

## Milk quality and cheese diversification

J.J. (Diarmuid) Sheehan<sup>†</sup>

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

**Abolition of EU milk quotas in 2015 is projected to result in a 2.75 billion litre increase in Irish milk production by 2020. Although cheese offers vital market opportunities for this increased milk production, traditional cheese markets such as Cheddar, are predicted to grow more slowly than for other semi-soft and semi-hard cheese types. Innovation is now focused on achieving greater diversity in cheese types manufactured on Irish commercial plants and on development of new products with specific properties for target markets. This innovation is best illustrated by the current Teagasc – Irish Dairy Board collaboration. This review considers the relative influence of milk quality on diversification of the portfolio of cheeses manufactured from a seasonally-produced Irish milk supply with particular reference to milk microbial profile and to milk enzyme complement for the manufacture and ripening of non-Cheddar cheese varieties.**

*Keywords:* Cheese; cheese diversification; microbial profile; milk enzymes; milk quality

### **Cheese: An Important and Growing Sector**

Abolition of EU milk quotas in 2015 is projected to result in a 2.75 billion litre increase in Irish milk production by 2020, equivalent to an increase of approximately 50% (Anon. 2012). This is anticipated to enhance the value of primary production in Ireland by about €700 million along with further downstream benefits in the form of increased dairy products, export earnings

and employment (Anon. 2012). However, it also poses significant challenges in processing of an increased milk pool and in leveraging greater share of existing dairy markets through the development of new products of consistent quality.

Cheese has been targeted as a vital end-product for an increased milk pool due to continued increases in global cheese consumption, high end-use versatility, potential for significant added value, and

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<sup>†</sup>Corresponding author: Diarmuid Sheehan; Tel: +353 25 42232; Fax: +353 25 42340;  
E-mail: diarmuid.sheehan@teagasc.ie

as a profitable outlet for surplus milk fat (Wilkinson, Meehan and Guinee 2000). Surprisingly, cheese has not historically been a major component of the Irish dairy product mix and its share in milk utilisation has always lagged well behind that of European competitors. However, this is fast changing as evidenced by the growth in natural cheese production to 185,600 tonnes in 2012 (CSO 2013) and the anticipated growth to reach about 215,000 tonnes by 2020. Crucially, it is an export-led industry with 92.8% of production exported in 2011, equating to 167,000 tonnes (CSO 2013).

#### **Cheese and Milk: Diversification of an Existing Industry**

Traditional cheese markets such as Cheddar are predicted to grow more slowly than for other semi soft and semi hard cheese types and are also coming under increased price pressure. Such trends underline the need for research and innovation to achieve greater diversity in cheese types manufactured on Irish commercial plants and to develop new products with specific properties for target markets. Two particular routes have evolved in diversification of the industrial scale cheese portfolio; (i) production of novel hybrid cheeses combining the characteristics of diverse cheese types such as Dutch type and Swiss types with Cheddar type processes (Sheehan *et al.* 2007; Sheehan, Wilkinson and McSweeney 2008) and include Egmont (New Zealand) and Dubliner cheese (Ireland), or (ii) investment in dedicated continental or brine salted cheese plants to produce eye type and similar continental cheese types. Examples of changes from traditional Cheddar manufacture includes use of thermophilic cultures and heterofermentative mesophilic cultures,

elevated maximum scald temperatures ( $\geq 50$  °C), brine salting, altered ripening temperatures and profiles, reduced salt and salt-in-moisture levels and a greater emphasis on open texture (similar to Tilsit type cheeses) as well as optimisation of eye characteristics.

Given the complexities of a highly dynamic biological system that is cheese and in light of the changing profile of cheeses produced under a programme of diversification, selected aspects of milk quality may need to be reconsidered. Consistent cheese quality can only be realised with an in-depth understanding of how to consistently manufacture and ripen diverse high quality cheese-types using a seasonal milk supply. In particular, as stage of lactation of milk advances the cheese making quality of milk diminishes with implications for cheese quality and consistency (Guinee and O'Brien 2010). This creates particular demands on having sufficient levels of science and technology to minimise the impact of seasonal variations in milk compositional, physicochemical, microbial and biochemical profiles on the quality and consistency of a more diverse cheese range.

