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Review

Pre-treatment of cheese milk: principles and developments

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Abstract – Classically, very few pre-treatments are applied to milk for cheese-making, with some cheese varieties simply made from raw whole milk, but most made from pasteurised milk of which the composition (e.g., fat:protein ratio) may have been standardized. However, there has been consistent interest in more novel and sophisticated strategies for pre-treatment of cheese-milk. Approaches explored include the use of alternative processing technologies (e.g., membrane filtration, high-pressure treatment, homogenisation, heat treatments more severe than pasteurisation) or addition of sources of protein or milk solids (e.g., milk powders, whey protein products) or enzymes. The principal reasons for such pre-treatments of cheese-milk are: (1) to control the microbiology of the raw milk and the resulting cheese better than is possible by pasteurisation (e.g., inactivation or removal of spores, control of non-starter lactic acid bacteria); (2) increasing the yield of cheese, e.g., through heat- or pressure-induced incorporation of whey proteins, or enhancing sensory properties of reduced fat cheese by direct addition of microparticulated whey proteins; (3) manipulation of cheese ripening, e.g., reducing the likelihood of off-flavour development by inactivation of enzymes or accelerating ripening through increasing enzyme-substrate interactions; or (4) improving the texture and other functional properties, e.g., melting. Finally, the considerations for manufacture and ripening of different cheese varieties, or sub-classes of specific varieties (e.g., low-fat cheese) will clearly differ and add to the complexity of the technological options available. This article will review the key principles for pre-treatment of cheese-milk, as summarised briefly above.

milk / cheese / heat treatment / membrane separation / high pressure / homogenisation / standardisation

摘要 – 干酪原料奶预处理的原理及其进展。本文综述了干酪生产原来奶预处理的原理及其进展。传统干酪生产很少使用原料乳预处理技术, 许多品种的干酪直接用生鲜乳加工, 即使大多数以巴氏杀菌乳生产的干酪也只是对其组成 (脂肪和蛋白质的比率) 进行标准化。但是, 干酪用原料乳预处理作为一种新兴的技术已经引起广泛关注, 人们不断开发出来新的方法, 如加工技术 (膜过滤、高压处理、均质、比巴氏杀菌有效的热处理) ; 添加蛋白质、乳固形物 (如乳粉、乳清蛋白), 或外源酶。对干酪用原料乳进行预处理的主要原因有 : (1) 控制原料乳中的微生物, 使干酪质量优于巴氏杀菌干酪 (如去除芽孢或使其失活、控制非发酵剂乳酸菌) : (2) 增加干酪的产量, 如通过热处理或加压促进乳清蛋白与酪蛋白的融合, 或者通过直接添加微粒化的乳清蛋白来改善低脂干酪的感官性质 ; (3) 控制干酪成熟, 如减少由于酶失活而引起干酪产生异味的可能性, 或者通过提高酶与底物相互作用程度来加速干酪的成熟 ; (4) 改进质构及功能性, 如融化性。由于不同种类干酪或某一种类干酪的亚类 (如低脂干酪) 生产和成熟过程所要考虑的因素完全不同, 也增加了所选技术的复杂性。

奶 / 干酪 / 热处理 / 膜分离 / 高压 / 均质 / 标准化

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Résumé – Pré-traitement du lait de fabrication de fromage : principes et avancées. Classiquement, très peu de pré-traitements sont appliqués au lait de fabrication de fromage : quelques variétés de fromage sont faites simplement à partir de lait entier cru, mais la plupart d'entre elles sont fabriquées à partir de lait pasteurisé dont la composition (par exemple rapport matière grasse/protéine) a pu être standardisée. Cependant, un intérêt constant s'est manifesté pour des stratégies nouvelles et plus sophistiquées pour le pré-traitement du lait de fromagerie. Les approches explorées incluent l'utilisation de technologies de traitement alternatives (par exemple filtration membranaire, haute pression, homogénéisation, traitements thermiques plus sévères que la pasteurisation) ou addition de sources de protéines ou de matière sèche de lait (par exemple poudres de lait, protéines de lactosérum) ou d'enzymes. Les principales raisons de tels pré-traitements du lait de fabrication fromagère sont : (1) de contrôler la microbiologie du lait cru et du fromage résultant, mieux que ce qui est possible par pasteurisation (par exemple inactivation ou retrait des spores, contrôle des bactéries lactiques non levain) ; (2) d'accroître le rendement en fromage, par exemple en induisant l'incorporation de protéines de lactosérum par traitement thermique ou haute pression, ou d'améliorer les propriétés sensorielles du fromage allégé en matière grasse par l'addition directe de protéines de lactosérum microparticulées ; (3) d'orienter l'affinage du fromage, par exemple en réduisant la probabilité de développement de défauts de flaveur par inactivation d'enzymes ou en accélérant l'affinage en accroissant les interactions enzyme-substrat ; ou (4) d'améliorer la texture et les autres propriétés fonctionnelles, par exemple la fonte. Finallement, les facteurs à considérer pour la fabrication et l'affinage des différentes variétés de fromage, ou classes de variétés spécifiques (par exemple fromages allégés) vont différer clairement et augmenter la complexité des options technologiques disponibles. Cet article passe en revue les principes clés pour le pré-traitement du lait de fabrication de fromage tels que résumés brièvement précédemment.

lait / fromage / traitement thermique / séparation par membrane / haute pression / homogénéisation / standardisation

1. INTRODUCTION

For centuries, milk for cheese-making was subjected to no pre-treatment before curdling, and many cheese varieties worldwide are still made from raw milk, particularly, but not exclusively, artisanal cheeses. However, predominantly for reasons of safety, but also consistency of quality, and manipulation of product characteristics, most cheese-making today involves the treatment of milk by one or more processing steps prior to addition of coagulant and starter culture.

