that which occurs during chewing, cutting, slicing, shredding or compression, is dependent on many factors; cheese composition, degree of protein hydration and protein hydrolysis (Metzger et al., 2001; Chevenan et al., 2006; McCarthy et al., 2016; Everett and Auty, 2017). During ripening, cheese hardness and chewiness typically decrease (Sheehan and Guinee, 2004; Van Hekken et al., 2007). Other cheese parameters also change over ripening, including cohesiveness, springiness and adhesiveness, the evolution of these trends with ripening time are less clear (Chevanan et al., 2006; Upreti et al., 2006). Generally, changes in textural properties are related to the biochemical changes that occur over ripening e.g., proteolysis, protein hydration.

Variations in make procedure affect cheese composition and micro-and macrostructure and can, thereby, exert large effects on texture (Metzger et al., 2001; Guinee et al., 2002; Ong et al., 2012).
3.3.2. Shreddability

LMPS Mozzarella, due to its structure, shreds well which is regarded by manufacturers as a key attribute of Mozzarella for the preparation of frozen pizza (Jana and Mandal, 2011). Cheese becomes unsuitable for shredding after a number of weeks of storage as it becomes sticky and develops a tendency to clump and form balls (Guinee et al., 2001). Calcium reduction significantly increases the stickiness of Mozzarella and, consequently, is detrimental to the shreddability of cheese owing to sticking and balling (De Angelis and Bobbetti, 2011).

3.3.3. Flavour

Flavour may be defined as the combination of taste and aroma, and the flavour profiles of different cheese varieties have been extensively reviewed and studied (Buchin et al., 1998; McSweeney and Sousa, 2000; Barron et al., 2004; Thierry et al., 2017). However, multiple factors contribute to flavour; volatile compounds which are detected by either the orthonasal (odour) or retronasal (aroma) olfactory receptors, non-volatile compounds resulting from the breakdown of proteins or lipids are detected on the tongue (taste), and texture, as well as food structure breakdown and mouthfeel all contribute to the flavour perceived by the consumer (Le Quéré, 2004). Flavour development during ripening is heavily influenced by biochemical reactions, i.e. proteolysis and lipolysis (McSweeney and Sousa, 2000), which are mediated by the microflora present in the cheese, e.g., starter cultures, NSLAB. Although the impact of lipolysis on cheese flavour is less significant than that of proteolysis for many cheese varieties, it is nevertheless important in achieving a balanced flavour profile. Aromatic free fatty acids contribute directly to cheese flavour. Short- and medium-chain FFAs (4 to 12 carbon) contribute more to flavour than long-chain FFAs (>12 carbon chain), as the latter possess high perception thresholds and are not easily detected in the mouth, while detection of the former is much more rapid (Thierry et al., 2017). Excessive breakdown of milk fat has been associated
with rancidity, and the production of even moderate levels of certain short chain FFAs has been linked with rancid flavour (Fox and McSweeney, 1995). The contribution of proteolysis to flavour is due to predominantly the formation of free amino acids (FAAs) and short- and medium-length peptides, which contribute to savoury flavour in cheese (Sousa, 2001; Smit et al., 2005; Ardö, 2006). However, hydrolysis of β-casein and the production of above threshold levels of hydrophobic low molecular weight peptides can result in a bitter flavour in cheese (McSweeney and Sousa, 2000). Bitterness is of greater concern in reduced-fat cheese, due to the higher concentration of casein (and hence, β-casein) (Drake et al., 2010).

The flavour of cheese changes with age, from mild-flavoured when young (e.g., fresh Mozzarella) to a more complex flavour profile after 1 to 2 years of ripening e.g., Parmesan. LMPS Mozzarella has a mild and milky flavour (Johnson et al., 2009; McMahon, 2016). This is because of the short ripening time (typically < 4 weeks) and the limited extent of proteolysis and lipolysis.

Fat acts as a reservoir of flavour (Lyndsay, 1991), and without it, cheese flavour may become unbalanced and lacking typical ‘cheesiness’. Interestingly, fat can mask the perception of bitter hydrophobic peptides in cheese (Madsen and Ardö, 2001). Fat also plays a key role in the release of flavour in the mouth during mastication. Upon ingestion, cheese temperature rapidly increases to 37°C at which value dairy fat in cheese is fully liquid. According to Lopez (2006), the lubricating effect of fat contributes to the release of flavour and overall mouth-feel during consumption. In the absence of fat, cheese becomes more difficult to masticate and has poor flavour release (Delahunty et al., 1996). Delahunty et al. (1996) reported that that the volatile compounds 2-butanone and 2-heptanone are released more rapidly from a reduced-fat cheese matrix compared with a full-fat cheese matrix, and that flavour perception is affected not only by the profile of volatile compounds, but also by the rate of release of these compounds. This suggests that the flavour perception of reduced-fat cheese differs notably
from that of full fat cheese. Cheese flavour is also influenced by other factors including; cheese composition, storage time and conditions (Guinee and McSweeney, 2007; Ardö et al., 2017; Thierry et al., 2017).

3.3.4. Sensory

Sensory analysis is commonly used to characterise cheese flavour, in relation to different attributes and their intensities. However, the use of trained sensory panels is expensive and time consuming to establish. Consequently, there has been a move towards developing alternative methodologies that are less expensive and time consuming e.g., flash profile analysis, a descriptive free choice means of profiling where semi-trained panellists are free to use their own vocabulary to compare the whole product set, or ranking descriptive analysis, which allows samples to be arranged by intensity of a given attribute (Dairou and Sieffermann, 2002; Bragato Richer et al., 2010).

The acceptability of cheese to the consumer is impacted by a number of factors, e.g., the sensory properties, usage, convenience, price and nutritional values. The sensory properties of cheese are dependent on the texture and rheological properties, the appearance and flavour (Delahunty and Drake, 2004). The majority of sensory studies focussed on cheese have been on unheated cheese, analysing the correlations between composition, biochemical reactions (i.e., proteolysis and lipolysis), and the rheological properties with the flavour and sensory acceptability of various cheeses (Dimos et al., 1996; Drake et al., 2010; Foegeding et al., 2003; Pinho et al., 2004).
3.4. Functional characteristics of heated cheese

3.4.1. Flow

The term heat-induced flow can be used to describe the flow or spread of cheese on heating under defined conditions, e.g., at 280 °C in a convection oven for 4 min. It is routinely measured using empirical assays, including the Schreiber (Park et al., 1984) or Price-Olson (Olson and Price, 1958) methods. The Schreiber test typically involves placing a disc of cheese (e.g., 4.75 cm, diameter; 5 mm, height) in a convection oven at 280°C for 4 min. Results are expressed as the % increase in diameter of the cheese disc following heating (McCarthy et al., 2016). This test simulates the effects of cooking cheese on a pizza or lasagne. The Price-Olson method involves heating a cylinder of cheese (12 g; 2.2cm in diameter, 5 cm long) in an enclosed tube in an oven at 180°C for 7.5 min (Costa et al., 2010). This test simulates the cooking of cheese enclosed in a food, e.g., in a pasta bake, pie or toasted sandwich. Rheological attributes, such as loss tangent (ratio of loss modulus-to-storage modulus, G'/G'') as measured using low-strain shear oscillation is used to describe the viscoelasticity of cheese (Guinee et al., 2015). The change is given as the loss tangent as a function of temperature on heating from 20 to 95 °C and has been used to describe the increase in fluidity of the cheese on heating.

The heat-induced flow of cheese tends to increase during ripening/storage (Rynne et al., 2004; McCarthy et al., 2016), due to the associated increases in protein hydration and proteolysis over time (Metzger et al., 2001; Guinee et al., 2002; Rynne et al., 2004). Mozzarella cheese undergoes moderate flow upon heating (McMahon, 2016; Guinee, 2016b). Free fat and moisture act as lubricants within the melting cheese mass and thereby facilitate heat-induced flow; moreover, free fat reduces moisture loss, scorching and excessive dehydration of the cheese (Rudan and Barbano, 1998; Rudan et al., 1999). Consequently, the contents of fat and moisture are important compositional factors affecting the ability of cheese to flow and become fluid on heating.
3.4.2. Extensibility

Extensibility and stringiness are important attributes of heated Mozzarella and many methods have been proposed to measure this. Certain methods are somewhat simplistic and subjective, such as the fork test and three-pronged-hook probe tensile test and have become less used in favour of more objective methods. Apostolopoulos (1994) developed a test measuring the vertical extensibility of molten cheese using a heated model pizza. This method reflects real-life usage of pizza cheese and its extensibility, the test conditions; however, are somewhat ill-defined, as cheese weight and precise probe speed are omitted. Guinee and O’Callaghan (1997) modified the previous method to involve the horizontal extension of the pre-sliced pizza base with molten cheese. A pizza base was cut into two equal halves, covered with shredded cheese and baked. The cooked pizza was then placed on the platform of the stretching apparatus and the sides clamped in place. The two halves of the pizza were stretched apart until the extended strings of cheese broke completely; cheese stretchability was defined as the distance required to induce complete string breakage. This method is applicable to the real-life consumption of pizza and gives a meaningful insight into the stretching behaviour of cheese on pizza; however, the equipment required is cumbersome. All of the above methods describe, with varying accuracy, the stretching behaviour of cheese or cheese as a topping on a pizza base. However, cheese stringiness cannot be studied in isolation, as both the ability of cheese to stretch and form strings, and the toughness of the cheese, or resistance offered to the extension force, must be considered when discussing cheese stretchability. A study by Fife et al. (2002) characterised the stretchability of non-fat and LMPS Mozzarella cheese stretch using a three-pronged hook-shaped probe. A number of different descriptors (melt strength, stretch quality and stretch length) were obtained from the stretch profile of the cheese to give a more detailed description of the stretch performance of cheeses. This method was an improvement.
on the inaccuracies of the fork test and gave a more detailed description of cheese stretchability. McCarthy et al. (2016) described a method for the measurement of uniaxial vertical extensibility of molten cheese using a TPA texture analyser which describes both the ability of the cheese to stretch, and the work required to do so. This method, although similar to the one developed by Guinee and O’Callaghan (1997), generates data reflective of the stretching behaviour of pizza cheese.

3.4.3. Browning/blistering

Browning and blistering are also key factors affecting Mozzarella cheese. Browning to a certain degree is desirable in Mozzarella. However, the presence of reducing sugars such as lactose or galactose may encourage excessive browning on the cheese surface during cooking (Johnson and Olsen, 1985; Ma et al., 2013a). Ma et al. (2013a) found that Mozzarella cheese containing 0.54% (w/w) galactose demonstrated significantly higher browning upon cooking than a control cheese containing 0.02% (w/w) galactose. Blistering and scorching of the cheese surface are defects often associated with a lack of sufficient free oil. During heating, moisture rapidly evaporates from the unprotected cheese surface resulting in pockets of air forming just below the cheese surface, creating a ‘bubbling’ effect. These pockets become dehydrated and scorch easily (Rudan and Barbano, 1998; Ma et al., 2013b). Skin formation can also occur as the cheese surface becomes dehydrated, forming a tough, dried out skin on the surface. Rudan and Barbano (1998) found that the application of a hydrophobic barrier (vegetable oil) to the cheese surface impeded moisture evaporation during heating, and thereby prevented skin formation and scorching.
3.4.4. Free fat

Free fat exudes to a greater, or lesser, degree from the surface of heated cheese. Free fat creates a protective hydrophobic barrier to evaporation, ensuring that the cheese remains hydrated throughout cooking. This prevents skin formation on the cheese surface along with excessive browning and scorching. Skin formation can result in a cheese that is tough and has poor flow properties upon heating (Rudan and Barbano, 1998, Rudan et al., 1999). In contrast, an excessive degree of oiling-off can result in pools of free fat collecting on the surface of the cheese and creates a greasy, undesirable mouthfeel, which can be unappealing to consumers (McMahon and Oberg, 2017). Free fat or the extent of oiling off is measured by a relatively simple method whereby cheese is weighed and heated in a tube, distilled water and methanol added, centrifuged in order to separate the fat phase and the resultant fat column measured and expressed as a percentage of weight or total fat (Kindstedt and Rippe, 1990). The degree of oiling-off has been linked to the composition of the cheese, i.e., its fat content (Rudan et al., 1999; Kindstedt et al., 1993) along with the storage time and extent of proteolysis (Tunick et al., 1993, 1995; Yun et al 1993).

3.4.5. Sensory

The properties of heated cheese are of particular relevance in cooked dishes such as pizza. Nevertheless, comparatively little information is available on the sensory properties of heated cheese and the factors affecting them. Piggott et al. (1998) evaluated the sensory properties of unheated- and heated- (fried and grilled) Halloumi cheese. The results showed that heating the cheese had a significant effect on flavour as determined by the panel (e.g., the ability of the panel to discriminate the saltiness of the cheeses disappeared upon heating). The study also revealed a statistically significant relationship between the headspace volatiles and sensory
scores of the cheeses (e.g., the detection of pulegone, a terpene and carvone compounds correlated with the panel mean score for ‘minty’).

Ganesan et al. (2014) conducted sensory analysis on unheated- and heated- LMPS Mozzarella with varying degrees of salt reduction. This study demonstrated that in unheated Mozzarella, a salt reduction of up to 33% (w/w) was possible without a detrimental impact on the overall liking of cheese, while in the heated cheese a salt reduction of 25% (w/w) did not affect the overall liking. However, this study was conducted on Mozzarella cheese with a starting salt content of 1.80% (w/w), which is somewhat higher than the average salt content of LMPS Mozzarella, 1.66% (w/w) (Agarwal et al., 2011).

4. Reduced-fat Mozzarella cheese

4.1. Challenges associated with reduced-fat cheese

A reduction in fat content in Mozzarella impacts on many properties of the cheese; its composition, biochemical properties, functionality, texture and flavour (McMahon and Oberg, 1998; Rudan et al., 1999; Sheehan and Guinee, 2004; Drake et al., 2010). Reduced-fat Mozzarella tends to have an unbalanced flavour profile with a higher proportion of the flavour compounds present formed as a result of proteolysis rather than lipolysis. Moreover, fat reduction results in slower release of flavour compounds in the mouth, resulting in an unpleasant eating experience for the consumer (Drake et al., 2010).