The objective of this review is to consider issues associated with milk quality and to determine the relative influence of milk quality issues on diversification of the portfolio of cheeses manufactured from a seasonally-produced Irish milk supply.

#### **Milk Quality – Microbiological**

Milk quality may be defined under a broad range of characteristics notably; microbial (both pathogenic and non pathogenic bacteria); chemical; compositional; physicochemical; enzymatic; and issues of adulteration. The relative influence of these quality parameters on cheese diversification may be considered as follows:

### *Microbial safety*

Raw milk can potentially contain pathogenic bacteria such as *Salmonella* spp., *Listeria* spp., *E. coli*, *Campylobacter* spp., *Mycobacterium bovis* and *Brucella* spp. (Rea, Cogan and Tobin 1992; Jayarao and Henning 2001). However, as in current Cheddar production, the newer cheeses produced at industrial scale cheese also use pasteurised milk thus posing no greater risk to public health. However, consideration of pathogenic bacteria is necessary where diversification entails cheese manufacture from raw/unpasteurised milk (e.g., at farmhouse scale) or where diversification is focused on production of speciality cheese-types where, even though milk may be pasteurised, there is greater scope for contamination of cheeses with pathogenic bacteria through high frequency of handling individual cheeses, particularly in the development of smear-type cheeses where a complex and diverse microbial system evolves on unpackaged cheese surfaces, often with high pH levels during ripening in humidified atmospheres (Sheehan 2007).

### *Microbial quality*

Microbial populations present in raw milk can influence cheese quality dependant on microbial profile and microbial load of the raw milk, ability of milk microflora to survive pasteurisation, hygiene practices and build-up of microflora within the cheese manufacture plant, and additionally starter culture activity and acidity profiles, cheese manufacture technology, cheese compositional parameters, and ripening temperature/environments.

### *Psychrotrophic bacteria*

Growth of psychrotrophic bacteria in milk can result in production of bitter hydrophobic peptides liberated from the C-terminal region of  $\beta$ -casein and in  $\alpha_{s1}$ -casein through

the activity of proteinases (Lemieux and Simard 1991; McSweeney 2007). These enzymes, produced by psychrotrophs such as *Pseudomonas fluorescens* and *P. putrefaciens*, are heat stable and thus unaffected by pasteurisation temperatures consequently allowing for bitter flavours to accumulate during cheese ripening (Lemieux and Simard 1991). Similarly pseudomonads may also promote lipolysis of fat resulting in negative flavour attributes (McSweeney 2007).

### *Coliforms*

Poor hygiene or the use of unpasteurised milk can result in the presence of coliforms such as *Enterobacter*, *Escherichia*, *Citrobacter*, and *Serratia* which are strongly associated with early gas defects and may also produce off-flavours. Early gas defects in cheese are manifest within the first 24–48 h of manufacture and may occur prior to or during brining/salting of the cheeses. These defects are manifested as numerous small holes in the cheese and, as well as coliforms, are also associated with growth of yeasts and sometimes heterofermentative lactic acid bacteria in the cheese. These microbes produce  $H_2$  and/or  $CO_2$  gas aerobically or anaerobically as a by-product of lactose utilisation (Sheehan 2011).  $H_2$  is poorly soluble in the aqueous phase of curd and therefore even small quantities can cause serious gas problems (Sheehan 2011).

### *Yeasts*

Yeasts developing during cheese manufacture and ripening processes can originate from raw milk; however, they are heat sensitive and are killed by pasteurisation. Where hygiene practices are inadequate, potential contamination of a cheese plant may occur, particularly on surfaces of manufacturing equipment and in the air by yeasts which can result in gas blowing

in hard, semi-hard and soft cheeses. There has also been a resistance reported of the dominant yeasts associated with dairies (e.g., *Debaryomyces hansenii*, *Candida versatilis*, *Torulaspora delbrueckii*) to commercial sanitisers and cleaning compounds, and it is possible that these yeasts may colonise equipment during cleaning and sanitation cycles (Tudor and Board 1993; Viljoen 1998; Sheehan 2011)