Perhaps the simplest and earliest technological intervention, driven by safety concerns, was the pasteurisation of milk, first carried out in vats or kettles at temperatures around 63–65 °C (low-temperature, long-time, LTLT, pasteurisation) and more recently in continuous-flow plate heat exchangers at 72–74 °C for 15–30 s (high-temperature, short-time, HTST, pasteurisation). For a high proportion of cheese varieties, pasteurisation is the sole treatment applied to the cheese-milk. Pasteurisation also inactivates some enzymes, reverses shifts in the mineral balance of milk induced by cold storage, and influences the microflora of non-starter lactic acid bacteria (NSLAB) in the final cheese. It is primarily for the latter reason that many cheese-makers prefer to continue to use raw milk, as the contribution of NSLAB bacteria to cheese flavour is felt to be unacceptably impaired by pasteurisation. There has also been interest for some time in the application of heat treatments more severe than pasteurisation, which will result in significant denaturation of whey proteins and their resulting incorporation into cheese curd, with significant effects on cheese yield and composition.

In several varieties, or industrial production settings, the consistency of composition of cheese may also be controlled by standardisation of the incoming milk, generally in terms of manipulation of the ratio of fat to total protein or casein, by centrifugal separation and proportional mixing of cream whole milk and/or skim

milk, by membrane filtration, or by addition of sources of milk protein.

In addition, in recent years, there have been studies of application of a number of new approaches to processing of cheese-milk, including the application of technologies new either to cheese production or to food processing in general, to achieve certain product or process benefits; these include use of homogenisation, treatment at high pressures, and addition of exogenous enzymes other than those required strictly for coagulation of the milk.

This review will provide an overview of the broad approaches to pre-treatment of cheese-milk mentioned briefly above (see summary in Tab. I). A particular focus will be given to recent studies of the applications of novel and innovative processing strategies.

2. PRINCIPLES OF CHEESE-MAKING

The fundamental physico-chemical principle under-pinning the conversion of milk to a cheese curd is the destabilisation of the casein micelles to such an extent that a gel is formed which, when cut, syneresis in a manner which can be accelerated by mechanical stirring and/or mild heating (for general reviews on cheese manufacture and ripening, see [38–41]). This yields curd particles which can readily be recovered from the bulk aqueous serum phase of the milk, i.e., whey. In parallel, cultures of lactic acid bacteria added almost concurrently with the coagulant begin to grow and metabolise lactose to lactic acid, lowering the pH of the curd and ultimately playing a key role in the development of cheese flavour, through the release upon death and lysis of a range of catabolic enzymes which hydrolyse or otherwise convert milk constituents entrapped in the curd to a range of flavour and aroma compounds. Within a short time of the addition of coagulant and culture, the curd and whey are separated and the curd is pressed to enable fusion into a solid visco-elastic mass; this is then stored under conditions which allow the requisite biochemical reactions to be catalysed by the cheese micro-flora, which principally consists of the starter bacteria mentioned, but may also include other (non-starter) lactic acid bacteria and, in some varieties, yeasts and moulds deliberately added. During the storage, or ripening, period, enzymes within the coagulant and also arising from the milk itself break down the coagulated casein matrix, resulting in changes in cheese texture.

The broad principles outlined summarise the principle of manufacture of what are called rennet-coagulated cheeses, which represent the vast majority of both cheese varieties and actual tonnage of cheese produced globally and are the only cheese varieties dealt with in this review. The huge diversity of cheese varieties, from Pamesan (dry, crumbly and pungent) to Camembert (soft and mould-encrusted) results from surprisingly minor alterations in these core principles, such as the start of culture (bacterial and mould, if used), the extent to which whey is removed by stirring, cooking or pressing (greater whey removal leads to lower-moisture, harder, cheese) and the conditions during ripening (particularly temperature and relative humidity).

To consider the impact of milk processing technologies on cheese, it is important to consider what the milk contributes to cheese, as it is clear that much of cheese-making relies on external agents added to the milk (coagulant and cultures) and operations applied to the resulting curd. The key constituents, properties and populations of milk which are of interest to the cheese-maker are summarised in Table I.

Clearly, there are numerous potential advantages to applying more ambitious technological strategies for processing

Table I. Milk constituents, properties and populations of interest for cheese-making.

Parameter	Significance for cheese-making	Reasons and strategies for manipulation
Casein	Forms rennet gel, synereses to yield cheese curd	Increase level (addition of milk protein source) either of total protein, or relative to fat content (standardisation), or change properties (enzyme addition)
Fat	Contributes to cheese texture and yield	Increase or decrease fat content relative to total composition or protein level (standardisation); incorporate more directly into cheese curd through interactions with protein (homogenisation); accelerate release of volatile flavour compounds (enzyme addition)
Whey protein	Normally largely lost in whey	Incorporate into curd to increase yield (heat treatment, high-pressure treatment)
Water	Major constituent of cheese; level may be characteristic of particular varieties	Increase level to increase yield (heat treatment, high-pressure treatment) but may impair quality
Bacteria	Raw milk microflora either intact (raw milk) or pathogens and many spoilage and non-starter bacteria eliminated, but not spore-forming species (pasteurised milk)	Increase efficiency of removal of spore-forming bacteria for some species (membrane filtration)

cheese-milk, in terms of manipulating yield, composition, microbiological quality, and even biochemistry of ripening. However, in many cases actual implementation of new strategies has met with significant hurdles or barriers to implementation, and this review will consider these challenges alongside the possible advantages of each approach discussed.

3. HEAT TREATMENTS OTHER THAN PASTEURISATION

As outlined above, milk for cheese manufacture is heated to eliminate pathogenic bacteria, to minimise damage to caseins by proteolytic bacteria on storage or to incorporate heat-denatured whey proteins in curd, thereby improving cheese yield [7]. Furthermore, more severe heat treatment of milk may be applied to inactivate spores from *Clostridium tyrobutyricum* by 4 log cycles and thus minimise the late blowing gas defect during cheese ripening [124].