Cheese colour becomes more translucent and cheese loses its typical white opaqueness upon fat reduction (Rudan et al., 1999). Fat reduction affects Mozzarella cheese functionality, and attributes such as heat-induced-flow and stringiness are impaired (McMahon, 2016). A study by McCarthy et al. (2016) found that a fat reduction of 30% (w/w) in Cheddar cheese was sufficient to increase cheese toughness significantly and make stretching more difficult.
Fat in cheese suppresses moisture loss through heat-induced evaporation, by creating of a hydrophobic barrier (Guinee, 2015). Studies on Cheddar and Mozzarella (Guo et al., 1997; Guinee et al., 2000; McCarthy et al., 2017d) have found that the flow properties of heated cheeses increases as the levels of intact casein decreases, and the levels of proteolysis, soluble calcium and casein hydration increase.

4.2. Approaches applied to overcome the effects of fat reduction

Studies have investigated different approaches to counteract the adverse effects of fat reduction on the functional e.g., heat-induced flow, stretchability and stringiness, and sensory properties e.g., creaminess, succulence, of unheated and heated cheese, (Table 3). These include: addition of fat replacers e.g., lecithin, Simplesse, Dairy-Lo, exopolysaccharide-producing cultures (McMahon et al., 1996; Drake et al., 1999; Costa et al., 2010); homogenisation of cheesemilk (Drake et al., 1995; Rowney et al., 2003); and reduction in calcium content (Guinee et al., 2002; Sheehan and Guinee, 2004; McCarthy et al., 2017d). Fat replacers can be divided into two categories; fat substitutes or fat mimetics (Akoh, 1998). Fat substitutes generally originate from fat and are either chemically synthesised or derived from fats or oils by enzymatic modification. These compounds can typically be used to substitute for fat in foods on a one-to-one basis and reduce the caloric content of the food. Examples include emulsifiers, medium-chain triacylglycerols and structured lipids (Akoh, 1998). Fat mimetics are typically carbohydrate- or protein-based substances (e.g., starch, whey, cellulose), and function by binding water in cheese; however, they cannot fully replace fat on a weight basis. Fat mimetics also cannot replace the non-polar functions of fat, such as its flavour-carrying ability or indeed functionality of fat (Banks, 2004).
The above approaches have resulted in varying degrees of success. Calcium reduction can be used to counteract the negative impact of fat reduction on physical cheese attributes e.g., excessive hardness through a reduction in the continuity of the casein network. Calcium crosslinks contribute to rigidity in the casein network, which along with the increased volume fraction of the protein network in reduced-fat cheese, lead to increased hardness and poor functionality. On reducing the calcium available to bind with the casein network, the continuity and integrity of the network is impaired, resulting in a softer curd. Calcium reduction can be achieved through direct acidification of the cheese milk with lactic acid, acetic acid or citric acid (McMahon, 2016). The pH of the curd/whey mixture is subsequently reduced which causes de-mineralisation in the curd, as calcium is solubilised and lost to the whey (McMahon et al., 2005). Feeney et al. (2002) found that Mozzarella cheese directly acidified with a lactic acid solution had higher levels of proteolytic activity, due in part to the reduction in calcium content which increased the susceptibility of the casein matrix to hydrolysis by residual chymosin. Sheehan and Guinee (2004) found that starter culture addition in conjunction with milk pre-acidification for Mozzarella manufacture resulted in reduced-calcium content with significant increases in levels of proteolysis and protein hydration in cheese. Reduction in available calcium addresses the impact of fat reduction in a number of ways: it results in a softer curd immediately following manufacture and during storage, it promotes proteolysis during cheese ripening which enhances cheese texture and functionality by reducing hardness and increasing heat-induced flow (Sheehan and Guinee, 2004; McCarthy et al., 2017d). However, there has been little work on the sensory properties and consumer acceptance of reduced-fat cheeses with reduced-calcium content.

Fenelon and Guinee (1997) found that the use of a whey-based fat substitute, Dairy-Lo™, in the production of reduced-fat Cheddar resulted in impaired chymosin activity, which in turn led to cheeses with higher levels of moisture and MNFS and a softer texture. Although fat
replacers can reduce the calorific content of foods, they do not counteract the unbalanced flavour of reduced-fat cheese; hence, their use in cheese production is limited (Drake et al., 2010).

5. Reduced-salt cheese

5.1. Effects of salt reduction on the composition and biochemical properties of cheese

Salt reduction affects cheese composition (overall salt content, ratio of salt to moisture) and the biochemical processes that occur during ripening, e.g., proteolysis. Salt has an inhibitory effect on proteolysis (Mistry and Kasperson, 1998; Rulikowska et al., 2013; McCarthy et al., 2016), although the magnitude of this effect is also impacted by the degree of casein hydration (Paulson et al., 1998), final moisture content (Pastorino et al., 2003) and increased interaction of calcium with the para-casein network (Guinee and Fox, 2004).

Reduced-salt cheese tends to have higher moisture content than full-salt cheeses (Rulikowska et al., 2013; Ganesan et al., 2014; McCarthy et al., 2016) along with reduced levels of protein hydration (Paulson et al., 1998). Protein hydration is inversely linked to expressible serum. Paulson et al. (1998) reported that unsalted, non-fat Mozzarella at day 24 expressed serum, while salted Mozzarella at the same age failed to express any serum, indicating that the para-casein network of the salted Mozzarella had absorbed the available serum. Creamer et al. (1985) suggested that salt addition promoted calcium solubilisation from para-casein in cheese. Hence, a reduction in salt may result in higher calcium retention in the protein matrix, which in turn may contribute to increased protein-protein interactions, a more aggregated para-casein network, and lower casein hydration. A reduction in the hydration of the casein network negatively impacts the heat-induced flow of the cheese and ability of the cheese to stretch, both of which are very important attributes for LMPS Mozzarella.
5.2. Effects of salt reduction on the functional properties

Unsalted cheese tends to be soft, pale, opaque and very adhesive (Paulson et al., 1998; Guinee and Fox, 2004). This is because of the associated effects of salt reduction on gross composition, and protein hydration (Guinee and Fox, 2004). Rulikowska et al. (2013) found that reducing salt content of Cheddar cheese from 3.0%, w/w to 0.5%, w/w resulted in reduced cheese firmness, fracture stress and strain; this was attributed to increases in the levels of moisture-in-non-fat substances (MNFS) and protein hydrolysis. Pastorino et al. (2003) found a significant positive correlation with increasing salt content and cheese firmness and brittleness in Muenster cheese. These authors also reported a reduction in cohesiveness and an increase in cheese adhesiveness with increasing salt content. A study by Paulson et al. (1998) reported that unsalted non-fat Mozzarella cheese performed poorly in melt tests compared to salted cheese, while unsalted cheese also had free serum on the cheese surface during heating.

McCarthy et al. (2016) studied the effects of salt reduction from 1.9% w/w to 1.2% w/w and 0.9% w/w on the rheology and cooking properties Cheddar cheese with 3 different fat contents (33% w/w, 22% w/w, 16% w/w). The study showed that the effect of salt reduction varied with the level of salt reduction and with fat content. Interestingly, in 33% fat cheese the reduction of salt resulted in a decrease in heat-induced flow while in the 22% and 16% fat cheese there was a significant increase in heat-induced flow. It was also observed by the authors that the most significant impact of salt reduction occurred from 1.9% w/w to 1.2% w/w, with a further reduction to 0.9% w/w having little, or no, effect. A similar trend was observed in Mozzarella cheese in a study by Ganesan et al. (2014). The authors reported that the heat-induced flow of LPMS Mozzarella cheese increased with salt reduction in the range from 1.8 to 0.95% (w/w) but decreased on further reduction to 0.7% (w/w); cheeses with 1.8% (w/w) (control) and 0.7% (w/w) had similar flow. In contrast to flow, an incremental reduction of
salt content from 1.8 to 0.7% (w/w) led to a progressive decrease in extensibility (stretch) of the cheese heated to 65 °C. At 2 weeks post manufacture, Mozzarella with a 25% reduction in salt content had the least resistance to stretch, while after 8 weeks the cheese with 50% reduction in salt was the most fluid and least elastic of the cheeses (Ganesan et al., 2014).

5.3. Approaches applied to overcome the effects of salt reduction

Salt replacers have been used with limited success as a means of reducing the quantity of sodium added to cheese. Fitzgerald and Buckley (1985) investigated the use of a number of salt replacers; magnesium chloride, potassium chloride and calcium chloride in a ratio of 1:1 with sodium chloride. The addition of magnesium or calcium chloride salts resulted in cheeses that were unacceptable to consumers due to flavour and textural defects, while a 1:1 mixture of potassium chloride and sodium chloride resulted in a cheese comparable to the control with added sodium chloride. However, these authors found that the complete substitution of sodium chloride with other salts such as potassium chloride, calcium chloride or magnesium chloride resulted in overly-soft cheeses with distinctive bitter off-flavours. El-Bakry et al. (2011) reported the successful total replacement of sodium chloride with potassium chloride in imitation cheese without any adverse effects on the heat-induced functionality of the cheese. However, the use of potassium chloride as a salt replacer has limited application, due to concerns over its potential toxicity at certain levels for people with renal failure or hyperkalaemia (Doorenbos and Vermeij, 2003).

In composite foods such as pizza, salt partitioning into zones of high- and low-concentration has been used as an approach to reduce the overall salt content of the food without negatively impacting the perception of saltiness (Guilloux et al., 2015). Previously, Fenelon et al. (1999) developed a novel process for enhancing the quality of half-fat Cheddar cheese, involving the blending of full-fat, and reduced-fat, curd particles after whey drainage.
The resultant reduced-fat blended cheese had significantly lower fracture stress and firmness that reduced-fat cheese made by the conventional manufacturing procedure; otherwise, there no significant between the cheeses with respect to composition, appearance or sensory characteristics. It is probable that the compartmentalization of fat and/or salt in regions of ‘high’ and ‘low’ content using combinations of the above approach may have potential in developing non pasta-filata reduced-fat, reduced-salt cheeses with enhanced quality. However, such an approach may not be successful for the manufacture of reduced-fat, reduced-salt pasta-filata cheeses, as the plasticization process applied during manufacture (Fig. 2) transforms the curd structure into a uniform molten mass (Fenelon et al., 1999).

6. Objectives

The objectives of the current study were to investigate:

(i) the interactive effects on fat and salt reduction on the composition, rheology, melting, and sensory properties of Mozzarella cheese (Chapter 1),

(ii) the effect of reducing calcium content on the properties of reduced-fat, reduced-salt cheese (Chapter 1), and

(iii) the effects of fat and salt reduction on the texture profile attributes, volatile compounds and sensory characteristics of unheated and heated Mozzarella cheese after 15 and 35 days of ripening (Chapter 2).
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Chapter 2

Interactive effects of salt and fat reduction on composition, rheology and functional properties of Mozzarella-style cheese*

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Abstract

Owing to the risks associated with excessive dietary intake of fat and sodium, there is an increased consumer demand for food products, including cheese, with reduced fat and salt contents. Research to date has focused mainly on the separate effects of reducing fat and salt. The primary objective of this study was to evaluate the combined effects of reducing fat from ~22% to 11% and salt from ~1.7% to 1.0%, on the composition, rheology and melting properties of Mozzarella cheese. A secondary objective was to investigate the effect of reducing the calcium content of the reduced-fat, reduced-salt cheese as a means of improving its properties. Reducing fat and salt content led to higher levels of protein and moisture, lower contents of moisture-in-non-fat substances, fat-in-dry matter and salt-in moisture. These changes coincided with lower water binding capacity, lower primary proteolysis and increased hardness and chewiness in the unheated cheese, and with a lower flow and higher work to extend (EW) the heated cheese. Lowering the calcium content of the reduced-fat reduced-salt cheese increased the levels of moisture and moisture-in-non-fat substances, and reduced the protein content and the calcium-to-casein ratio; simultaneously, the unheated cheese had a higher water binding capacity, a reduced hardness and chewiness, while the heated cheese had higher flowability and a lower EW compared to the reduced-fat, reduced-salt cheese. Despite the mitigating effects of calcium reduction, the reduced-fat, reduced-salt, lower-calcium cheese was, nevertheless, firmer, harder, more cohesive and chewy, flowed less and had a higher EW than the full-fat, full-salt cheese.
1 Introduction

Pizza consumption continues to expand globally, especially in the US where sales value was estimated at US $38 billion in 2014 (Nickle and Pehrsson, 2013; Hynum, 2014; Rhodes et al., 2014). Simultaneously, the production of Mozzarella, the principal cheese used in pizza, continues to expand, for example by 1.3% per annum in the USA between 2010 and 2014, from 1.55 to 1.62M tonnes (Statista, 2015). Nevertheless, pizza at high consumption rates can contribute significantly to dietary fat and sodium, especially in the youth (Moshfegh et al., 2012; Drewnowski and Rehm, 2013; Powell et al., 2015). Excessive intakes of fat, saturated fat and sodium are undesirable because the associated health risks, such as obesity, hypertension, heart attack, stroke, cancer and diabetes (He and MacGregor, 2009; Moshfegh et al., 2012). In response, there is an increasing demand for cheeses with reduced levels of salt and fat. Consequently, there has been a significant focus on the separate effects of reducing fat (Rudan et al., 1999; van Hekken et al., 2007) and salt (Paulson et al., 1998; Ma et al., 2013) on the quality of Mozzarella cheese. Generally, fat reduction is associated with an increase in rigidity, elasticity and hardness and a loss of meltability. Various approaches have been used to improve the quality of reduced-fat Mozzarella, including a reduction in the calcium content of the cheese (Metzger et al., 2001) and homogenization or microfluidization of milk or cream (Tunick et al., 1993; Rudan et al., 1998; van Hekken et al., 2007). While extensive research has considered the effects of altering salt content of Cheddar cheese (Rulikowska et al., 2013), comparatively little information is available on the influence of salt on the quality of Mozzarella. Unsalted Mozzarella was found to have lower water-holding capacity and lower heat-induced flowability than the corresponding salted cheese (~1–2% NaCl; Guo et al., 1997; Paulson et al., 1998). The current study on cheese was undertaken as a work package within the FP7 EU funded project ‘novel processing approaches for the development of food products low in fat, salt and sugar reduced’ (acronym ‘PLEASURE’). An overall ambitious goal was to
achieve, *inter alia*, comparable sensory properties in the ‘reduced’ fat and salt cheeses with equivalent products of ‘normal’ composition, thus setting the scene for deployment of novel technological adaptations and textural restructuring where needed. This translated into the experimental objectives of the present study, i.e. to investigate the effects of the simultaneous reduction of fat and salt, from ~22% to 11% and from 1.7% to 1.0%, respectively, on the compositional, biochemical and functional properties of Mozzarella-style cheese, and to explore the effect of reducing the calcium content, and hence the degree of calcium-induced cross-linking of casein, as a means of normalizing the characteristics of the reduced-fat, reduced-salt cheese.