#### *Non-starter lactic acid bacteria (NSLAB)*

Non-starter lactic acid bacteria (NSLAB) are adventitious bacteria that gain access to cheese via the ingredients used and/or the production and ripening environment. They occur as heterogeneous populations with cell densities exceeding  $10^6$  cfu/g cheese during the ripening process (Swearingen, O'Sullivan and Warthesen 2001). Non-starter lactic acid bacteria primarily consist of facultatively heterofermentative (mesophilic) lactobacilli (FHLs) as well as *Pediococci*, *Enterococci*, and *Leuconostocs* (Beresford *et al.* 2001; Beresford and Williams 2004). FHLs are capable of growth at pH ranging from 5.5 to 6.2, in 4–6% salt and temperatures from 2 °C to 54 °C (Lynch *et al.* 1996). Defects caused by microorganisms that affect the quality of cheese include odour and flavour defects, biogenic amine (BA) formation, gas formation, and secondary fermentations. Controlling the strains, and the proportions thereof, is emerging as a key issue to minimise cheese defects (McSweeney 2007)

FHLs, salt tolerant and mesophilic lactobacilli cause gas blowing in Cheddar-type and Swiss and Dutch-type cheeses (Sheehan 2011). This issue is more pronounced in raw milk cheeses due to high levels of NSLAB in comparison to cheese made from pasteurised milk (Sheehan 2011). Gas production occurs from the fermentation of residual lactose

and galactose to CO<sub>2</sub> during ripening by FHLs such as *Lactobacillus brevis* and *Lactobacillus fermentum*. Salt-in-moisture (S/M) levels influence starter activity with low starter activity leading to high levels of residual lactose. S/M level also affects lactose utilisation and lactate production by NSLAB (Turner and Thomas 1980; Beresford and Williams 2004).

NSLAB in Swiss-type cheese are mainly composed of FHL that begin growth at the beginning of ripening (Gagnaire, Thierry and Léonil 2001) and eventually reach  $\sim 10^8$  cfu/g (Thierry *et al.* 1998). Demarigny *et al.* (1996) reported *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *L. brevis* in Swiss-type cheese and as the cheese ripened *L. paracasei* began to dominate the NSLAB flora. *L. brevis* are present in pasteurised milk but at lower levels due to pasteurisation and competition by other NSLABs such as *L. paracasei* (Daly, McSweeney and Sheehan 2010). FHL are often inoculated into cheese milk in artisanal cheese manufacture in Switzerland to control PAB activity and reduce secondary fermentation (Baer and Ryba 1999). Jimeno Lazaro and Sollberger (1995) showed that FHL strains such as *L. rhamnosus* and *Lactobacillus casei* with high peptidolytic activity and fast autolysing nature are present in significant numbers in Swiss-type cheese, and inhibited the growth of PAB and reduced the prevalence of split defects. Those authors attributed the inhibition of the PAB by FHL to citrate metabolism. FHL metabolise citrate to acetate, formate and CO<sub>2</sub>, acetate and formate appear to have an inhibitory effect on PAB growth. Similarly, diacetyl inhibits the growth of PAB and is produced by *L. rhamnosus* (Jimeno *et al.* 1995). Martley and Crow (1996) demonstrated citrate utilisation by *L. plantarum* in Emmentaler and showed in cheese with citrate-fermenting

organisms that less propionic acid was formed compared to cheese manufactured without citrate-fermenting organisms.

NSLABs capable of BA formation include *Lactobacillus buchneri*, *Lactobacillus curvatus*, *L. casei* and *Lactobacillus acidophilus* while certain strains of starter lactobacilli such as *Lactobacillus bulgaricus* and *Lactobacillus helveticus* are also capable of BA formation, although this has become less of an issue due to screening for decarboxylase activity (McSweeney 2007).