Heat treatment of milk at conditions more severe than those used for conventional pasteurisation results in denaturation of whey proteins, interactions between whey proteins and casein micelles, and transfer of soluble calcium, magnesium and phosphate to the insoluble colloidal state. Casein micelles are very stable at high temperatures, although changes in zeta potential, size, hydration of micelles and some association-dissociation reactions do occur under severe heat treatments [37,126–129]. Denaturation of whey proteins exposes side chain groups originally buried in the native structure, particularly reactive thiol groups, and the unfolded proteins may self-aggregate or interact with casein micelles, through interactions with κ-casein. Ionic strength, pH and the concentrations of calcium and

protein influence the extent of denaturation of the whey proteins [24,100,129]. The extent of association of denatured whey protein with casein micelles is dependent on the pH of the milk prior to heating, levels of soluble calcium and phosphate, milk solids concentration and mode of heating (direct or indirect).

For cheese-makers, the principal interest has been in increasing yield by exploiting this heat-induced association of caseins with whey proteins, while attempting to minimise undesirable changes in cheese quality. The effects of heat treatment at temperatures between 72 and 140 °C for holding times between 15 s and 5 min on whey protein denaturation prior to incorporation into cheese were reported by Law et al. [80]. Heat treatment of milk at ~ 110 °C for 60 s increased protein recovery in curd by 10% [6]. Where facilities to heat to temperatures > 100 °C are not available, increasing milk pH prior to heating increases levels of whey protein denaturation [9].

In the cheese vat, high heat treatment of milk prolongs rennet coagulation times and reduces the strength of rennet gels [5, 26], leading to impaired syneresis [10, 92, 115]. The adverse effects on coagulation are attributed to the inhibition of hydrolysis of κ-casein by chymosin due to the β-lactoglobulin/κ-casein complex at the micelle surface impairing the accessibility of κ-casein to the coagulant [55, 59, 139], to reduced reactivity of renneted micelles with attached denatured whey proteins to aggregation, or to a reduction in the concentration of micellar calcium [139, 142]. Reduced shrinkage of the *para*-casein-whey protein network promotes increased water-binding [145], leading to poor coagulation and syneresis properties, increased set-to-cut times, soggy curds with poor matting ability, ragged curd chips and poor curd fusion during cheese manufacture [48, 92]. Gel-forming properties of high-heat-treated milks may be partly restored by ultrafiltration of milk to higher protein levels prior to cheese manufacture, reducing pH and increasing milk temperature during coagulation, increasing the level of added rennet and/or by the addition of $CaCl_2$ to the cheese-milk [54, 55] or by pH cycling [129]. Acidification of heated milks reduces charge repulsion and increases solubilisation of colloidal calcium [22]. Acidification to pH 5.8 or pH 6.2 prior to renneting of strongly heated milks increases cheese yield [5, 8, 10] and pH cycling (acidification to pH 5.5, overnight storage at 4 °C and adjustment to pH 6.2) prior to renneting has been shown to increase moisture and protein contents in Cheddar cheese [70] although that study did not report on cheese texture and sensory properties.

High heat treatment increases cheese yield and retention of whey protein and moisture in cheese and reduces the level of calcium and phosphorus due to reduced dry matter and increased whey protein content [7–9, 129]. Flavour intensity is reduced in cheeses made from strongly heated milk [5, 8, 48] but bitterness was largely eliminated by a reduction in rennet quantities used [6].

Guinee et al. [48] attributed decreased cheese firmness with increased heating severity of milk to increased cheese moisture and decreased protein content. Adjustment of pH to 6.2 prior to renneting resulted in Cheddar cheese with a texture comparable to control cheese [5, 8] and Banks et al. [9] reported that manipulation of pH prior to heating had a significant effect on melt characteristics of a Cheddar-type cheese.

Calvo et al. [23] reported greater breakdown of β-casein during ripening of Cheddar-type cheese manufactured from milk heated to 110 °C for 30 s than in cheese made from pasteurised milk, but Benfeldt et al. [12] attributed reduced hydrolysis of β- and α_{s2}-caseins in Danbo cheese made from milks heated

to 90 °C for 60 s to thermal inactivation of the plasminogen activation system and heat-induced interactions between the plasminogen activation system and β-lactoglobulin. Guinee et al. [55] reported higher levels of primary proteolysis and reduced levels of smaller peptides and amino acids in cases of increased levels of whey protein denaturation in cheeses manufactured from UF retentate produced from strongly heated milk.

From studies of other varieties, severe heat treatment of milk resulted in a more open microstructure in Quarg [76], while Camembert-type cheese produced from severely heated milk had a higher yield and flavour, body and texture attributes similar to that of control cheeses made from pasteurised milk [43].

4. CARBON DIOXIDE TREATMENT OF CHEESE-MILK

There has been interest for some time in the use of carbon dioxide (CO_2) as a treatment for milk for preservation and technological reasons, due to its solubility in milk and inhibitory effect against a broad spectrum of micro-organisms [61]. It has been shown that addition of CO_2 to raw milk decreased proteolysis, due to effects on both microbial growth and concomitant protease production and inhibited plasmin activity due to pH reduction; lipolysis was also retarded, probably due to reduced microbial growth [90].

A small number of studies have considered the possible use of such treatment prior to cheese-making. Nelson et al. [103] injected CO_2 into milk to a level of 1600 ppm after pasteurisation, which reduced pH to around 5.9, and made Cheddar cheese using normal levels of coagulant and starter addition. Milk treated with CO_2 had lower whey pH at drainage, shorter total make time, and altered yield due to increased losses of calcium and fat, and increased salt retention. When cheese made using CO_2 addition was ripened, cheese retained CO_2 and treated cheese showed accelerated proteolysis, perhaps due to changes in substrate availability of increased retention or activity of chymosin [104].

5. HIGH-PRESSURE TREATMENT OF CHEESE-MILK

High-pressure (HP) treatment of food has progressed in a relatively short space of time from a research subject of academic curiosity limited by perceived huge expense of use at industrial scale to a commercially realistic processing option, albeit for specific niche applications such as oysters, meat, guacamole and fruit products, including juices and smoothies. While these applications have grown rapidly within the last ten years, applications for dairy products have been notably absent; however, within the last year applications for treatment of processed cheese spreads (Spain) and functional and fermented dairy products (New Zealand) have been launched. The relative slowness of transfer of research in dairy products to market applications does not reflect a lack of potential interest; in contrast, whereas, for many of the products listed above, HP treatment results in microbial or enzymatic inactivation without loss of nutrients or flavour attributes (the principal advantage of using HP for these products), the effects of pressure on milk are far more complex, and often unique.