2 Materials and methods

2.1 Cheese manufacture

Mid-lactation milk was obtained on three occasions over a 6-week period from the autumn-calving Friesian herd at the Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark. The milk was standardized to the required protein-to-fat ratios for the control (full-fat; 0.94) and reduced-fat (3.36) cheeses, pasteurised at 72°C for 15s, cooled to 36°C, and pumped into jacketed, cylindrical, stainless steel 500-L vats with automated variable-speed cutting and stirring (APV Schweiz AG, CH-3076 Worb 1, Switzerland). The milk was inoculated with frozen cultures *Streptococcus thermophilus* (TH4) and *Lactobacillus helveticus* (LHB02) at weight a ratio of 2:1 and at a level recommended by the supplier (Chr. Hansen, Little Island, Cork, Ireland). After a 40-min inoculation period, chymosin (single strength Chy-Max® plus, 200 IMCU; Chr. Hansen, Hoersholm, Denmark) was diluted 1 in 10 with distilled water and added at level of 0.14mL/kg milk based on a protein level of 3.16g/100g. Following gelation, the gels were cut at a similar strength (*G*′, 40Pa) measured using low amplitude strain oscillation rheometry (Guinee et al., 2000), healed for 10min, and
the curd–whey mixture was cooked at a rate of 0.25°C/min to a scald temperature 42°C for the full-fat cheese and 38°C for the reduced fat cheese. The whey was drained at a pH of 6.0 for the full-fat and 6.1 for the reduced-fat cheese and the drained curds formed into a consolidated mass which was cut into slabs. The slabs were piled at pH 5.6 and milled at pH 5.1. Subsequently, the curds were salted at a rate of 4.21 and 2.06 (% w/w) for the full-salt and reduced-salt variants of the full-fat (FFFS and FFRS) and reduced-fat (RFFS and RFRS) cheeses. The salted curds were held for 20 min while turning at 5 min intervals to ensure uniform salt distribution. The curds were then transferred to the stretching unit (Automatic Stretching Machine, Model d; CMT, S. Lorenzo di Peveragno CN, Italy) and plasticized using water at 78–80°C at a water to curd ratio of ~1.4, as described by Guinee et al. (2000). The hot curd (58°C) was moulded into rectangular 2.3-kg blocks, which were cooled by holding the blocks in a dilute brine solution (10% w/w NaCl; 0.2% w/w Ca) at ~3 to 2°C for 30 min, allowed to surface dry at room temperature for 20 min, vacuumed packed and stored at 4°C for 75 days. The method for manufacture the reduced-fat, low-calcium full-salt and reduced cheeses (RFFSLCa, RFRSLCa) was the same as the reduced-fat full salt and reduced-salted cheese, except for the following differences: the milk was acidified to pH 5.7 at 29°C by adding the required quantity of diluted lactic acid 11% w/v, the whey was drained at pH 5.6 at ~35 min after scalding to 38°C, the curds were milled and salted at 5.65 and 2.24%, respectively, for the full-salt (RFFSLCa) and reduced-salt (RFRSLCa) variants. Preliminary studies were undertaken to establish the required salting levels for the different cheeses.

2.2 Sampling of cheese

Cheeses were sampled for composition at 7 day, for pH, water-soluble N (WSN), expressible serum, firmness, texture profile analysis and heat-induced-flow at 1, 7, 15, 35, 50
and 75 days. The work to extend (stretch) the hot molten cheese (EW) was measured at 35, 50 and 75 days only, due to unavailability of measuring equipment at earlier times.

2.3 Analysis of cheese

2.3.1 Composition

Cheese was cut into cubes (2.5 cm$^3$), shredded in a Hallde RG-350 machine (AB Hallde Maskiner, Kista, Sweden) using the raw food grating disc (K), and grated to particle size of <1 mm, in a Braun blender (34209 Melsungen, Germany). The grated cheese was analysed in triplicate at 7 days for moisture, fat, total protein, ash, calcium, phosphorous and salt using IDF standard methods (Guinee et al., 2000). The pH was measured at each of the sampling points using a cheese slurry prepared by macerating a blend of the grated cheese (20 g) and heated (40°C) distilled water (12 g).

2.3.2 Proteolysis

A water-soluble extract of the cheese was prepared from a slurry of cheese and water at a weight ratio of 1:2 (Fenelon and Guinee, 2000), and a 3 ml sample of the filtrate was analysed for WSN using macro-Kjeldahl method (IDF, 1993). Urea–polyacrylamide gel electrophoresis (urea–PAGE) was performed on a PROTEANs II xi cell vertical slab gel unit (Bio-Rad Laboratories Ltd., Hemel Hempstead, Herts, UK), using a separating and stacking gel system, as described by Rynne et al. (2004). The sample buffer (pH 8.7) was prepared by dissolving 0.75 g Tris (hydroxymethyl)- methylamine, 49 g urea, 0.7 ml mercaptoethanol and 0.15 g bromophenol blue in distilled H$_2$O, made up to a final volume of 100 ml. Cheeses were dissolved based on a defined quantity of protein (4.25 g/l sample buffer) and also on a defined quantity of cheese (16 g/l sample buffer). The dissolved samples were incubated at 55°C for 60 min and filtered through glass wool to remove fat deposits. The gels (1 mm thick) were pre-
run at 280 V for 40 min prior to sample loading. Gels were run at 280 V for 30 min, for the stacking gel, and at 300 V for the separating gel until the tracking dye was approximately 1 cm from the end of the plates. Gels were stained in Coomassie Blue G250, destained in acetic acid (10%)/methanol (25%) mixture, and rinsed in distilled water.

2.3.3 Texture profile analysis and firmness

Six cube-shaped samples (2.5 cm) were cut from each cheese, wrapped tightly in tin foil and stored refrigerated (4°C) overnight. Each cube was taken from the refrigerator and immediately compressed, in a direction perpendicular to the direction of fibre orientation, to 60% of its original height in two consecutive bites at a rate of 60 mm/min on a TAHDi texture profile analyser (TPA) (Stable Micro Systems, Goldalming, Surrey, England) at room temperature. A typical TPA (force/distance) profile is shown in Fig. 1. The following parameters were calculated from the TPA profile: hardness, defined as the maximum force recorded in bite 1 (height H1); cohesiveness, ratio of area during compression in bite 2 to area in bite 1 (A2/A1); adhesiveness, area of the negative peak formed when the cross-head (plunger) is withdrawn from the sample after the first bite (A3); springiness, as the ratio of the distance travelled by the plunger in bite 2 (C) to the distance travelled during bite 1 (B); and chewiness, product of hardness × cohesiveness × springiness (H1×A2/A1×C/B).

Firmness was measured in a single-bite compression test, in which each cheese cube was compressed to 30% of its original height. None of the cheese samples fractured during compression; firmness, was calculated as the force (height) at full compression.
Fig. 1 A typical force-distance profile by TPA on compressing a cube-shaped sample of Mozzarella-style to 40% of its original height cheese at a rate of 60 mm/min at 8°C. A1 refers to area under the force-distance curve in compression (bite) 1, A2 to the area under the curve in bite 2, A3 to the area of the negative force on de-compressing after bite 1, B to the compression distance in bite 1, and C to the compression distance in bite 2.

2.3.4 Non-expressible serum

Levels of expressible cheese serum (ES) expressed during centrifugation at 12,500×g for 75 min at 25°C, were measured as described by (Guinee et al., 2002). Expressed serum and fat were collected in a Duran bottle (Duran Group GmbH, Mainz, Germany) and stored at 4°C until the fat layer had solidified. The fat layer was punctured and the subnatant serum was then collected and weighed to give the level of expressible serum. The level of non-expressible serum was then calculated by subtracting the weight of expressible serum per 100 g cheese from the percentage moisture, and expressing as gram per gram of protein (NESP g/g protein).
2.3.5 Functionality of heated cheese

Heat-induced melting was measured by expressing a change in dimension (e.g. diameter, length) as a % of the original dimension on heating using (1) a modified Schreiber method and (2) a modified Olson and Price method (Guinee and O’Callaghan, 2013). The former is indicative of the melting behaviour of cheese when exposed as a topping (e.g. on pizza) during oven heating, and the latter when the cheese is covered, or largely covered, during cooking (e.g. as a cheese slice in hamburger).

The work required to extend the molten cheese (EW) was measured using uniaxial extension on a TA-HDi Texture Analyser (Fig. 2). Grated cheese (60 g) was filled into a plastic container (9 cm × 5.5 cm × 4 cm) containing a comb, placed in a microwave oven (Whirlpool MW201, Fonthill Industrial estate, Dublin 22, Ireland) set at 750 W, and heated for 55 s to 90 to 95°C, to mimic cooking temperatures on a pizza pie. The plastic container with the molten cheese was immediately placed in the measuring cell of the TAHDi texture profile analyser. The comb was attached to the cross-head, which was programmed to pull the comb, and thereby extend the molten cheese, to a distance of 380 mm at a rate 1 cm/s. The temperature, as measured using a non-contact infrared thermometer (Optex Thermo-Hunter PT-3S; Graham and White Instruments Ltd, St Albans, Hertsfordshire, UK), decreased from ~90°C for the molten cheese mass on removal from the oven to ~35–45°C of the strings at maximum extension. Using the resultant force-distance curve (Fig. 2), the EW (in millijoule), defined as the force (in newton) by distance (in millimetre), was calculated as the area beneath the curve.
Fig. 2 A typical force-distance curve obtained on extension of hot molten cheese (94°C) by 380mm at a rate of 10 mm/s. The top inset shows the plastic container (A) with the extension device consisting of a shaft (B) that connects to the cross-head of the TA-HDi Texture Analyser and a comb (C) embedded in the molten cheese, and the bottom inset, the molten cheese undergoing uniaxial extension.

2.4 Statistical analysis

Three replicate cheesemaking trials were undertaken, each consisting of six treatment cheeses (FFFS, FFRS, RFFS, RFRS, RFFSLC, RFRSLC) varying in fat, salt and calcium content. A randomised complete block design applied to the six treatments and three blocks (replicate trials) was used for analysis of the response variables relating to cheese composition at 7 days, and, in some cases, to compare different treatments after a given ripening time. Analysis of variance (ANOVA) was undertaken using SAS version 9.3 (SAS Institute, 2004). The level of significance was established at P<0.05. Tukey’s multiple-comparison test was used for paired comparison of treatment means and the level of significance was determined at P<0.05.
A split plot design was utilised to measure the effects of treatment, maturation time and the interaction thereof on the response variables as measured at specific intervals throughout ripening. Analysis of variance for the split plot design was carried out using a general linear model (GLM) procedure of SAS 9.3 (SAS Institute, 2004). Differences of statistical significance (p<0.05) were established using Fisher’s least significant difference.

3 Results

3.1 Cheese composition

The gross compositions of the treatment cheese are shown in Table 1. The composition of FFFS complied with the definition of LMPS Mozzarella as specified by the FDA (CFR, 2014) and is similar to that reported previously for commercial Mozzarella (Guinee et al. 2000). Based on the specification for low-moisture part skim Mozzarella (30≤fat-in-dry matter<45 g/100g; 45 < moisture ≤52 g/100g), the fat content based on the median fat-in-dry-matter (FDM) value (37.5 g/100g cheese) at the minimum and maximum moisture levels are 18.0 and 20.6 g/100g, respectively. EU legislation specifies a 30% reduction in fat content for compliance to the term reduced-fat (EU Regulation EC No 1924 2006). All the experimental reduced-fat cheeses, both full-salt and reduced-salt, had fat levels <12 g/100g, and can therefore, be classified as reduced fat.

Reducing the fat content of the full-salt and reduced-salt cheeses resulted in significant increases in moisture and protein, and decreases in FDM and moisture-in-non-fat substances (MNFS). Comparison of corresponding pairs of cheese (FFFS and FFRS, RFFS and RFRS, and RFFSLCa and RFRSLCa) showed that apart from its effect on salt-in-moisture content, reducing salt content had otherwise little impact on the composition of the 7-day-old cheese, probably because of the small difference in salt content between the full-salt and reduced-salt levels (~1.8% versus 1.1%). However, the FFRS cheese had a higher fat content and lower
protein content than the FFFS cheese; this may be due to differences in fat losses during the kneading/stretching process in hot water rather than to the impact of salt *per se*.