The ability of NSLABs and FHLs to survive pasteurisation or alternatively to be injured by pasteurisation but still capable of metabolic activity and thus capable of generating defects in diverse cheese types during ripening is of significance. *Lactobacilli* are generally not described as thermophilic although some authors report some thermo resistance especially when assays involve milk (Jordan and Cogan 1999). Ladero *et al.* (2011) reported that while particular strains of *L. brevis* did not survive pasteurisation other strains of *L. buchneri* and *L. curvatus* were partially resistant showing a reduction on treatment of ~2 logs. Consideration is required of initial bacterial load in milk and also of lactobacilli which are non-culturable post pasteurisation but which may remain metabolically active during cheese ripening and whose enzyme complement may have the ability to generate defects in cheese.

#### *Clostridia in cheese*

Any diversification strategy needs to consider the diet of herds producing milk for new cheese types. In particular, silage fed herds results in spores of *Clostridium tyrobutyricum* or *Clostridium butyricum* in cheese milk. Other butyric acid bacteria species known to contribute to late gas defects via spore germination include

*Clostridium sporogenes*, and *Clostridium beijerinckii* (Sheehan 2011). Where such milks are used to manufacture Swiss or Dutch-type cheeses germination of spores and growth of clostridia can occur during ripening resulting in the fermentation of lactate to acetate, butyrate, CO<sub>2</sub> and H<sub>2</sub> with late gas blowing defect in the cheeses. These cheese types are particularly susceptible to spore germination due to their anaerobic environment as well as higher ripening temperatures (in excess of 20 °C) in the case of the Swiss-types. The low salt and acid content also assists in spore germination. Spores enter milk via faecal contamination of cow's udders and are capable of surviving high temperature pasteurisation (Sheehan 2011). Good hygiene practices, with respect to both milk and manufacturing equipment, combined with microfiltration or bactofugation of cheese milk reduces the possibility of contamination. Enzymes such as lysozyme could be added to the cheese milk or the use of bacteriocins such as nisin may be considered in preventing contamination with clostridia spores. Nitrates were traditionally added for preservation purposes (McSweeney 2007; Sheehan 2011).

### **Milk Quality – Chemical Residues and Safety**

Chemical residues may relate to residues present within the lactating animal which are transferred to the milk produced (Power *et al.* 2013), or may relate to residues from external sources (Danaher and Jordan 2013). Antibiotic residues will inhibit starter activity during cheese manufacture.

Antibiotics are used to treat bacterial infections including mastitis (infections of the mammary gland) in lactating cows or, as slow release preparations, in dry cow therapy. In cases of insufficient

withdrawal periods or increased or incorrectly administered dosage, antibiotic residues will occur in milk (Marth and Ellickson 1959; Honanen-Buzalski and Reybroeck 1997). As well as public health concern (emergence of antibiotic-resistant strains, impact on human intestinal flora, potential for allergic reactions) when present in cheese milk, antibiotic residues pose technological problems due to their partial or total inhibition of the growth of starter cultures and acid production (Marth and Ellickson 1959; Honanen-Buzalski and Reybroeck 1997).

It is standard practice at large scale cheese manufacture plants to detect for the presence of antibiotics in cheese milk. However, such facilities may not always be available at farmhouse or artisanal level and this may have implications for cheese manufacture in cases where milk has not been withheld for a sufficient period of time where treatment for mastitis or other infection has been administered.

The concentration of antibiotics required to inhibit different starters depends on the strain used and on the antibiotic type. In general, lactic acid bacteria are more sensitive to penicillin than to cloxacillin (Marth and Ellickson 1959). Lactococci are more sensitive to streptomycin and tetracycline and more resistant to penicillin than *S. thermophilus* and thermophilic lactobacilli. *Lactobacillus delbrueckii* subsp. *lactis* and *L. helveticus* are less resistant to penicillin than most strains of *L. casei* and *L. delbrueckii* subsp. *bulgaricus* and *Propionibacterium freudenreichii* are less resistant to penicillin than lactobacilli (Marth and Ellickson 1959; Cogan 1972). Antibiotics may also influence the associative growth between two species when growing together (Marth and Ellickson 1959).