HP treatment principally exerts effects on macromolecules with complex structures, changing their structure and properties; as milk proteins are an enormously complex protein system, it is perhaps not surprising that dramatic changes occur under pressure, with repercussions

for a whole range of milk and dairy product properties. The principal heat-induced change in milk proteins, denaturation of whey proteins, as discussed earlier, also occurs under pressure, with β-lactoglobulin being more susceptible to denaturation, at pressures above 100 MPa (the typical units of pressure, where 1 MPa is 10 times atmospheric pressure), than α-lactalbumin, which is significantly denatured only at or above 400 MPa [65].

However, arguably the most interesting effects of pressure concern the casein micelles, which change little at normal milk processing temperatures, except for the interactions with whey proteins mentioned. Depending on the pressure applied, duration and temperature of treatment and pH of milk, HP treatment of milk can result in aggregation of casein micelles (around 250 MPa) or significant dissociation (at pressures above 400 MPa). These phenomena have been shown to be due to the extent to which reassociation occurs after varying extents of pressure-induced disruption of the cohesive forces maintaining the structural integrity of casein micelles, e.g., the extent of solubilisation of colloidal calcium phosphate (CCP) [65].

Such fundamental changes in the characteristics of the basic building blocks of milk gels have predictably profound effects on the cheese-making properties of milk. Rennet coagulation time, for example, may be reduced by treatment at 100–300 MPa, but increased following more severe treatments [113, 150]. Huppertz et al. [67] showed that HP treatment increased wet curd yield, by up to 25% after treatment at 600–800 MPa, due to both incorporation of denatured whey protein and increased moisture retention. The combined effects of heat and pressure on rennet coagulation have also been reported [87], and it has been shown that HP treatment can modulate the negative effects of excessive heat treatment on cheese-making properties of milk [68].

There have been contradictory reports on the effect of HP treatment on acidification of milk by lactic acid bacteria, with Pandey et al. [113] reporting a reduced rate of pH change in HP-treated milk relative to raw or pasteurised milk, but Huppertz et al. [66] reporting the reverse, which they suggested may be due to increased availability of substrates for bacterial growth due to pressure-induced dissociation of casein micelles.

While a number of authors have thus described the changes in rennet coagulation properties following HP treatment, there have been relatively few studies in which actual cheese is produced from HP-treated milk [110, 123, 136]. Of these, a number also concentrate mainly on cheese composition, yield and textural properties. One of the earliest studies on Cheddar cheese reported that, while HP treatment increased the yield of Cheddar cheese, textural defects resulted from increased incorporation of moisture into cheese [31]. San Martín-González et al. [122] produced Cheddar cheese from milk treated at 483 or 676 MPa at a range of temperatures and found pressure- and temperature-dependent increases in cheese yield and moisture content, and increased cheese hardness.

A number of studies of the biochemical properties of cheese made from raw, pasteurised or HP-treated goats' milk have indicated that the latter had higher levels of incorporated β-lactoglobulin (β-lg), and altered rates of proteolysis of caseins and profiles of free amino acids during ripening [20, 137] and altered profiles of organic acids [19], while levels of lipolysis in cheese made from HP-treated milk were closer to those in raw milk cheese than in that made from pasteurised milk [17]. The same authors have reported on the textural and microstructural properties of the three types of cheese [21], and have reported that the microbiological quality of cheese made from HP-treated milk was similar to that

of that made from pasteurised milk [18]. There have also been reports that HP treatment of milk can improve the acceptability of reduced-fat cheese [101].

A recent paper has indicated that treatment of raw milk at 500 MPa for 10 min reduced an innoculum of *Listeria monocytogenes* to undetectable levels in both milk and cheese, without significant effects on cheese composition [86]. Another potentially interesting application of HP in cheese involves inactivation of bacteriophage in milk or whey [102].

In terms of potential industrial application, HP treatment involves packaging product into sealed flexible containers (e.g., bags) and immersing them in a pressure-transmitting medium (typically an emulsion or alcohol:oil mixture) in a chamber within which the pressure can be raised to the requisite level and maintained for the desired time, with controlled rates of compression and decompression; the manipulation of pressure is typically attained by use of a piston or high-pressure pumps. This is clearly a batch system and currently available commercial systems treat up to around 300 kg of product per cycle; the limitations of scale for large cheese factories are thus a possible barrier to implementation. Some semi-continuous plants have been developed where liquids such as milk could be pressure-treated directly by a piston within a chamber called an isolator; connection of several such chambers in sequence but operating out of phase allows semi-continuous operation, but such systems do not seem to have been adopted by the food industry. This, and the very substantial capital investment for HP treatment systems, makes it critical that very attractive advantages for the cheese-maker will need to be proven before commercial adoption is likely (see Patel et al. [114] for a discussion of the hurdles involved in commercialisation of HP in the dairy industry).

6. HOMOGENIZATION OF CHEESE-MILK

Since its presentation by Auguste Gaulin at the Paris World Fair in 1899, the homogenizer has become a standard tool in the dairy industry. The primary aim of homogenization of milk is to reduce the size of the fat globules, thereby delaying their creaming rate [69]. In raw milk, fat globule size commonly ranges from ~ 0.2–15 μm, and homogenization generally aims to reduce the maximum to < 2 μm. For this purpose, two-stage valve homogenizers are commonly used, which operate at pressure of ~ 20 MPa. More recently, novel homogenization devices, e.g., high-pressure homogenizers and microfluidisers, which can operate at pressures of several hundred MPa and achieve greater reductions in fat globule size, have been developed [69]. In cheese-making, homogenization of cheese-milk can be of interest for the purpose of preventing creaming of fat globules, reducing fat losses in the whey or controlling development of free fat in the cheese [71, 112, 119].