Reducing the calcium content of the RFFS and RFRS cheeses resulted in significant increases in the contents of moisture and MNFS and pH, and a concomitant reduction in level of protein.
Table 1 Effect of reducing salt, fat and calcium on 7 day-old Mozzarella cheese

<table>
<thead>
<tr>
<th>Composition</th>
<th>FFFS</th>
<th>FFRS</th>
<th>RFFS</th>
<th>RFRS</th>
<th>RFFSLCa</th>
<th>RFRSLCa</th>
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<tr>
<td>Moisture (% w/w)</td>
<td>47.6A</td>
<td>48.0B</td>
<td>50.4Ay</td>
<td>50.1Ay</td>
<td>56.7ax</td>
<td>55.8ax</td>
</tr>
<tr>
<td>Protein (% w/w)</td>
<td>25.4Ab</td>
<td>23.4bb</td>
<td>32.1Aax</td>
<td>32.7Aax</td>
<td>28.2ay</td>
<td>29.4ay</td>
</tr>
<tr>
<td>Fat (% w/w)</td>
<td>21.5bA</td>
<td>23.1Aa</td>
<td>11.4Abx</td>
<td>11.0Bx</td>
<td>9.0ay</td>
<td>10.6ax</td>
</tr>
<tr>
<td>Salt (% w/w)</td>
<td>1.61Aa</td>
<td>1.03bb</td>
<td>1.78Aax</td>
<td>1.13Bax</td>
<td>1.77ax</td>
<td>0.94bx</td>
</tr>
<tr>
<td>Ca (mg 100 g⁻¹)</td>
<td>761Aa</td>
<td>655Bb</td>
<td>942Ax</td>
<td>1009Ax</td>
<td>551Ay</td>
<td>506Ay</td>
</tr>
<tr>
<td>Ca (mg g⁻¹ protein)</td>
<td>30.0Aa</td>
<td>28.0Aa</td>
<td>29.4Aax</td>
<td>30.9Aax</td>
<td>19.6Ay</td>
<td>17.2by</td>
</tr>
<tr>
<td>MNFS (% w/w)</td>
<td>60.7Aa</td>
<td>62.5Aa</td>
<td>56.9Abx</td>
<td>56.2Bby</td>
<td>62.3Ax</td>
<td>62.4Ax</td>
</tr>
<tr>
<td>S/M (% w/w)</td>
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<td>2.14bb</td>
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<td>1.69by</td>
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<tr>
<td>FDM (% w/w)</td>
<td>41.06bA</td>
<td>44.41Aa</td>
<td>22.94Bbx</td>
<td>22.15Bbx</td>
<td>20.68ax</td>
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<tr>
<td>NESP (g g⁻¹ protein)</td>
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<td>1.51Aa</td>
<td>1.51Aay</td>
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<td>5.20Aay</td>
<td>5.29Aay</td>
<td>5.76ax</td>
<td>5.66ax</td>
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</table>

¹Cheese code: Treatment cheeses, described in detail in Materials and Methods, included the following: full-fat, full salt (FFFS); full-fat, reduced-salt (FFRS); reduced-fat, full-salt (RFFS); reduced-fat, reduced-salt (RFRS), reduced-fat, full-salt with low calcium content (RFFSLCa); and reduced-fat, reduced-salt with low calcium content (RFRSLCa).

²Presented data represent the means of three replicate trials for each of the treatment Mozzarella cheeses.

³Abbreviations: Ca, calcium; MNFS, moisture-in-non-fat substances; S/M, salt-in-moisture; FDM, fat-in-dry matter; NESP, non-expressible serum.

Values within a row not sharing a common lowercase superscript differ significantly for the statistical effect of salt content on the full-fat cheeses (FFFS, FFRS), the reduced-fat cheeses (RFFS, RFRS), and reduced-fat cheese with low calcium level (RFFSLCa, RFRSLCa) (P < 0.05).

Values within a row not sharing a common uppercase superscript differ significantly for the effect of reducing fat content on the full-salt (FFFS, RFFS) and reduced-salt (FFRS, RFRS) (P < 0.05).

Values within a row not sharing a common uppercase superscript differ significantly for the effect of reducing calcium content on the reduced-fat full-salt (RFFS, RFFSLCa) and reduced-fat reduced-salt (RFRS, RFRSLCa) cheeses (P < 0.05).
3.2 Proteolysis

3.2.1 Urea-polyacrylamide gel electrophoresis (PAGE)

The urea-PAGE gel electrophoretograms of the six different cheeses applied to the gel on the basis of a fixed weight of protein or a fixed weight of cheese are shown in Figs. 3 and 4, respectively.

For all cheeses, αs1-casein was degraded slowly during ripening mainly into αs1-CN (f24-199), but also to breakdown products of lower, αs1-CN (f102-199), and of higher electrophoretic mobility such as αs1-CN (f121-199), αs1-CN (f33-), αs1-CN (f104-199), as identified according to Mooney et al. (1998). Comparatively little hydrolysis of β-casein was noted, even though low intensity bands of γ3-, γ2- and γ1-caseins and β1-CN (f1-192) were present at all ripening times at intensities depending on the cheese treatment. When loading the gel with a fixed weight of cheese (Fig. 3), the electrophoretogram reflects the differences in protein content and its significance in relation to rheology melt characteristics and sensory properties. The levels of intact αs1-casein and β-casein in 1-day-old cheeses increased as the fat content was reduced, as indicated by the higher intensity of these bands in the RFFS and RFRS cheeses compared to FFFS and FFRS cheeses. This trend, which is expected because of the inverse relationship between fat and protein content (Table 1), is less evident at later times, especially for αs1-casein at ≥35 days, owing to differences in the rates of degradation of αs1- and β-caseins between the different cheeses.

The extent of proteolysis in the different cheese treatments, without the confounding effect of differences in total protein content due to variation in compositions of the different cheeses, can be seen from the electrophoretogram with a fixed weight of protein (Fig. 3). Reducing the salt content of the full-fat cheese led to a slight increase in the degradation of αs1-CN, as reflected by the slightly higher intensities of αs1-CN (f24-199) in the FFRS cheese at 35 and 50 days compared to corresponding FFFS cheese. In agreement with the trend in WSN, salt
reduction had no notable effect on the casein breakdown in the RF cheeses (RFFS, RFRS). Reducing salt content resulted in higher levels of β-CN (f1-192) but otherwise had little impact on the extent or pattern of β-casein degradation. Reducing the fat content of the 35 and 50-day-old FS and RS cheeses resulted in higher levels of intact α_s1-CN and lower concentration of α_s1-CN (f24-199).

Reducing the calcium content of the RFFS and RFRS cheeses resulted in notable increases in the degradation of α_s1-CN at all times, especially at 50 days where essentially all the native α_s1-CN had been degraded. Similarly, degradation of β-casein was more extensive in the reduced-calcium cheeses (RFFSLCa, RFRSLCa) at 35 and 50 days, especially in the RFRSLCa in which significant accumulation of the peptide β-casein (f1-192) occurred.
Fig. 3 Urea-polyacrylamide gel electrophoretograms of Mozzarella-style cheeses (lanes 1-6) loaded with a fixed weight of protein (4.25 mg protein per lane) after ripening at 4°C for 1 or 7 days (a) and for 35 or 50 days (b). The cheeses, defined in Table 1, include: FFFS, lane 1; FFRS, lane 2; RFFS, lane 3; RFRS, lane 4, RFFSLCa, lane 5; and RFRSLCa, lane 6. Sodium caseinate (lane C), loaded at 4.25 mg protein per lane, was used as control, unhydrolyzed casein. Proteins and peptides 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-*); 11, αs1-CN (f104-199).
Fig. 4 Urea-polyacrylamide gel electrophoretograms of Mozzarella-style cheeses (lanes 1-6) loaded with a fixed weight of cheese (16 mg protein per lane) after ripening at 4°C for 1 or 7 days (a) and for 35 or 50 days (b). The cheeses, defined in Table 1, include: FFFS, lane 1; FFRS, lane 2; RFFS, lane 3; RFRS, lane 4; RFFSLCa, lane 5; and RFRSLCa, lane 6. Sodium caseinate (lane C), loaded at 4.25 mg protein per lane, was used as a control unhydrolyzed casein. Proteins and peptides 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-*); 11, αs1-CN (f104-199).
3.2.2 Water-Soluble Nitrogen

The level of WSN, which is indicative of the degree hydrolysis of the para-casein by residual chymosin or plasmin, increased in all cheeses during ripening (Fig. 5).

The level of WSN in the FF (FFFS, FFRS) and RF (RFFS, RFRS) cheeses was significantly affected by ripening time, but not by salt content. In contrast, WSN in the RFLCa (RFFS, RFRS) cheese was affected by salt content, with the mean level in the RFRSLCa cheese over the ripening period being significantly lower than that of the RFFSLCa cheese (Table 2).

Reducing fat content significantly decreased the mean level of WSN in the FS cheese over ripening period but did not significantly affect the level in the RS cheese. The latter result however contrasts with urea PAGE which showed less intact αs1-casein in the RFRS cheese compared to the RFFS cheese. Calcium reduction significantly increased the mean value of WSN over ripening in the RFFS cheese, but not in the RFRS cheese. Moreover, ANOVA indicated that the mean WSN level in the RFRSLCa cheese over the ripening was significantly higher than that of the RFFS cheese and FFFS cheese.
Fig. 5 Changes in water-soluble nitrogen (WSN) in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include: a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
Table 2 Statistical significances (P-values) for changes in different compositional and rheological parameters of Mozzarella-style cheese on reducing fat in full-salt and reduced-salt cheeses, salt in full-fat and reduced-fat cheeses, and calcium content in reduced-fat full-salt and reduced-fat reduced-salt cheeses\(^1\,2\,3\)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pH</th>
<th>NESP</th>
<th>WSN</th>
<th>Cohesiveness</th>
<th>Adhesiveness</th>
<th>Hardness</th>
<th>Springiness</th>
<th>Gumminess</th>
<th>Chewiness</th>
<th>Firmness</th>
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<tr>
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<td>&lt;0.001</td>
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<tr>
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### Reduced-fat, full-salt cheese

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### Reduced-fat, reduced-salt cheese

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1. There was 1 degree of freedom (df) for fat level, salt level and calcium level, 5 for ripening time and 5 for the interactions.

2. ANOVA was carried out using a general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC), where the effects of treatment and replicates were estimated.

3. Abbreviations: NESP, non-expressible serum; WSN, water soluble nitrogen.
3.3 Changes in non-expressible serum

The level of non-expressible serum (NES), expressed as gram per gram of protein (NESP) is an index of the water-holding capacity (WHC) of the cheese (Guo et al., 1997), with higher levels of NESP corresponding to a higher WHC (Guinee et al. 2002). NESP is an important attribute in cheese intended for cooking, as it affects the ability of the matrix to retain moisture during heating and, hence, the cooking properties, such as flowability, stretchiness and fluidity (Guo et al., 1997).

The level of NESP in the FF cheeses was significantly affected by salt content, ripening time and interaction of ripening time and salt content, with the mean level over the 75-day ripening period being significantly higher in the FFRS than in the FFFS cheese (Table 2, Fig. 6). Hence, ANOVA of the data at individual ripening times indicated that the levels of NESP in FFFS were lower than those of the FFRS at times ≥15 days. In contrast to the FF cheese, the NESP levels in the RF and RFLCa cheeses were not influenced by salt content but were significantly affected by ripening time and the interaction between ripening time and salt content.

The level of NESP in the FS and RS cheese was significantly affected by fat content and the interaction of fat content and ripening time (Table 2, Fig. 6), with the mean level in the RF variants (RFFS, RFRS) over the 75-day ripening period being significantly lower than that of the corresponding FF variants (FFFS, FFRS). Moreover, ANOVA indicated that the level of NESP in the FFFS cheeses was significantly higher than that in the RFFS cheese at ripening times ≥35 days, while the level in the FFRS were higher than those in the RFRS cheese at times ≥15 days.

Reducing the calcium content of the RFFS and RFRS cheeses significantly increased the mean level of NESP over the 75-day ripening period and at all ripening times.
Fig. 6 Changes in levels of non-expressible serum in Mozzarella-style cheese during ripening at 4°C. The cheeses, defined in Table 1, include: a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
3.4 Rheology and texture profile analysis

The changes in firmness (force on compression of cheese cube to 30% original height in a single compression stroke or bite at 8°C) and different texture profile parameters (hardness, adhesiveness, cohesiveness, chewiness and springiness) on 40% compression in a two-bite test) during ripening are shown in Figs. 7, 8, 9 and 10. None of the cheeses fractured during compression.

The firmness, hardness, chewiness, adhesiveness and cohesiveness in FF and RF cheeses were significantly affected by salt content, ripening time, and, in most cases, by the interaction of salt and ripening time (Table 2). Consistent with the age-related increase in casein hydrolysis, the mean values for all of these parameters decreased significantly in all cheeses during ripening, apart from adhesiveness which increased.

Reducing salt content of the full-fat (FFFS) and reduced-fat cheeses (RFFS, RFFSLCa) resulted in significant decreases in the mean values for hardness and chewiness over the 75-day ripening period (Table 2), but did not affect cohesiveness. Conversely, the mean adhesiveness of these cheeses over the ripening period increased with reduction in salt content.

Firmness and all TPA parameters of the full-salt (FS) and reduced-salt (RS) cheeses were significantly affected by fat content and by the interaction of fat content and ripening time in the case of firmness, hardness, adhesiveness and chewiness (Table 2). Reducing fat content significantly increased the mean firmness, hardness, chewiness and cohesiveness of both the FS and RS cheeses over ripening. In contrast to the above, the mean adhesiveness of both the FS and RS cheeses over the ripening period increased significantly as the fat content was reduced (Fig. 9).

Reducing calcium content of the RFFS and RFRS cheeses significantly reduced the mean values for firmness, hardness and chewiness over the 75-day ripening period. In contrast the mean adhesives of the RFFS cheese increased, while that of the RFRS decreased, as the
calcium content was reduced. As indicated by the significant interactive effects between calcium content and ripening time, the response of the above variables to ageing differed depending on calcium level, and on salt content as discussed above (Table 2). Similar to the trend noted for influence of salt content, cohesiveness in both the RFFS and RFRS cheeses was unaffected by calcium level in the range studied.
Fig. 7 Changes in hardness in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include: a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
Fig. 8 Changes in chewiness in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include: a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
Fig. 9 Changes in adhesiveness in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include: a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
Fig. 10 Changes in firmness in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include: a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
3.5 Meltability

The age-related change in the flowability of the heated cheese was measured using modifications of the Schreiber (Sch-flow, Fig. 11) and Price-Olson (PO-flow, data not shown) methods, as described by Guinee and O’Callaghan (2013). These tests simulate, respectively, the extent of flow or spread of cheese in cooking applications where cheese is exposed to the oven atmosphere (such as pizza and cheese bruschetta), or not exposed, as in closed sandwiches and burgers.

The Sch-flow and PO-flow of the FF and RF cheeses were significantly affected by salt content, by ripening time and by the interaction of salt and ripening time (Table 3). The flow of all cheese increased significantly during ripening, as the level of proteolysis increased. This increase was generally most pronounced during the first 15 days during which the αs1-casein was extensively hydrolysed (Fig. 11).