During Cheddar-type cheese manufacture lower levels of antibiotic residue

result in a reduced rate of acidification particularly in the drain to salting period which necessitates longer manufacture times, may influence moisture contents and may result in higher cheese pH. Elevated levels of antibiotic residues can result in up to a complete cessation of acidification after renneting and thus an abnormally high cheese pH. Cheeses may have an uneven texture and pasty body with abnormal flavours described as yeasty, rancid or fermented (Whitehead and Lane 1956). Antibiotics also inhibit the growth of non-starter lactic acid bacteria which may reduce flavour intensity particularly in raw milk cheeses (Walsh, McSweeney and Fox 1996).

In brine salted cheeses, antibiotic residues inhibit starter growth and acidification resulting in poor curd syneresis, soft curd particles with an excessive whey content and overall in a curd with an elevated moisture content. Coliforms, which are not inhibited by antibiotics like penicillin, can increase in numbers and produce gas which forms numerous holes in the curd (Jacquet 1953). In Swiss-type cheeses manufactured with propionic acid bacteria, abnormal fermentations occur including the butyric acid fermentation with development of abnormal eyes, slits, cracks, brown spots discolouration and putrefaction. Development of wet, slimy surfaces with a strong off odour may also occur (Wessner 1953; Mayra-Makinen 1995).

### **Milk Quality and Enzyme Activity**

Plasmin content of milk varies with advancing stage of lactation (Richardson and Pearce 1981). Varying milk and cheese plasmin levels have been linked with alterations in cheese ripening patterns and in cheese quality. High levels of plasmin in milk and its associated elevated levels of



proteolysis give longer rennet gelation times and a markedly lower gel firmness and coincide with a more porous, open structured rennet gel and less connectivity between the particles and clusters making up the gel matrix (Guinee and O'Brien 2010). Addition of plasmin or addition of mastitic milk which had largely similar effects to addition of exogenous plasmin increased rates of primary proteolysis overall quality of smear ripened cheese (O'Farrell *et al.* 2002). Where milk production is seasonal, cheeses manufactured from early lactation milk have poorer eye development and this may be partly attributed to a lower concentration of plasmin in the milk (Lawrence, Heap and Gilles 1984).

Cleavage of  $\beta$ -casein by plasmin leads to the formation of  $\gamma$ -caseins and proteose peptones (Gordon *et al.* 1972; Bastian and Brown 1996; Somers and Kelly 2002). Plasmin is the dominant indigenous proteinase in milk (Sousa, Ardö and McSweeney 2001) and its properties have been reviewed in detail by Bastian and Brown (1996) and Kelly and McSweeney (2003). Plasmin has relatively high heat stability (Kaminagowa, Mizobuchi and Yamuachi 1972) and a pH optimum of 7.5 (Grufferty and Fox 1988). The contribution of plasmin to primary hydrolysis of caseins is more pronounced in cheeses where high cooking temperatures are used during manufacture (Steffen *et al.* 1987; Sousa *et al.* 2001; Somers and Kelly 2002).

Cheeses manufactured using high cook temperatures, e.g., Swiss, Swiss Cheddar-hybrid types and grana type cheeses, have elevated levels of plasmin activity compared to Cheddar-type cheeses due to increased plasminogen activation resulting from inactivation of plasmin inhibitors and inhibitors of plasminogen activators being lost in the whey during cheesemaking (Farkye and Fox 1990; Somers and Kelly