Due to the reduction in fat globule size on homogenization, the total surface area of the fat globules increases and the amount of original fat globule membrane material is by far insufficient to fully cover the newly-formed surface [69]. As a result, other surface-active components of milk, primarily caseins and, to a lesser extent, whey proteins, become adsorbed onto the surface of the newly formed globules [69, 146]. Thus fat globules in homogenized milk almost resemble casein-covered emulsion droplets. The adsorption of caseins onto the fat globules has the following implications for cheese-making characteristics of milk:

(1) casein surface area in milk is increased, but the amount of micellar casein is reduced;
(2) two types of particles with a casein micelle surface layer exist: native casein

micelles and casein-covered fat globules;
(3) when adsorbed, casein micelles tend to spread over the surface of the fat globule and hence increase in effective surface area but with reduced surface-density of κ-casein.

The rennet coagulation time (RCT) of unhomogenised milk is generally lower than that of homogenized milk [57, 62, 135, 149]. This is probably related to the larger casein surface area in homogenized milk, as well as the lower surface density of κ-casein. The former increases the probability of interactions between particles, whereas the latter, for a subclass of particles, reduces the amount of κ-casein that needs to be hydrolyzed before micellar flocculation is induced. Conflicting reports exist on the influence of homogenization on the rate of rennet-induced gel formation; both homogenization-induced increases [57, 62, 149] and decreases [135] therein have been reported. Zamora et al. [149] reported that, on high-pressure homogenization (100–330 MPa), the rate of gel formation may be either increased or decreased, with no clear trend as a function of homogenization pressure, but large differences in the pH of samples hinder a clear and unequivocal interpretation of these data.

Negative aspects of homogenization occur in the subsequent stages of cheese-making, i.e., the syneresis of the *para*-casein matrix and the fusion of the *para*-casein micelles into a strong and cohesive network. Cheese curd from homogenized milk shows poor syneresis [32, 46, 62] and, as a result, has high moisture content. Furthermore, cheese curd prepared from homogenized milk is also often characterized by a coarse and brittle structure [46, 135, 149].

The reason for the impaired syneresis of curd prepared from homogenized milk can be traced back to the role of fat globules in the *para*-casein matrix. In curd from unhomogenised milk, milk fat globules are, on average, of the volume of several thousand *para*-casein micelles and are distributed throughout the matrix individually or in clusters with areas of up to tens of micrometers [50]. Native fat globules do not interact with the *para*-casein micelles in the curd matrix and do not participate in syneresis. Hence, they act as plasticizers, with their spatial distribution determining the length scale over which syneresis in the *para*-casein matrix occurs, and prevent the excessive syneresis of the *para*-casein matrix that is, for instance, observed in cheese curd made from skim milk [50, 116]. In homogenized milk, the much smaller fat globules are distributed at a considerably smaller length scale, thereby reducing the scale over which syneresis can occur. Furthermore, unlike their unhomogenized counterparts, homogenized milk fat globules do interact with the *para*-casein matrix, thus reducing the overall effectiveness of the syneresis process because less of the *para*-casein micelle surface is available for interaction with other micelles [46, 50]. The already reduced amount of micellar casein present in homogenized (see above) is likely to contribute to this phenomenon.

Cheese made from homogenized milk is generally characterized by an increased moisture content [47, 71, 72, 84, 143], which can lead to deviations in ripening profiles. In addition, if cheese from homogenized milk is not heat-treated sufficiently, excessive lipolysis may occur, due to the fact that the membrane of homogenized fat globules is more permeable for lipase than the native milk fat globule membrane [72, 146].

7. MEMBRANE SEPARATION OF CHEESE-MILK

Membrane separation processes are commonly applied to separate a liquid under a pressure gradient through a semi-permeable membrane into two liquid

streams of different composition, the permeate (which flows through the membrane) and the retentate (which concentrates those substances which do not pass through the membrane in a reduced volume of fluid). These processes are applied in dairy processing for an ever-increasing range of applications, e.g., concentration, demineralization, protein separation, or removal of bacteria. Four types of membrane filtration can be distinguished [77, 93, 94]:

(1) *ultrafiltration (UF)*, which selectively separates macromolecules having a molecular mass of 1000–200 000 g·mol^{-1};
(2) *microfiltration (MF)*, which selectively separates particles and macromolecules with a molecular mass greater than 200 000 g·mol^{-1};
(3) *nanofiltration (NF)*, which selectively separates molecules with a molecular weight ranging from 200–1000 g·mol^{-1};
(4) *reverse osmosis (RO)*, which separates solutes with a molecular mass smaller than 150 g·mol^{-1}.

Of these technologies, UF, and, to a lesser extent, MF can be used as a pretreatment for cheese-milk. For an extensive and detailed overview of membrane processing in cheese technology, the reader is referred to the reviews of [96–98].

7.1. Ultrafiltration

Essentially, UF enables concentration of casein content and recovery of whey proteins for cheese manufacture [51, 53]. UF of milk at pH 6.6–6.8 concentrates mineral salts bound to casein micelles in the same proportion as proteins and increases buffering capacity, which affects acidification, pH, rennet coagulation and rheological characteristics of curd [98]. Acidification before or during UF [53] and/or salt addition to retentate leads to solubilisation of colloidal calcium in the permeate.

In cheese-making, three types of UF retentate can be distinguished:

(1) *Low-concentration UF retentate*: Milk is concentrated a maximum of 2-fold prior to cheese-making, mainly for standardization of protein level. Advantages of this application are increased manufacturing efficiency, reduced rennet requirements and increased cheese yield.
(2) *Medium-concentration UF retentate:* Milk is concentrated a minimum of 2-fold and a maximum of 5-fold. This application is presently of little commercial interest.
(3) *Liquid pre-cheese:* Milk is concentrated to a composition similar to that of the cheese variety to be made, followed by addition of starter culture and subsequent setting with rennet. Processes based on this technology have been developed successfully for the manufacture of some softer cheese varieties, e.g., Camembert, Feta and blue cheese.