Using both methods, the mean flow of the FF cheeses over the 75-day ripening period increased significantly as the salt content was reduced. The difference in flow between the FFFS and FFRS was greatest at 1 day (25% and 44%, respectively) and remained essentially constant between 15 and 75 days, at which time the respective flows for the latter cheeses were 55% and 65%. While a similar trend was noted for the RF cheese, the difference in the magnitude of the means over the 75-day ripening period or the values at any specific ripening time were relatively small, for example ~34% vs. 37% at 75 days, and would be of little practical significance (Guinee, 2015). Reducing the fat content of the FS and RS cheeses resulted in notable decreases in the mean flow over the entire ripening period, with the percentage reduction being most pronounced for the Sch-flow (49% and 52% for the FS and RS, respectively) than for the PO-flow (36% and 29% for the FS and RS, respectively). Moreover, ANOVA indicated that the flow at all ripening times (Fig. 11) was significantly
lower in the RF cheeses, for example from ~34% in the RFFS cheese vs. 55% for the Sch-flow in the FFFS at 75 days.

The Sch-flow and PO-flow of the RFFS and RFRS cheeses was significantly influenced by calcium level and by its interaction with ripening time (Table 3). The mean Sch- and PO-flow of the RFFS and RFRS cheeses over the ripening period increased significantly as the calcium content was reduced (Fig. 11). Despite the increase in flow of the RF cheeses on reducing calcium content, the flow of the RFFSLCa and RFRSLCa cheeses was, nevertheless, notably lower than that of the FFFS and FFRS cheeses at all times.
Fig. 11 Changes in Schreiber flow (Sch flow) in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include a) full-fat and reduced-fat cheeses with different salt levels: FFFS (●), FFRS (○), RFFS (■) and RFRS (□); and b) reduced-fat cheeses with different salt and calcium levels: RFFS (■), RFRS (□), RFFSLCa (▲) and RFFSLCa (△). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
Table 3  Statistical significances (P-values) for changes in cooking properties of heated Mozzarella-style cheese on reducing fat in full-salt and reduced-salt cheeses, salt in full-fat and reduced-fat cheeses, and calcium content in reduced-fat full-salt and reduced-fat reduced-salt cheeses\textsuperscript{1,2,3}

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\textsuperscript{1}There was 1 degree of freedom (df) for fat level, salt level and calcium level, 5 for ripening time (except in the case of EW where there were 2) and 5 for the interactions (except in the case of EW where there was 2).

\textsuperscript{2}ANOVA was carried out using a general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC), where the effects of treatment and replicates were estimated.

\textsuperscript{3}Abbreviations: Flow-Sch and Flow-PO denote % flow of heated cheese as measured using modified Schreiber and Price-Olson methods, respectively; EW: Work required for uniaxial extension of the molten cheese by 380 mm.
3.6 Energy required extend/stretch (EW)

The work required to uniaxially extend (EW) the molten cheese was measured as an indicator of the toughness of the melted Mozzarella on pizza. The EW of the molten FF and RF cheeses was significantly affected by salt content, by ripening time and by the interaction of salt and ripening time in the case of the latter (Fig. 12; Table 3). The mean EW of the RS cheeses over the ripening period was lower than that of the corresponding FS cheeses. The EW of all cheeses, apart from the FFRS cheese, decreased significantly between 35 and 75 days, with the percentage decrease being highest for the reduced-fat cheeses.

The EW of the full-salt (FS) and reduced-salt (RS) cheeses were significantly affected by fat content, and by the interaction of fat content and ripening time (Table 2). Reducing the fat content significantly increased the mean EW of both the FS and RS cheeses over ripening, with the difference between FF and RF variants diminishing as ripening time progressed.

The EW of the reduced-fat cheeses was significantly influenced by calcium level, with the mean EW for the RFFSLCa and the RFRSLCa over the ripening period being lower than those of the RFFS and RFRS cheeses. However, the magnitude of the calcium effect on EW was small compared to the effect of fat. Hence, the mean EW of the RFFSLCa or RFRSLCa cheeses over the ripening period was significantly higher than that of the corresponding FFSS and FFRS cheeses. Nevertheless, the difference between the above cheeses (RFFSLCa vs. RFFS, and RFRSLCa vs. FFRS) diminished between 35 and 75 days, at which time the EW of all four cheeses was similar. In contrast, the EW of the RFFS and RFRS were significantly higher than that of the FFSS and FFRS at all ripening times.
Fig. 12 Changes in EW (work required to uniaxially extend molten cheese by 380mm) in Mozzarella cheeses during ripening at 4°C. The cheeses, defined in Table 1, include a) full-fat and reduced-fat cheeses with different salt levels: FFFS ( ), FFRS ( ), RFFS ( ) and RFRS ( ); and b) reduced-fat cheeses with different salt and calcium levels: RFFS ( ), RFRS ( ), RFFSLC ( ) and RFRSLC ( ). Presented values are the means of 3 replicate trials; error bars represent standard deviations of the mean.
4 Discussion

Consistent with earlier studies for Cheddar (Guinee et al., 2000) and Mozzarella (Rudan et al., 1999; Paulson et al., 1998), reducing fat content resulted in a higher level of protein and lower content of MNFS. However, reducing the calcium content of reduced-fat cheese resulted in a significant increase in the content of MNFS, and reduction in both the level of protein and calcium-to-protein ratio. The latter effects, which concur with those reported by others for Mozzarella (Guinee et al., 2002; Uperti et al., 2006), are consistent with a higher degree of casein hydration (Table 1, Fig. 6), which in model systems has been found to be inversely correlated to casein bound calcium (Carr et al., 2002). The general absence of an effect of salt content on composition supports the findings of Paulson et al. (1998) whose data show no relationship between salt content and moisture or MNFS of non-fat Mozzarella, in the range 0.14–2.18% salt. However, it is at variance with studies on Cheddar cheese (Rulikowska et al., 2013) which reported that moisture content decreased on average by \(-0.14\%\) per 0.1% increase in salt content in the range 0.14% to 2.0%.

The level of primary proteolysis in the control FFFS cheese (WSN \(\sim\)10–12 g/100g of total N at 45–60 days), as monitored using urea-PAGE and level of WSN, is typical of that previously reported for Mozzarella stored at 4 or 8°C (Rudan et al., 1999; Feeney et al., 2001). The increase in proteolysis during ageing was affected by fat content, with fat reduction leading to a slower rate of degradation of \(\alpha_s\)-CN and development of WSN in the full-salt cheese, and salt reduction. The breakdown of \(\alpha_s\)-CN was inhibited by fat reduction, and accelerated by reduction in calcium level and, to a lesser extent, salt. Moreover, reduction in calcium level resulted in more extensive degradation of \(\beta\)-CN to \(\beta\)-CN (f1-192). These trends reflect the findings of previous studies for the effects of reducing fat (Rudan et al., 1999; Fenelon and Guinee, 2000), salt (Rulikowska et al., 2013) and calcium (Feeney et al., 2002) in various cheeses including Cheddar and Mozzarella.
The level of NESP in all cheeses during maturation, reflecting an enhanced water binding capacity of the protein (Guo et al., 1997; Guinee et al., 2002; Metzger et al., 2001; McMahon et al., 1999; Paulson et al., 1998), probably as a consequence of solubilisation of calcium phosphate (O’Mahony et al., 2006) and protein hydrolysis (Guo et al., 1997). The reduction in NESP as fat level was reduced concurs with the reduction in MNFS content, and may reflect a higher degree of casein aggregation in the reduced-fat cheese. The level of NESP was not influenced by the altering salt content, except in the FF cheese where the mean level over the 75 day ripening period increased as salt level was reduced. This trend differs from that reported by Paulson et al., (1998) who found that ES in non-fat Mozzarella decreased as the salt and salt-in moisture levels in non-fat Mozzarella were increased from 0.14% to 0.85%, and 0.23% to 1.40%, respectively. These inter-study differences may relate to differences in composition, especially factors that affect protein hydration such as protein-to-moisture ratio and calcium-to-casein ratio (Strange et al., 1994; Bouchoux et al., 2009); the calcium-to-protein ratio of the RF cheeses in the current study (29–31 mg/g in the RF cheeses and 17–20 mg/g in the RFLC cheeses) was notably higher than that (12–13 mg/g) in Paulson et al. (1998). The large increase in NESP in the RF cheese on reducing the calcium content is commensurate with the decrease in the extent of casein crosslinking by calcium (e.g., attached to phosphoserine groups and acidic amino acid residues) and the inverse relationship between the hydration of casein and para-casein and calcium content in model dilute protein dispersions (Creamer, 1985; Carr et al., 2002). Hence, a similar trend has been reported previously for Mozzarella (Guinee et al., 2002).

The firmness, hardness and chewiness generally decreased, while adhesiveness increased, to varying degrees during ripening depending on levels of fat, salt and calcium. Similar trends for the effect of maturation have been reported for firmness and hardness in Mozzarella (Yun et al., 1993) and other internal bacterial ripened cheeses such as Cheddar and
Gouda which do not undergo moisture loss during maturation (Creamer and Olson, 1982; Fenelon and Guinee, 2000). This trend is expected as the latter parameters are indices of the stress-bearing capacity of the casein network, which is attenuated by the decrease in the content of intact casein and by hydration of the protein strands and the attendant increase in the ratio of viscous-to-elastic character. In contrast, adhesiveness, a measure of work necessary to overcome the attractive forces between the cheese and compression plate (O’Callaghan and Guinee, 2004), generally increased during ripening, a trend that is consistent with the increase in the hydration which is expected to reduce the cohesive (attractive) forces with the calcium phosphate para-casein network. Reducing salt content had a similar, though smaller, effect to ageing, i.e. lower firmness, hardness and chewiness, and higher adhesiveness. Chevanan et al., (2006) found that the response of hardness, chewiness and adhesiveness to salt level in Cheddar cheese depended on concentrations of lactose and calcium phosphate; a trend similar to that of current study was noted in Cheddar denoted as ‘high calcium phosphate and high lactose’. Reducing calcium content had similar, but notably larger effects than salt, namely reductions in firmness and chewiness, an increase in adhesiveness, and no effect on cohesiveness. Similar trends were reported by Chevanan et al. (2006) for hardness and adhesiveness and may be attributed to a lower degree of calcium cross-linking of the casein strands and the concomitant increase in casein hydration in the reduced-calcium cheeses (Guinee et al., 2002). However, the current results contrasted with those of Chevanan et al. (2006) who found that lowering calcium content significantly reduced cohesiveness to an extent influenced by levels of lactose and salt-in-moisture. The inter study differences in relation to the latter parameter may be associated with differences in the type of cheese (Mozzarella and Cheddar), fat and moisture content of cheese, and the levels of salt content and calcium-to-casein ratio which varied from 2.5% to 1.7% and 26.2 to 20.6 mg/g protein in the study of Chevanan et al. (2006) compared to 1.7% to 1.0% and 30.9 to 17.2 mg/g protein in the current study.
In contrast to the effects of ageing, reducing fat content significantly increased the mean firmness, hardness, chewiness and cohesiveness of both full-salt and reduced-salt cheeses over ripening. This trend, which concurs with the findings of Rudan et al. (1999) and Drake et al. (1999), is consistent with the higher concentration of intact protein and, hence, volume fraction of casein network in the reduced-fat cheeses (Guinee, 2015). Hence, as a corollary adhesiveness decreased as the fat content was reduced in full-salt and reduced-salt cheeses because of the concomitant reductions in the levels of MNFS and casein hydration.

The heat-induced flow of all cheeses increased during ageing. This trend, which has been extensively reported for various cheese types including Mozzarella and Cheddar, has been attributed to the reduction in intact casein content, solubilisation of calcium and increases in casein hydration (Guo et al., 1997; Guinee et al., 2000). Similarly, the flow was increased by reduction in the levels of salt or calcium level. Similar trends were reported for the effect of salt by Ma et al. (2013) and for calcium by Pastorino et al. (2003) and O’Mahony et al. (2006). This effect, more pronounced with calcium, is likely to be associated with attenuation of the casein network by greater hydrolysis of the casein on reducing salt and by the reduction in the concentration of calcium induced crosslinking on reducing calcium content (Guinee, 2015). In contrast to the effects of reducing salt or calcium level, fat reduction significantly reduced the flow of FS and RS cheeses. This trend corroborates the findings of previous studies (Rudan et al., 1999; Guinee et al., 2000), and is associated with a strengthening of the cheese matrix, due to the attendant increase in the volume fraction of the casein network, the reduction in the lubrication effect of fat and protective effect of free oil in suppressing moisture evaporation from the melting cheese surface (Rudan et al., 1998; Guinee, 2015).

The EW of the molten Mozzarella decreased with ripening time and with reduction in calcium content. The effects of ripening time and calcium are consistent with the associated increase in casein hydrolysis and decreases in the levels of intact casein (Addeo and Masi,
1992; Ak and Gunasekaran, 1995) and degree of casein crosslinking which would attenuate the tensile strength of the para-casein fibres. Hence, as a corollary, the increase in casein concentration with fat reduction coincided with an increase in the mean EW of the FS and RS cheeses. Reducing salt content led to a significant decrease in the mean EW of the RF cheese but not in the FF cheese. A tentative explanation for this effect is a concentration-dependent, salt-induced change in ultrastructure (Paulson et al., 1998).

5. Conclusion

Simultaneous reduction of fat from ~22% to 11% and salt from 1.7% to 1.0% in Mozzarella cheese resulted in higher levels of moisture and protein, and lower levels of MNFS, FDM and SM. Compared to the FFFS cheese, the RFRS cheese had lower mean levels of non-expressible serum, WSN, adhesiveness, heat-induced-flowability and higher mean levels of firmness, hardness, chewiness, cohesiveness and EW over the 75-day ripening period. These effects, apart from salt-in-moisture content, were mainly due to the effect of fat. While salt reduction reduced firmness and chewiness and increased the mean adhesiveness of the FF cheese (and in some cases the RF cheese) over the 75-day ripening period, the magnitude of the changes caused by reducing salt level was notably smaller than that affected by reducing fat content. Reducing the calcium content of the RFRS cheese by ~50%, from ~1,000 to 506mg/100g by pre-acidification of the cheese milk, significantly increased the level of moisture, MNFS and NESP, and reduced the protein content and calcium-to-casein ratio. These changes counteracted the adverse effects of fat reduction to greater or lesser degrees by reducing its calcium-to-casein ratio from ~30 to 17 mg/100g. Nevertheless, the RFRSLCa cheese was firmer, harder, more cohesive, gummy and chewy, less adhesive, and flowed less and required greater energy to extend on heating compared to the FFFS or FFRS cheese.
Acknowledgments The research leading to these results has received funding from the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement KBBE-2011-5-289536 (PLEASURE).