2002). Collin *et al.* (1987) observed different kinetics in the production of  $\gamma_1$ -casein in comparison to  $\gamma_2$ - and  $\gamma_3$ -caseins in the ripening of Comté cheese, but did not specify the temperature to which the curd was cooked during manufacture. Delacroix-Buchet and Fournier (1992), on increasing cook temperature from 52 °C to 56 °C during manufacture of Gruyère type cheese, observed a significant increase in levels of  $\gamma_2$ - and  $\gamma_3$ -caseins during ripening, but no significant change in levels of  $\gamma_1$ - or  $\beta$ -casein. Somers and Kelly (2002) reported increased plasmin-induced proteolysis of  $\beta$ -casein to  $\gamma$ -caseins in miniature cheeses cooked to 55 °C compared to those cooked at 48 °C. Sheehan *et al.* (2007) reported that increasing cook temperature from 47 to 53 °C had no significant effect on mean levels of  $\beta$ - or  $\gamma_1$ -casein, resulted in a numerical, though not statistically significant increase, in levels of  $\gamma_2$ - and  $\gamma_3$ -caseins and in a significant interaction between the effects of cook temperature and time on levels of  $\gamma_3$ -casein. The increased proteolysis of  $\beta$ -casein to  $\gamma$ -caseins was attributed to increased plasmin activity (Bastian and Brown 1996) due to an increased rate of plasminogen activation during ripening, possibly due to inhibitors of plasminogen activators and plasmin being lost in the whey during cheesemaking (Farkye and Fox 1990) or to thermal inactivation of inhibitors of plasminogen activators. Farkye and Fox (1990) reported increased plasmin activity in cheese on increasing cook temperature from 31 to 52 °C.

#### *Cathepsin D*

Similar to chymosin, the indigenous milk acid proteinase cathepsin D is an aspartic proteinase with a low pH optimum and coagulates milk at high concentrations (Hurley *et al.* 2000). It also hydrolyses  $\alpha_{s1}$ -casein to  $\alpha_{s1}$ -CN (f24-199) (McSweeney,

Fox and Olson 1995). Low levels of cathepsin D activity in cheese may be masked by that of chymosin (McSweeney *et al.* 1995; Somers and Kelly 2002). However, its activity has also been reported in cheeses manufactured without chymosin or with inactivated chymosin (Visser and de Groot Mostert 1977; Noomen 1978; Lane *et al.* 1997; Wium, Kristiansen and Qvist 1998; Larsen *et al.* 2000; Hurley *et al.* 2000).

Igoshi and Arima (1993) isolated an acid proteinase from Emmental and Gruyère cheeses and concluded that it was cathepsin D rather than chymosin as it did not hydrolyse  $\kappa$ -casein at a greater rate than  $\alpha_{s1}$ -casein. Similarly, Cooney *et al.* (2000) reported increased hydrolysis of  $\alpha_{s1}$ -casein during ripening of Swiss-type cheeses made from milks of increasing somatic cell count and proposed that cathepsin D could be responsible.

Cathepsin D has been shown to be relatively stable under high cook temperatures and at least partially survives pasteurisation at 72 °C  $\times$  15 s (Larsen *et al.* 2000; Hayes *et al.* 2001) although it is completely inactivated at 70 °C  $\times$  10 min (Kaminagowa *et al.* 1972). Hayes *et al.* (2001) also showed a 45% survival of cathepsin D after heating in skim milk at 55 °C for 30 min to simulate conditions during the manufacture of high cook cheeses. Draper and Zeece (1989) showed that the heat stability of bovine cathepsin D increased with decreasing pH, with a corresponding increase in enzyme activity.

Cathepsin D remains at least partially active in high cook cheeses where chymosin maybe partially or totally inactivated. However, further studies are required to determine the affect of varying cook temperature during cheese manufacture on the relative activity of cathepsin D in cheese during ripening, particularly where chymosin may not be totally inactivated and there is a need for further research

to compare the relative activity and extent of inactivation of the two enzymes during cheese manufacture.

### Conclusions

Cheese provides a significant opportunity for utilisation of the projected increased milk pool arising from abolition of milk quotas. However, this requires a high level of innovation, increased diversification in cheese type and a move away from traditional cheese markets such as Cheddar, which are predicted to grow more slowly than for other semi-soft and semi-hard cheese types. This shift from Cheddar-type cheeses to cheeses with elevated cooking temperatures (Swiss, Italian-types), higher moisture and pH levels (Swiss and Dutch -types), greater diversity in microbiota (Smear-types and increased ripening temperatures (Swiss and Italian-types) has places significant importance on the quality and profile of cheese milk. This review considers the role of milk microbial profiles, enzyme compliments and the potential for chemical residues to have significant influence on the consistency of diverse cheese types manufactured from milk of varying quality.

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Received 23 October 2013