During UF, milk runs pressure tangentially across a membrane with a molecular weight cut-off (MWCO) of 1000–200 000 g·mol^{-1}; for cheese-milk, MWCO is generally < 20 000 g·mol^{-1}. Compounds with a molecular mass greater than the MWCO of the membrane, e.g., globular fat, caseins, whey proteins and micellar salts, are selectively concentrated in the UF retentate, whereas those with a molecular smaller than the MWCO, e.g., lactose, serum salts and peptides, are found largely in the UF permeate at their original concentration. This has two major implications for cheese-making properties of milk:

(1) The inter-micellar mean free distance is reduced considerably, from ~ 200 nm in unconcentrated skim

milk [146] to < 10 nm in skim milk concentrated to a casein content of ~ 20% [75]. The reduced inter-micellar mean free distance forces the micelles to interact more frequently with each other as a result of collisions induced by Brownian motion [27, 144].

(2) The buffering capacity of the milk is increased considerably [96, 98]. This increase in buffering capacity is primarily due to the increased concentrations of proteins and micellar minerals in the UF retentate, both of which are key contributors to the buffering capacity of milk, particularly in the pH region 5.5–7.0 [121].

As a result of these changes, the cheese-making properties of UF retentates differ from those of unconcentrated milk in several aspects. Rennet coagulation time is not affected by concentration if the same amount of rennet is added to unconcentrated or UF concentrate [42, 93, 98], indicating that the proportion of κ-casein hydrolyzed at the point of flocculation is lower in UF retentate than in unconcentrated milk, as is indeed observed experimentally [25, 75, 140]. Following 4-fold concentration, hydrolysis of ~ 50% of κ-casein is required to induce gel formation [25] whereas, at ~ 7 fold concentration, hydrolysis of only 20% of κ-casein is required [75]. The rate of gel firming can also be enhanced by concentration [97,98], although recent studies by Karlsson et al. [75] showed that, following ~ 7-fold concentration (casein content ~ 20%, w/w), the rate of gel formation of the UF retentate was lower than that of the unconcentrated milk. The reduced coagulation and flocculation rate was related to a high zero-shear viscosity of milk reducing the rate [75].

This emphasises the fact that the rate of gel formation is strongly influenced by both casein concentration and the degree of hydrolysis of the κ-casein and that optimization is required to maximize the rate of gelation of UF retentates. When cheese-milk was partially supplemented with UF retentate, RCT decreased [113] and increasing protein level resulted in reduced gelation time and increased firming rates [53].

Microstructural analysis by confocal laser scanning microscopy of rennet-induced gels of ~ 7-fold concentrated UF retentate revealed that larger rennet-induced casein aggregates are formed in unconcentrated milk than in UF retentate [75]. The degree of macroscopic syneresis, i.e., separation of whey from the gel on a macroscopic level, is considerably less for curd prepared from UF retentate than for curd prepared from unconcentrated milk [75, 117]. Microsyneresis, i.e., re-arrangement of the protein network on a microstructural level, was observed in renneted unconcentrated milk as well as 7-fold concentrated UF retentate, but occurred at a considerably later time-point in the latter [75]. This delay in microsyneresis in a rennet gel from UF retentate is probably due to the fact that micelles therein are still partially covered by κ-casein at the onset of gelation and thus have a low affinity for binding and subsequent rearrangement and fusion [28, 141]. Further studies on the microstructure of rennet gels prepared from retentate concentrated 2–5 fold by UF are recommended to further our understanding of this area.

Although dependant on concentration factor, adjustment of manufacture protocols, etc., increasing milk protein levels in Cheddar cheese manufacture by use of low-concentration UF results in increased moisture-adjusted yield and actual cheese yield, increased cheese protein, salt-in-moisture, calcium and phosphorous contents and decreased moisture levels [51, 79]. An optimum degree of concentration for making hard cheese varieties of ~ 1.7:1 has been recommended [35, 36, 79].

Cheese made from UF retentate is often characterized by a long time required

to reach the desired pH and an acidic taste [96–98] which is related to the higher buffering capacity of a UF retentate. Furthermore, flavour development in hard and semi-hard cheese made from UF retentate is generally slow, which has been related to a reduced rate of proteolysis of caseins during ripening of such cheese [11,98], probably resulting from retention of inhibitors of chymosin and plasmin in the UF retentate [11].

UF of milk to 4–4.5% protein reduced moisture-in-nonfat-substance (MNFS) levels in cheese made from late-lactation milk to levels similar to those for mid-lactation milk with no significant difference in proteolysis or flavour and with enhanced texture [16]. Increased starter inoculum and reduced cook and Cheddaring temperatures resulted in similar composition and moisture-adjusted yields to control cheeses with improved texture and increased proteolysis in Cheddar cheese manufactured from UF milk [125].

Homogenisation of cream in milks supplemented with UF skim milk to 5.93% protein resulted in Cheddar cheeses of improved functionality and texture, yields, solids and fat recovery [112]. Protein losses in whey were reported to be similar to those for control cheeses [51] or lower from retentate cheese when expressed as kg component lost per kilogram cheese obtained [79].

7.2. Microfiltration

While applications of MF in cheesemaking are by no means as widely studied as those for UF, there have been some recent developments that are worth noting. First of all, MF may be used for partial microbial decontamination of cheese-milk, as outlined in Figure 1. The "Bactocatch" process [97,98] involves microfiltration of the skim milk using 1.4 μm pore size at 37 or 50 °C to concentrate the microbes in milk in the UF retentate, which, together with the cream, is subjected to a heat treatment, e.g., a UHT treatment at 115–120 °C. The retentate containing bacteria and somatic cells (accounting for ~ 5% of the skim milk stream or ~ 0.5% if a second MF process is incorporated) may be added to the cream prior to heat treatment; however, thermostable enzymes present may have deleterious effects on subsequent cheese quality [94].