References


Chapter 3: Sensory quality of unheated and heated Mozzarella-style cheeses with different fat, salt and calcium levels*

* Published in International Journal of Dairy Technology.

Abstract

The current study evaluated the effects of reducing fat from 23% to 11% and salt from 1.6 to 1.0% on the texture profile attributes, volatile compounds and sensory characteristics of unheated and heated (95°C) Mozzarella-style cheeses after 15 and 35 days of storage at 4°C. Reducing fat content significantly reduced the sensory acceptability, mainly because of associated increases in firmness, rubberiness, chewiness and reduction in fat flavour and cheese flavour in the unheated and heated cheeses. Reducing salt content had relatively minor effects. Reducing calcium content counteracted some of the negative sensory attributes associated with reduced-fat cheese.
1. Introduction

The acceptability of cheese to the consumer is influenced by many factors including sensory properties, usage properties, convenience of use, price and nutritional value. The sensory properties in turn depend on flavour constituting taste and aroma, texture and rheological properties and appearance (Delahunty and Drake, 2004). Most of the studies on the sensory analysis of cheese have examined the relationship between sensory properties of the unheated cheese and its compositional-based properties (e.g., gross composition, levels of proteolysis, lipolysis and/or volatile compounds) or rheological properties. Previous studies (Vangtal and Hammond, 1986; Virgili et al., 1994; Pinho et al., 2004 and Hou et al., 2013) have shown correlations between volatile profiles, cheese composition, free fatty acid content and their flavour and aroma profiles in a variety of cheeses, including Swiss-type, Parmigiano-Reggiano, Terrincho and Cheddar investigating the correlations between a number of cheese attributes.

Lee et al. (1978) reported significant correlations between sensory parameters hardness, springiness and adhesiveness and the corresponding attributes as measured using texture profile analysis (TPA). Similarly, Chen et al. (1979) using linear regression analysis reported significant correlation between hardness, cohesiveness, chewiness and adhesiveness as measured by sensory panel and instrumentally, across a range of cheese types including Muenster, Provolone, Cheddar, Mozzarella and others. Studies by McEwan et al. (1989) and Lawlor and Delahunty (2000) have demonstrated the influence of texture and flavour in cheese selection by consumers. Using a selection of different cheese including Mahon, Cambozola, Gruyère and Wensleydale, Lawlor and Delahunty (2000) found seven groups of consumers with differing preferences based on cheese type. Overall, the sensory attributes that scored highly for cheese preference were ‘fruity’ odour, ‘balanced’ and ‘sweet’ flavour and ‘firm’ texture.
The properties of heated cheese are of particular relevance in cooked dishes such as pizza, lasagne and pasta. Nevertheless, comparatively little information is available on the sensory properties of heated cheese and how these are affected by composition or other characteristics of the unheated cheese. Piggott et al. (1998) investigated the sensory properties of unheated fried and grilled samples of retail Halloumi cheese. While assessors could easily discriminate between the unheated cheeses for saltiness, this difference disappeared during heating due to the emergence of a more creamy, milky and fatty flavour character. Lee et al. (1978) reported a significant correlations between melting temperature (temperature at which the melting cheese mass underwent a notable decrease in viscosity) and the sensory scores for hardness and chewiness of unheated cheese, a range of different cheese types Mozzarella, Cheddar and Muenster.

The primary objective of the current study was to evaluate the effect of reducing the levels salt and fat on the sensory properties of 15 and 35 day-old Mozzarella cheeses and the effect of calcium reduction on the sensory properties of reduced-fat cheese. A secondary aim was the investigate relationships between sensory characteristics of the cheese and concentration of volatile compounds, texture profile characteristics and instrumental meltability characteristics.
2. Materials and Methods

2.1. Cheese Manufacture

Mozzarella cheeses with fat, salt and calcium levels were manufactured in triplicate trials, as described by Henneberry et al. (2015). In each trial, 6 variants of Mozzarella cheese were produced: full-fat, full-salt (FFFS); full-fat, reduced salt (FFRS), reduced-fat, full-salt (RFFS); reduced-fat, reduced-salt (RFRS), reduced-fat, full-salt, low-calcium (RFFSLC); and reduced-fat, reduced-salt salt, low-calcium (RFRSLC). The cheeses were stored at 4°C for 15 and 35 day.

2.2. Texture Profile Analysis (TPA)

Six cube-shaped samples (2.5 cm) were cut from each cheese, wrapped tightly in tin foil and stored refrigerated (4°C) overnight. Each cube was taken from the refrigerator and immediately compressed in two successive bites, in a direction perpendicular to the direction of fibre orientation, to 60% of its original height in two consecutive bites at a rate of 60 mm/min on a TAHDi Texture Profile analyser (TPA) (Stable Micro Systems, Goldalming, Surrey, England) at room temperature. The following parameters were defined from the resultant force/time curve as described and defined by Gunasekaran and Ak (2003): hardness, cohesiveness, adhesiveness, resilience and chewiness.

2.3. Flow and Stretchability of heated cheese

The flow was measured by expressing a change in dimension (e.g., diameter, length) as a % of original dimension heating using (i) a modified Schreiber method and (ii) a modified Olson and Price method (Guinee and O’Callaghan, 2013). The former (denoted Sch-flow) is
indicative of the melting behaviour of cheese when exposed as a topping (e.g., on pizza) during oven heating, and the latter, denoted Flow-PO, when the cheese is covered, or largely covered, during cooking (e.g., as a cheese slice in hamburger).

The work required to extend the molten cheese (EW) was measured using uniaxial extension on a TA-HDi Texture Analyser, as described by Henneberry et al. (2015).

2.4. Sensory Evaluation

Naïve assessors (n=25, age range of 21-48 years) were recruited in University College Cork, Ireland. All were Mozzarella cheese consumers and evaluated twelve different Mozzarella cheeses varying in fat, salt and calcium content (Table 1) (Henneberry et al., 2015) utilising sensory Hedonic descriptors. Sensory analysis was carried out in panel booths conforming to international standards (ISO 8589: 2007). All samples were blast frozen to -35°C and then stored at -20°C until required. Unheated samples were held at refrigeration temperatures overnight (4°C), before monadic presentation to the assessor panel at ambient temperatures (~21°C) and coded with a randomly selected 3 digit code. For the heated samples, ~30 g of cheese was placed in a polyamide/polyethylene bag, heat sealed then placed in water-bath at 95°C for 10 min until it reached a core temperature of 95°C. The cheese was immediately served to assessors. No more than six samples were presented at any individual sensory session. Each assessor was provided with deionised water and instructed to cleanse their palates between tastings. Additionally, each assessor was asked to indicate their degree of liking on a 10 cm line scale ranging from 0 (extremely dislike) at the left to 10 (extremely like) at the right and rating subsequently scored in cm from left. A Ranking Descriptive analysis (RDA) (Bragato Richter et al., 2010; Dairou and Sieffermann, 2002) was then carried out on the same samples used for the affective testing. All six samples were presented
simultaneously and tested in one sitting with short breaks included for the heated cheeses (every 3 cheeses) so that they could be assessed while still hot. The order of the presentation of all test samples was randomized to prevent first order and carryover effects and all samples were presented in duplicate.
Table 1 Cheese composition, biochemical and textural attributes

<table>
<thead>
<tr>
<th></th>
<th>FFFS</th>
<th>FFRS</th>
<th>RFFS</th>
<th>RFRS</th>
<th>RFFSLC</th>
<th>RFRSLC</th>
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<tr>
<td><strong>Composition at 15 days</strong></td>
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<td></td>
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<tr>
<td>Moisture (% w/w)</td>
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<td>48.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>50.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Protein (% w/w)</td>
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<td>23.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>23.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.6&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.1&lt;sup&gt;cd&lt;/sup&gt;</td>
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<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>655&lt;sup&gt;d&lt;/sup&gt;</td>
<td>942&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>551&lt;sup&gt;e&lt;/sup&gt;</td>
<td>506&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Protein hydration (g water/g protein)</td>
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<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>WSN (% of total N)</td>
<td>5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>236&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>50&lt;sup&gt;e&lt;/sup&gt;</td>
<td>139&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Adhesiveness (Nmm)</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Resilience (-)</td>
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<td>0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Biochemical and textural parameters at 35 days</strong></td>
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<td>Protein hydration (g water/g protein)</td>
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<td>WSN (% of total N)</td>
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<td>6.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>136&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Chewiness (N)</td>
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<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Cohesiveness (-)</td>
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<td>0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Adhesiveness (Nmm)</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

1Presented data represent the means of three replicates for each of 6 treatment Mozzarella cheeses: full-fat full salt (FFS), full-fat reduced salt (FFRS), reduced-fat full salt (RFFS), reduced-fat reduced salt (RFRS), reduced-fat full salt low calcium (RFFSLCa) and reduced-fat reduced salt low calcium (RFRSLCa).

2The statistical effects of reducing fat, salt and calcium contents are indicated by lower case superscripts. Values not sharing a common superscript differ significantly, P < 0.05.

3Abbreviations: M/P, moisture-to-protein ratio; S/M, salt-in-moisture; Ca, calcium; WSN, water soluble nitrogen (% of total nitrogen)
2.5. Volatile analyses

For volatile analysis, 4 g of the sample was added to 20 ml screw capped SPME vial and equilibrated to 50°C for 20 min with pulsed agitation of 5 sec on 2 sec off at 650 rpm. The samples were analysed in duplicate. Sample introduction was accomplished using a CTC Analytics CombiPal Autosampler. A single 50/30um Carboxen™/divinylbenzene/polydimethylsiloxane (DVB/CAR/PDMS) fibre was used for analysis (Agilent Technologies Ireland Ltd, Little Island, Cork). The SPME fibre was exposed to the headspace above the samples for 25 min at depth of 1 cm. The fibre was retracted and injected into the GC inlet and desorbed for 1 min at 250°C. Injections were made on an Agilent 7890A GC with an Agilent HP-Innowax column (60 m x 0.25 mm x 0.5 μm) column using a multipurpose injector with a merlin microseal (Agilent Technologies Ireland Ltd). The temperature of the column oven was initially set at -10°C, held for 2 min, then increased at 25°C/min to 20°C, followed by increasing at 6°C/min to 110°C, then at 10°C/min to 250°C yielding at total GC run time of 42.2 min. The carrier was helium at a constant flow of 1.2 ml/min. The detector was an Agilent 5975C MSD single quadrupole mass spectrometer (Agilent Technologies Ireland Ltd). The ion source temperature and interface temperature were set at 230°C and 150°C, respectively, and the MS mode was electronic ionization (-70 V) with the mass range scanned between 35 and 250 amu. Compounds were identified using mass spectra comparisons to the NIST 2011 mass spectral library, and from an internal data base with known target and qualifier ions for each compound, a standard was used for some compounds that were not already in the internal database. An auto-tune of the GCMS was carried out prior to the analysis to ensure optimal GCMS performance. A set of external standards was also run at the start and end of the sample set and abundances were compared to known amounts to ensure that both the SPME extraction and MS detection was performing within specification.
2.6. Statistical analyses

ANOVA-Partial Least Squares Regression (ASLPR) was used to process the data for the unheated and heated cheeses, accumulated from sensory, volatile, compositional and instrumental texture profile and melting analysis of the cheeses. The APLSR plots and corresponding p value of regression coefficient correlations are presented in Figures 1 to 4. For each plot, principal component (PC) 1 versus PC 2 is presented; other PCs did not yield additional information. Close proximity of samples (names) to sensory attributes indicates correlation between the sample and the particular sensory attribute; the level of significance for correlation was set at P < 0.05.

3. Results and Discussion

3.1. Sensory analyses of unheated cheese

ANOVA-Partial Least Squares Regression (APLSR) Plots of all samples plotted against sensory variables, as determined through the affective hedonic assessment and the flash profile descriptive assessment, are presented in Figs 1 and 2 for unheated and heated Mozzarella. Tables 2 and 3a, b presents the P values of the regression coefficients for the relationships between sensory attributes and the sample (code) for the unheated and heated cheeses, respectively.
Fig. 1 ANOVA-Partial Least Squares Regression (ASLPR) plot for unheated Mozzarella-style cheeses, showing the affective, descriptive sensory and instrumental data. Where sensory and instrumental terms have the same name (e.g., sensory- and instrumental-Chewiness), the sensory term is denoted by the suffix S (e.g., Chewiness-S) and the instrumental term by I (e.g., Chewiness-I).
From Fig. 1 it can be seen that the unheated full-fat (FF) Mozzarella cheeses FFFS15, FFRS15, FFFS35 and FFRS35 were grouped on the left hand side of the plot and were positively (P < 0.05) correlated to the hedonic attributes, overall acceptability, liking of texture and liking of flavour (Table 2). FFFS15 and FFRS15 cheeses were also positively (P < 0.05) correlated to liking of appearance and liking of aroma. While FFRS15 cheese was positively correlated with firmness, rubbery and chewiness, FFFS35 and FFRS35 were negatively (P < 0.05) correlated with these same attributes (Table 2). The reduction in the intensity of these attributes between day 15 and 35 is consistent with the decrease in the magnitude of the corresponding instrumental parameters hardness, resilience and chewiness (Table 1) (Henneberry et al., 2015).

The reduced-fat (RF) cheeses RFRS15, RFFS15 and RFFS35 were negatively (P <0.05) correlated with liking of texture, liking of flavour and overall acceptability, while RFRS35 was also negatively correlated with the liking of texture, but not with liking of flavour and overall acceptability. All RF cheeses (RFRS15, RFFS15, RFFS35, and RFRS35) were positively correlated with the sensory textural descriptive attributes firmness, rubbery, and chewiness (apart from RFRS15); this trend is consistent with the findings of Madsen and Ardö (2001), who found that sensory firmness, hardness of Danbo cheese increased significantly as the fat content was reduced from 25 to 13% (w/w). Hence, unlike the FF cheeses, where increasing the ripening time from 15 to 35 days resulted in a change in correlation from positive to negative for these parameters, increasing the ripening time did not significantly reduce the high intensity (levels) of firmness, rubbery, and chewiness associated with the RF cheeses.
Table 2 P Values of regression coefficients (ANOVA values) for the relationships between sensory attributes and cheese code, as derived by Jack-knife uncertainty testing for unheated Mozzarella cheese samples

<table>
<thead>
<tr>
<th>Cheese code</th>
<th>Liking of Appearance</th>
<th>Liking of Aroma</th>
<th>Liking of texture</th>
<th>Liking of flavour</th>
<th>Overall Acceptability</th>
<th>Colour</th>
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<th>Firmness</th>
<th>Rubbery</th>
<th>Chewiness</th>
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</tbody>
</table>

Presented are P-Values (P < 0.05) from the estimated regression coefficients from ANOVA-Partial Least Squares Regression (APLSR). The Sign dictates weather the correlation is positively or negatively correlated.
The significantly higher intensities of sensory firmness, rubbery, and chewiness in the 35 day-old RF cheeses, compared to the FF cheeses, is primarily due to their higher protein content which contributes to the strengthening of the cheese matrix, making it less susceptible to deformation when subjected to stresses, such as those encountered during consumption. There was no association of the hedonic sensory attributes and the reduced-fat low-calcium (RFLC) cheeses, apart from RFFSLC35, which was negatively (P <0.05) correlated with liking of flavour and overall acceptability. Both RFRSLC15 and RFFSLC35 were not correlated with any textural sensory attributes, but RFFSLC15 was positively (P <0.05) correlated with rubbery and chewiness. RFRSLC35 was also negatively correlated to firmness.

While there was generally no relationship between cheese type and salt taste, the RFRS15 and RFRSLC15 cheeses were, surprisingly, found to be positively correlated with salt taste. The lack of correlation between salt taste and the corresponding FFRS15 cheese suggests that the perception of salt taste may be influenced by factors other than salt content, for example the matrix properties (e.g., structure, protein content and volume fraction), degree of protein hydrolysis and hydration (as indicated by WSN and NESP) and types and levels of volatile compounds (Tables 1, 5) (Djordjevic et al., 2004; Saint-Eve et al., 2009; Guinee and O’Kennedy, 2009). Moreover, it is possible that in the case of the full fat cheeses, fat flavour masks the perception of salt (Shamil et al., 1992; de Roos, 1997). A tentative explanation for the positive correlation of salt taste with RFRS15 and RFRSLC15 is that the higher concentration of native milk salts in the cheese (Ca, P) in the RFRS15 and RFRSLC15 cheeses could be contributing to saltiness and compensating for the reduction in salt content (Fitzgerald and Buckley, 1985).

Overall, full fat cheeses with full salt or reduced-salt levels (FFFS15, FFFS35, FFRS15, FFRS35) were most desirable as reflected by the positive correlation with hedonic attributes and the absence of a correlation, or the presence of a negative correlation, with
rubbery, firm and chewiness attributes. Moreover, FFFS and FFRS cheeses were negatively (P < 0.05) correlated to off-flavour at day 35. The reduced fat full salt (RFFS) and reduced fat reduced salt (RFRS) were the least desirable cheeses, being positively correlated with firmer, rubbery texture and chewiness attributes.

Generally, there was little correlation between cheese type and fat flavour, except for FFRS15 which showed a positive correlation and RFRS15 which was negatively correlated. There was no correlation between cheese type and fat flavour at 35 days, suggesting that other factors apart from fat content also influence the perception of fat flavour.

3.2. Sensory analyses of heated cheese

Heated Mozzarella cheese variants FFRS15, FFRS35 and FFFS35 were grouped on the lower right hand side of the ANOVA-Partial Least Squares Regression plot (Fig. 2) and were positively (P < 0.05) correlated with overall acceptability, liking of texture, liking of flavour, colour, oiling off, fluidity, succulence, cheese aroma intensity, fat flavour, cheese flavour intensity, cooked cheese flavour and negatively (P < 0.05) correlated to firmness (Table 3a, b). Additionally, FFRS15 cheese was positively (P < 0.05) correlated with glossiness, spreadability and cream flavour and negatively (P < 0.05) with chewiness and rubbery texture, while FFRS35 was positively (P < 0.05) correlated with liking of aroma, spreadability, stretchiness, stringiness and cooked cheese flavour and negatively (P < 0.05) with chewiness, rubbery texture and off-flavour. FFFS35 also correlated positively (P < 0.05) with liking of aroma, cooked cheese flavour and salt taste. FFFS15 was positively (P < 0.05) correlated with liking of flavour, colour, moistness, salt taste, cream flavour and fat flavour and negatively (P < 0.05) correlated with chewiness and rubbery texture. Conversely, the reduced-fat cheeses
were generally negatively (P < 0.05) correlated with liking of texture, liking of flavour, overall acceptability, fluidity, spreadability, moistness and other attributes (Table 3 a, b; Fig. 2).
Fig. 2 ANOVA-Partial Least Squares Regression (ASLPR) plot for heated Mozzarella-style cheeses, showing the affective, descriptive sensory and instrumental data. The instrumental parameters of the heated cheese included flow, as measured using modifications of the Schreiber (Sch-Flow) and Price-Olson (FlowPO) methods, and work to uniaxially extend the molten cheese (EW).
Reducing the calcium content of the reduced-fat cheeses (RFFSLC15, RFFSLC35, RFRSLC15, RFRSLC35) generally improved their sensory acceptability, as indicated by the absence of a positive correlation or the presence of a negative correlation between the LC cheeses and attributes such as firmness, chewiness, spreadability and rubbery texture, and the presence of a positive correlation or absence of a negative correlation with attributes such moistness, stringiness, cheese aroma intensity and fat flavour. Overall the more negative attributes were more associated with the reduced fat cheeses and the more positive attributes with the full fat cheeses, similar to the unheated cheese.
Table 3a P Values of regression coefficients (ANOVA values) for the relationships between sensory attributes and cheese code, as derived by Jack-knife uncertainty testing, for heated Mozzarella cheese samples

<table>
<thead>
<tr>
<th></th>
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<th>Liking of Texture</th>
<th>Liking of Flavour</th>
<th>Overall Acceptability</th>
<th>Colour</th>
<th>Oiling of visible free oil</th>
<th>Fluidity</th>
<th>Firmness</th>
<th>Succulence</th>
<th>Chewiness</th>
<th>Spreadability</th>
<th>Rubbery Texture</th>
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Presented are P-Values (P < 0.05) from the estimated regression coefficients from ANOVA-Partial Least Squares Regression (APLSR). The Sign dictates weather the correlation is positively or negatively correlated.
**Table 3b** P Values of regression coefficients (ANOVA values) for the relationships of sensory terms as derived by Jack-knife uncertainty testing for heated Mozzarella cheese samples

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Presented are P-Values (P < 0.05) from the estimated regression coefficients from ANOVA-Partial Least Squares Regression (APLSR). The Sign dictates weather the correlation is positively or negatively correlated.
3.3. Sensory and physical properties of the unheated cheese

Table 4 shows the P values for the relationships between cheese type and instrumental TPA parameters. The reduced-fat cheeses (RFFS15, RFFS35, RFRS15 and RFRS35) were positively correlated with instrumental hardness and chewiness. In contrast, the full-fat (FFFS15, FFFS35, FFRS15, FFRS35) and reduced-calcium (RFFSLC15, RFFSLC35, RFRSLC15, RFRSLC35) cheeses were either not, or negatively, correlated with the latter parameters. Generally, a similar relationship was found between cheese type and sensory hardness in the 35 day-old cheeses, and to a lesser extent in the 15 day-old cheeses. These trends highlight the effectiveness of calcium reduction as a means on enhancing the texture of unheated reduced-fat Mozzarella, aligning it more closely with full-fat Mozzarella. The general agreement between sensory and instrumental hardness and chewiness concurs with the results of previous authors.

Table 4 P values of regression coefficients (ANOVA values) for the relationships of instrumental analyses as derived by Jack-knife uncertainty testing for unheated and heated Mozzarella cheese samples

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<th>Hardness</th>
<th>Chewiness</th>
<th>Cohesiveness</th>
<th>Adhesiveness</th>
<th>Cooking properties</th>
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Presented are P-Values (P < 0.05) from the estimated regression coefficients from ANOVA-Partial Least Squares Regression (APLSR). The Sign dictates whether the correlation is positively or negatively correlated.
Instrumental cohesiveness is an index of the work required to overcome the internal structure and, thereby breakdown the cheese in two compressive bites (van Vliet, 1991); it thereby simulates the work required to rupture the cheese using the molars (Gunasekaran and Ak, 2004). A high value of cohesiveness is indicative of a rubbery product which recovers after deformation and, hence, requires a high level of work to degrade to a bolus ready for swallowing. Hence, the 35 day-old full-fat cheeses FFFS35 and FFRS35 were negatively correlated with instrumental cohesiveness and sensory rubberiness, while the corresponding RFFS35 and RFRS35 cheeses showed an opposite trend. This result is consistent with the higher volume fraction of the calcium phosphate para-casein network in the reduced-fat cheeses which necessitates an increased number of bites (compression between molars) to overcome the structure during consumption. Adhesiveness correlated negatively with the 35 day FFFS and FFRS cheese but positively with the corresponding RFFS and RFRS cheeses.

RFFS15, RFRS15, RFFS35, RFRS35 and RFFSLC35 were negatively correlated with adhesiveness while the FFFS15, FFRS15, FFFS35 and FFRS35 were positively correlated with adhesiveness.

3.4. Sensory and physical properties of the heated cheese

The instrumental flowability (Sch-flow, FlowPO) correlated negatively with the heated RFFS15, RFFS35, RFRS15 and RFRS35 cheeses, and positively or non-significantly with the FFFS, FFRS, RFFSLC and RFRSLC cheeses (Table 4). The observed trend is consistent with the lower fat content and moisture-to-protein ratio (M/P) of the RFFS and RFRS cheese; fat and moisture are both known to act as lubricants on the surfaces of fracture planes within the cheese, and thereby facilitate displacement of contiguous layers, when the cheese is subjected to stresses as during heating (Guinee et al., 2015). A similar trend to instrumental flowability
was noted for the (sensory) fluidity of the cheese, which represents the ease with which the product flows in the mouth. Both sensory stretchiness, representing the level of extension, and stringiness (the ability to the cheese to form strings) were positively correlated with the FFRS35 cheese and negatively with the corresponding RFFS35 cheese. Otherwise, there was no relationship between cheese type, on the basis of fat or calcium content, and sensory stretchiness, sensory stringiness or instrumental work to extend (EW).

3.5. Sensory and Flavour volatile analyses of the unheated and heated cheese

Fig 3 and 4 and Tables 5 and 6 present the P values for the regression coefficients for the aroma volatiles of unheated and heated Mozzarella cheese respectively. In total 30 volatile compounds were identified in the unheated and heated Mozzarella cheese; 11 ketones (acetone, 2-butaneone, 2,3-butanedione, 2,3-pentanediione, 2-hexanone, 2-heptanone, acetoin, 2-hydroxy 3-pentanone, 2-nonanone, 2-dodecanone, 2-pentadecanone), 7 alcohols (ethanol, 2-methyl 1-propanol, 1-pentanol, 3-methyl 1-butanol, 1-hexanol, 2-butoxy ethanol, phenyl alcohol), 4 aldehydes (3-methyl butanal, nonanal, benzaldehyde, benzeneacetaldehyde), 3 acids (acetic, butanoic, hexanoic), 3 terpenes (limonene, cymene, γ-terpinene), 1 furan (2-ethyl furan) and 1 phenolic compound (toluene). However, only the compounds that were statically different between the cheeses (unheated and heated) are discussed.
Fig 3 ANOVA-Partial Least Squares Regression (ASLPR) plot for unheated Mozzarella-style cheeses, showing the affective descriptive sensory data and volatile compound.
3.5.1. Unheated cheese