Average decimal reduction of bacteria is > 3.5 (10 to 50 cfu·mL^{-1} milk) and is > 4.5 for sporeforming bacteria, due to binding of bacterial spores to part of the cell wall resulting in larger apparent cell size [94]. Decimal reduction of pathogenic bacteria is 3.5–4.0 and somatic cells are totally removed [94]. However, milk produced using this process has been described by cheese-makers as 'too clean' and cheese prepared therefrom may lack flavour development as a result. MF results in reduced cheese NSLAB counts, with reduced intensity of cheese flavour and aroma, secondary proteolysis and levels of short-chain volatile acids in Swiss-type cheeses [13] and lower populations of heterofermentative lactobacilli, propionic acid bacteria and enterococci than from raw milk or from pasteurised milk with added MF retentate. However, Roy et al. [120] reported similar counts of *Lactobacillus casei* and lactococci in both MF and thermised milks but with a lower mesophillic spore count in the latter. Beuvier et al. [13] reported that MF treatment of milk resulted in lower levels of hydrolysis of β-casein in cheeses than from pasteurised milk; however, Roy et al. [120] proposed that heating of cream or skim milk during MF treatment increased hydrolysis of β-casein in Cheddar cheese, possibly due to activation of the plasminogen-plasmin activation system. Further optimization is required to achieve desirable ripening characteristics of such cheeses.

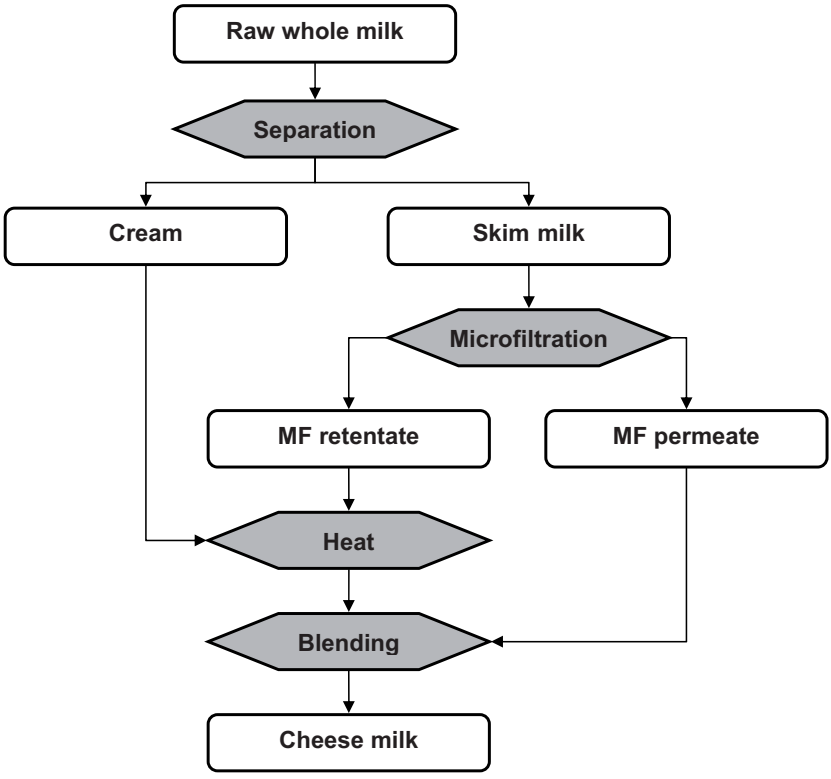

Figure 1. Process for decontamination of milk by microfiltration (adapted from [98]).

A second application of MF in cheesemaking is in standardization of the casein content of milk, rather than the total protein content that is standardized by UF. MF uses larger pore sizes and lower pressures than UF. Whey proteins are smaller molecules (3 to 5 μm) compared to casein micelles (15 to 600 nm) and can be separated by use of 0.1 to 0.2 μm pore size membranes [14]. This separation produces casein enriched retentate and permeates containing significant amounts of native state α-lactalbumin and β-lactoglobulin. MF also concentrates calcium phosphate in the micellar form [105]. Brandsma and Rizvi [14] showed in-process pH adjustment and microfiltration of acidified skim milk with 0.2 μm membrane to produce highly concentrated retentate with reduced Ca and whey protein content suitable for use in cheese manufacture.

Casein-enriched milk prepared by MF has been reported to have improved rennet coagulation properties [95, 133] and reduced loss of fat and fines in the whey [98]. Increasing MF concentration factor resulted in Mozzarella and Cheddar cheeses with increased moisture, protein and calcium contents, total solids recovery, actual and composition-adjusted cheese yields, proteolysis and flavour and decreased hardness. Increasing chymosin level and adjusting MNFS levels for milk concentrated 1.8 X resulted in Cheddar cheese similar to control cheese [14, 15, 105, 106]. Furthermore, the MF permeate that is produced is an ideal substrate for production of high quality whey

protein isolate or further fractionation of individual whey proteins, due to its neutral pH, and lack of residual coagulant or other proteases and lack of caseinomacropeptide [95].

Processes for the extensive concentration of milk by MF, to the point where whey removal subsequent to addition of rennet and starter is minimal or absent, have also been described [2, 15, 90]. The development of proteolytic and functional characteristics of Mozzarella cheese made from MF retentates has been reported to be slower than in control cheeses due to the absence of starter culture, lower levels of rennet used and inhibition of cheese proteolysis due to residual whey proteins in the MF retentates [2,15]. This problem may be ameliorated through increased rennet levels, standardisation of curd cook times and addition of starter culture [2, 15, 105].

8. STANDARDISATION OF CHEESE-MILK BY PROTEIN ADDITION

Standardisation of milk protein/casein levels, by concentration of milk solids or by producing a protein rich fraction which may be subsequently added to milk prior to cheese manufacture, may be used to reduce some negative defects associated with a seasonal milk supply [5, 52], such as variable protein/casein contents which result in poor curd-forming properties and in variations in yield and in composition and consistency of resultant cheeses [3, 83, 109]. Increased yield results from reduced losses of fat and casein particles in whey and better retention of whey proteins in the aqueous phase of cheese. Furthermore, standardisation of milk protein to higher than normal levels enables increased plant throughput without installation of extra cheese vats [52].

Protein standardisation may be achieved by: use of low-concentrated retentate (LCR) produced by UF or RO of cheesemilk; enrichment of casein by MF; or addition of phosphocasein powder (PC) or milk protein concentrate (MPC) [52], typically followed by cheese manufacture using conventional equipment [98]. Addition of denatured microparticulated whey proteins to cheesemilk may also be used to ameliorate defects associated with reduced fat cheese [34, 134].