FFFS15 and FFRS15 cheeses were positively (P <0.05) correlated with toluene (nutty, bitter, almond, plastic), limonene (citrus), cymene (woody) and nonanal (green, citrus, fatty, floral). FFFS15 was also positively (P <0.05) correlated with 2-butoxy ethanol. Toluene is likely a result of contamination from the atmosphere or migration from packaging into the cheese or cheesemilk, but may also be a result of the degradation of β-carotene from feed which is transferred into the milk (Villeneuve et al., 2013). Toluene is not uncommon in cheese (Dimos et al., 1996; O’Riordan and Delahunty, 2001). Limonene and cymene are terpenes that would also have originated from feed and were transferred into cow’s milk. Again both compounds have been previously identified in cheese (Buchin et al., 1998; Smit et al., 2005). Nonanal is an aldehyde derived from the metabolism of free amino acids or free fatty acids and is widely found in cheese (O’Riordan and Delahunty, 2001; Smit et al., 2005). 2-Butoxy ethanol has been found in cheese, although relatively uncommon (Arques et al., 2007), but is more likely a residue from cleaning or from packaging. At day 35 only toluene remained positively (P <0.05) correlated with FFFS and FFRS, however nonanal was still positively (P <0.05) correlated with FFFS. At day 15 FFFS was negatively (P <0.05) correlated with 2-ethyl furan (sweet, burnt, earthy, malty), but not negatively correlated with any volatile at day 35. 2-Ethyl furan is a furan and not typically found in cheese and is likely formed from Maillard reactions, but may also be the result of Strecker reactions or lipid oxidation (Vranová and Ciesarová, 2009). FFRS cheese was not negatively (P <0.05) correlated with any volatile at day 15, however, at day 35 was negatively (P <0.05) correlated with hexanoic acid (sweaty, cheeseey, sharp, goaty, bad breath) and 2-Heptanone (blue cheese, spicy, roqueforti). Hexanoic acid is widely present in cheese and is a direct result of lipolysis of milk fat (Curioni and Bosset, 2002). 2-Heptanone is a ketone derived from the β-oxidation of fatty acids during cheese ripening and is also widely found in cheese (Curioni and Bosset, 2002). The reduced fat cheese
RFRS15 was positively (P <0.05) correlated with 2-Butanone (sour milk, buttery, etheric), 2-Ethyl furan and benzeneacetaldehyde (honey-like, violet, rose, hyacinth) and negatively (P <0.05) correlated with 2-butoxy ethanol and nonanal. By day 35, RFRS was positively (P <0.05) correlated with hexanoic acid and negatively (P <0.05) correlated with 1-Hexanol (green, floral). 2-Butanone is a ketone and also derived from β-oxidation of fatty acids during cheese ripening and is also widely found in cheese (Curioni and Bosset, 2002). Benzeneacetaldehyde (phenylacetaldehyde) is also widely found in cheese and is the result of the metabolism of phenylalanine (Smit et al., 2005). 1-Hexanol is also common in cheese and is a result of the metabolism of free fatty acids (Curioni and Bosset, 2002). RFFS cheese at day 15 was positively (P <0.05) correlated with 2-Heptanone, Benzealdehyde (bitter almond, sweet cherry), Benzeneacetaldehyde, Hexanoic acid and negatively (P <0.05) correlated with Phenyl ethyl alcohol (unclean, rose, violet-like, honey, floral). At day 35 RFFS cheese was positively (P <0.05) correlated with Benzeneacetylaldehyde and negatively with 2-Butoxy ethanol. Both Benzaldehyde and Phenyl ethyl alcohol (2-Phenylethanol) are derived from the metabolism of phenylalanine and are common in cheese (Curioni and Bosset, 2002; Smit et al., 2005). RFFSLC cheese at day 15 was not correlated with any volatile compound, but at day 35 was positively correlated with 3-Methyl butanal (malty, powerful, cheese, green, dark chocolate). 3-Methyl butanal is a very common aldehyde in cheese and results from the catabolism of leucine. RFRSLC cheese at day 15 was positively correlated with nonanal and not negatively (P <0.05) correlated with any attribute, while at day 35 the cheese was positively (P <0.05) correlated with limonene and cymene. The FF cheeses at day 15 were positively (P<0.05) associated mainly with volatiles from feed and nonanal from fat or protein metabolism. This association was still strongly evident from FFFS cheese at day 35, but less so for FFRS cheese. The RF cheeses did not have any positive correlations that were similar at different salting contents between days 15 and day 35. No obvious negative correlations were evident in the
FF or RF cheeses. The low calcium cheeses had no negative correlations and any positive correlations were not consistent with salting levels or sampling times. The PCA biplot (Fig. 3), incorporating sensory and volatile data, discriminates the cheeses into defined groups. The FF cheeses are separated from all other cheeses on the positive side of the x-axis, with the RF cheeses grouped tightly together on the negative side of this axis. The RF low calcium cheeses were between the FF and RF but had a closer association to the RF cheeses.
Table 5 P Values of regression coefficients (ANOVA values) for the relationships of volatile compounds as derived by Jack-knife uncertainty testing for unheated Mozzarella cheese samples

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Presented are P-Values (P < 0.05) from the estimated regression coefficients from ANOVA-Partial Least Squares Regression (APLSR).

The Sign dictates whether the correlation is positively or negatively correlated.
3.5.2. *Heated cheese*

In the heated Mozzarella cheese, FFRS15 cheese was positively (P <0.05) correlated with 2,3-Butanedione (buttery, caramel, toffee), toluene and was not negatively (P <0.05) correlated with any volatile. FFRS35 was also positively (P <0.05) correlated with toluene and phenylethyl alcohol, and negatively with 2-Hexanone. 2,3-Butanedione (diacetyl) which is derived from carbohydrate metabolism and is widely found in cheese (Smit et al., 2005). FFFS15 was positively (P <0.05) correlated with 2,3-Butanedione, Nonanal, phenylethyl alcohol and at day 35 with toluene. FFFS was not negatively correlated with any volatile at either day. RFRS15 was only positively (P <0.05) correlated with Benzeneacetaldehyde and negatively with toluene, with no positive or negative correlations evident for RFRS35, RFFS15 and RFFS35. RFRSLC15 was positively correlated with acids; acetic acid (vinegar, peppers, green, floral, sour), butanoic acid (sweaty, buttery, cheesy, faecal, rancid) and hexanoic. Butanoic acid, derived from hydrolysis of milk fat, and acetic acid, mainly ensuing from lactose metabolism, are very common in cheese (Smit et al., 2005). RFRSLC, at either time point was not negatively correlated with any volatile. RFFSLC15 was not correlated with any volatile; however, at day 35 it was positively correlated with acetic acid and negatively with 2,3-Butanedione.
Fig 4 ANOVA-Partial Least Squares Regression (ASLPR) plot for heated Mozzarella-style cheeses, showing the affective, descriptive sensory data and volatile compounds
No clear trends were evident in positive correlations of volatile compounds amongst these heated cheeses, but that very little negative correlations were evident. The PCA biplot (Fig. 4) incorporating the volatile and sensory data clearly discriminates the cheeses into 3 groups; FF, RF and low calcium RF. The discrimination in these cheeses is very similar to that found in the unheated cheeses.
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Presented are P-Values (P < 0.05) from the estimated regression coefficients from ANOVA-Partial Least Squares Regression (APLSR). The Sign dictates whether the correlation is positively or negatively correlated.
4. **Conclusion**

Overall, the unheated and heated Mozzarella full-fat cheese variants had the highest sensory acceptance of the 6 cheeses at 15 and 35 days. In contrast, the reduced fat cheeses generally scored lowest for liking of texture, flavour and overall acceptability at 15 and 35 days. Reducing salt content in the range studied (1.7 to 1.0%, w/w) did not adversely impact on sensory properties of the cheese, but reducing fat in the range 21 to 10% (w/w) had a very definitive negative impact on the sensory quality. The overall sensory perception of the reduced-fat cheese was improved by reducing calcium levels but remained less acceptable than the full-fat cheese. The sensory properties and volatile profile of the unheated and heated cheeses were similar for each cheese type.

The sensory and volatile compound profile for the different cheese types when evaluated in the unheated or heated form showed were similar, clarifying the effect of heating on cheese sensory and volatile properties.

5. **References**


6. Acknowledgements

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Chapter 4:
General Discussion

The objectives of this thesis were to:

(i) establish the interactive effects of fat and salt reduction on the compositional, textural, functional and sensory properties of unheated and heated Mozzarella-style cheese and to (ii) investigate calcium reduction as a means of counteracting the negative impact of fat reduction on the textural, functional and sensory properties of Mozzarella-style cheese. An additional objective was to (iii) investigate the effect of heating on the sensory properties and volatile profile of the cheeses.

Cheese is a dairy product with historical economic value (Soares, 2013) that continues to be in high demand with consumers to the modern day. The continued increase in its popularity can be attributed to the versatility of cheese; it may be consumed as a food in its own right, or as an ingredient in composite food, e.g., lasagne, pizza, bruschetta. Mozzarella, a cheese commonly used as an ingredient in such foods continues to grow in production volumes (Statista, 2018) in line with the increase in convenience foods (Statista, 2019). The changing lifestyle of the modern consumer with limited free time is reflected in the increase in sales of convenience foods (Quick, 2013). In the last two decades the average preparation time for the main meal of the day has decreased from 60 minutes to 32 minutes (Quick, 2013). Of the convenience foods gaining popularity, pizza is one of the most significant as in 2014 the USDA named pizza a ‘primary key food’ in the USDA National Food and Nutrient Analysis Program as it contributed more than 14 nutrients of public health significance to the American diet (Nickle and Pehrsson, 2014).
The modern day consumer demands convenience but also choice and low fat alternatives, primarily due to heightened consumer awareness of links between high dietary fat intake and certain diseases, e.g., cardiovascular disease, stroke (He and MacGregor, 2009). The continued growth of the pizza cheese and convenience food markets therefore depends on the development of foods with reduced fat and salt that are acceptable to the consumer. Although there have been numerous studies investigating the effects of fat reduction and salt reduction in cheese, and means to overcome them (Sheehan and Guinee 2004; Ganesan et al., 2014; McCarthy et al., 2016) their success has been limited.

Previous studies have examined the effects of fat reduction in Mozzarella (Sheehan and Guinee, 2004), salt reduction in Mozzarella (Paulson et al., 1998; Ganesan et al., 2014) while other studies have investigated the interactive effects of fat and salt reduction in Cheddar cheese (McCarthy et al., 2015; 2016). To the authors knowledge, no studies have been conducted on the interactive effects of fat and salt reduction in Mozzarella cheese.

Various means of overcoming the negative effects of fat reduction on the textural, functional and sensory properties of Mozzarella and Cheddar cheese have been studied, including the use of fat replacers (McMahon et al., 1996), use of exopolysaccharide producing cultures (Costa et al., 2010), adjunct cultures (Drake et al., 1997) and reduction of calcium-induced casein-crosslinking (McCarthy et al., 2017). The negative impact of salt reduction on cheese flavour and texture has been predominantly tackled by means of substitution. El-Bakry et al. (2011) reported the successful total replacement of sodium chloride with potassium chloride without detrimentally impacting the heat-induced functionality of imitation cheese, while Fitzgerald and Buckley (1985) had reported a 50% replacement of sodium chloride with potassium chloride without textural or flavour defects in Cheddar cheese. Although the use of salt replacers has been shown to be successful in these studies, the threshold level for reduction of salt from the typical level of a commercial LMPS Mozzarella (1.66%, w/w) without...
detrimentally affecting the functional properties of the cheese or detection by sensory panel has not been established. A study by Ganesan et al. (2014) studied the effect of salt reduction on the textural, functional and sensory properties of both unheated and heated LMPS Mozzarella at a single timepoint. However, this study used a salt content (1.80%, w/w) which is somewhat higher than that of a typical commercial Mozzarella (1.66%, w/w) (Agarwal et al., 2011).

To the author’s knowledge, no work has been conducted on the effect of heating on the sensory profile and headspace composition of reduced-fat, reduced-salt Mozzarella cheese, a cheese that is typically consumed after heating.

In Chapter 1, a series of experimental cheeses were manufactured varying in fat, salt and calcium content. A full-fat (22%, w/w), full-salt (1.70%, w/w) (FFFS) cheese was produced as a control. Additionally, a full-fat, reduced-salt (FFRS), reduced-fat, full-salt (RFRS), reduced-fat, reduced-salt (RFRS), reduced-fat full-salt, low calcium (RFFSLC) and reduced-fat, reduced-salt, low calcium (RFRSLC) cheeses were made. The cheeses were analysed for composition, texture, heat-induced functionality, i.e., heat-induced flow and stretchability, proteolytic activity and any interactions between the reduction of fat and salt content. The same cheeses were also studied for their sensory properties in Chapter 2. A flash profile descriptive assessment and affective hedonic assessment were carried out to quantify the sensory profile of the cheeses, in addition to the volatile headspace analysis of the cheeses at two timepoints (15d and 35d) in both unheated and heated cheese. The relationship between instrumental measurement of texture profile analysis (TPA) and sensory scores was also studied for correlations.

Reducing the fat content from ~22% to 11% resulted in elevated moisture and protein levels, and lower levels of MNFS, FDM and SM. Reduced-fat, reduced-salt cheese had reduced protein hydration, WNS, adhesiveness, heat-induced flow, sensory liking of flavour and texture
and overall acceptability while firmness, hardness, chewiness, cohesiveness and EW were higher than in full-fat, full-salt cheese. Concurrently reducing salt content from 1.7% to 1.0% reduced cheese firmness and chewiness and increased adhesiveness, while there was no adverse impact on the sensory properties of unheated or heated cheeses. The magnitude of the effect of fat reduction was more significant compared with that of salt reduction. Reducing the calcium content by ~50% by pre-acidification of the cheese milk, significantly increased the moisture, MNFS and NESP, and reduced the protein content along with the calcium-to-casein ratio. Reducing the calcium content counteracted the negative impact of fat reduction on cheese quality; however, the functional and textural properties of this cheese were still inferior to full-fat, full-salt cheese.

Reducing salt content in the range studied (1.7% to 1.0%, w/w) did not adversely impact on sensory properties of the cheese, but reducing fat in the range 22% to 10% (w/w) had a distinctly negative impact on the sensory quality. The overall sensory perception of the reduced-fat cheese was improved by the reduction of calcium levels; however, this remained less acceptable than the full-fat cheese. The instrumental measurements for TPA attributes such as hardness, resilience and cohesiveness correlated well with sensory scores for attributes such as firmness, rubberiness and chewiness. The age-related changes in TPA attributes followed a similar trend to that seen in the sensory results for these attributes. The sensory properties and volatile profile of the unheated and heated cheeses were similar for each cheese type, clarifying the effect of heating on cheese sensory and volatile properties.

The study undertaken as part of this thesis revealed that there was scope for the reduction of salt content in LMPS Mozzarella in the range of 1.7%, w/w to 1.0%, w/w without detrimentally impacting consumer acceptance. An alternative approach to salt reduction in cheese could be employed whereby the addition of sodium chloride is simply restricted to a similar or greater extent to that of the current study. Curds could be segregated; some salted
and some unsalted before being layered at moulding to produce a cheese with overall reduced salt content containing zones of high and low salt concentration that would be perceived like a full-salt cheese. Guilloux et al. (2015) conducted a study on a composite food (pizza) where the various ingredients contained different levels of salt. The authors achieved a 30% reduction in the overall salt content of the pizza without negatively affecting the perception of saltiness. To the author’s knowledge, this approach has not yet been attempted in cheese.

The potential for the reduction of fat content and the viable means to overcome the challenges it presents is not quite so clear cut. Although the negative impact of fat reduction on the textural and functional properties was counteracted by calcium reduction, the cheese was not deemed to be as acceptable by the sensory panel. It is possible that a reduction in fat content of a smaller magnitude than that of the current study, coupled with a reduction in calcium content would result in a cheese with a considerable reduction in fat content and deemed to have a similar consumer acceptance to a full-fat version.
References


contribution of a mixture experimental design approach applied to pizza. J. Sensory Stud., 30: 484-498.