MPC has the same casein:whey protein ratio as milk [56]. MF of skim milk allows water, lactose, α-lactalbumin, β-lactoglobulin, soluble minerals and NPN to pass through the membrane in the permeate but retains casein in the retentate [105, 106]. MPC produced by spray drying of retentate from ultrafiltration of skim milk contains ~ 65% protein, 3% fat, 2% calcium and 20% lactose [138], while MPC made by ultrafiltration of skim milk and diafiltration contains very little lactose. SMP and skim milk condensed by evaporation contain ~ 51% and 14% lactose respectively [138]. High lactose content in cheese may promote undesirable fermentation, atypical flavour or white crystal defect during cheese ripening [1].

Commercial calcium caseinate produced by acid precipitation is rich in casein but low in minerals. Rennet coagulation time for cheese-milk supplemented with 6% calcium caseinate powder was significantly higher than for a similar level of supplementation with diafiltered microfiltered DMF or UF retentates [133]. Milk enriched with calcium caseinate before production of low-fat Cheddar produced curd which did not retain fat, possibly due to reduced calcium levels inhibiting coagulation and formation of adequate curd structure, and yields were lower than cheese from DMF powder [133]. PC and MPC have rennet coagulation properties similar to those of milk at similar protein concentration and ionic strength [33, 74, 78]. In a comparative study, increasing milk protein content through standardisation with UFR, MPC or PC resulted

in enhanced rennet coaguability, reduced gelation time and increased curd firmness but there was no significant effect of protein type [52].

Manufacture of reduced-fat Cheddar cheese from milk standardised with MPC doubled cheese yields per unit weight of milk, significantly increased total solid recoveries, reduced lactose contents and NSLAB counts, but had no significant effect on starter bacteria count, and gave lower levels of FAA and brothy and bitter scores than control cheeses [138]. Decreased levels of primary proteolysis were possibly due to reduced plasmin activity or to addition of chymosin on a volume basis, rather than on a casein basis. Increased moisture-adjusted yield may be due to a high level of whey protein denaturation in the MPC and thus a high level of protein recovery in the MPC-fortified cheese [52]. Standardisation of milk protein content using PC, UFR or MPC resulted in significantly increased fat recovery and yield of cheese per unit weight of milk, normalised to reference levels of casein and fat [52, 56]. However, as PC is a relatively new ingredient, limited availability could limit its use [52].

Microparticulated whey proteins (produced by microparticulation of whey protein concentrate under conditions of heat and shear) act as a non-interacting filler in the cheese matrix and can mimic the structural properties of fat [91] and may be used in manufacture of low-fat cheese [29]. Lucey and Gorry [89] reported that a commercial microparticulated milk protein had little effect on rennet coagulation; however, Fenelon and Guinee [34] and Guinee et al. [49] reported impaired rennet coagulation properties, with lower curd-firming rates and curd firmness after a fixed renneting time, possibly due to the denatured protein content or dilution of the casein, i.e., the active gel forming component. Addition of microparticulated whey protein resulted in reduced-fat Cheddar with increased moisture and MNFS contents, higher yields, probably due to hydrated whey proteins, lower firmness, but had little effect on or slightly reduced levels of proteolysis and flavour grades [34, 89].

9. TREATMENT OF CHEESEMILK WITH EXOGENOUS ENZYMES

Apart from coagulant, the most common reasons for incorporation of exogenous enzymes in cheese manufacture are: (i) acceleration of ripening, usually by addition of commercial proteinase or lipase preparations; (ii) enhancement of cheese flavour, by addition of peptidases and lipases; (iii) increasing cheese yield, e.g., by addition of transglutaminase or phospholipases. Enzymes (as individual enzymes or as mixtures) may be added to cheesemilk prior to cheese manufacture or during manufacture, alongside the starter or rennet [4]. However, addition of proteinases directly to cheesemilk with rennet or starter culture may prematurely hydrolyse casein, interfere with renneting, and reduce yield [148], and losses of ~ 90% of added enzyme at whey drainage are typical [81]. Encapsulation of free enzymes provides an alternative approach to enzyme addition and also enables protection from the outside environment and may allow for controlled release [44, 132]. One specific approach is the use of liposomes, which are microscopic lipid vesicles comprising an outer shell of phospholipid and an internal aqueous core [82, 118, 130, 147]. The potential advantages of liposomes over other methods of enzyme encapsulation for cheese applications are that they are made from materials naturally present in cheese, they protect casein from early hydrolysis during cheesemaking, and they partition well in the curd [148].

9.1. Use of phospholipase

During cheese manufacture, 85–95% of milk fat is entrapped in the cheese curd [107] with the remaining fat lost in the whey and, to a lesser extent, in the brine, if used. In manufacture of pasta filata-type cheese, the fat retention rarely exceeds 90% because of additional losses encountered in the hot stretching step [85]. A new enzymatic method for increasing cheese yield through treating milk with phospholipase prior to cheesemaking has been reported [60, 85, 107, 108]. Phospholipase A_1 (EC 3.1.1.32) hydrolyses the *sn*-1 ester bond of phospholipids, resulting in formation of the less hydrophobic and thus more water-soluble lysophospholipids and fatty acids. It is proposed that lysophospholipids released from the fat globule membranes act as surface-active agents in the cheese curd, which emulsify water and fat during processing and reduces syneresis. Furthermore, α_{s1}-casein and β-lactoglobulin interact with lysophopsholipids and also form surface-active lipoprotein complexes [85]. Phospholipases used are specific and have little activity towards di- or tri- glycerides; thus, flavour defects caused by the release of short chain fatty acids are avoided, because phospholipids mainly contain non-volatile palmitic, oleic and stearic acids.

There have been a small number of studies of the use of phospholipases in cheese applications; no effect of phospholipase treatment of cheese-milk on renneting properties of the cheese-milk has been reported as yet. Hydrolysis of milk phospholipids with a commercial preparation of fungal phospholipase A1 from *Fusarium venenatum* added to cheese-milk prior to renneting during manufacture of low-moisture part-skim Mozzarella cheese reduced fat losses in whey and cooking water and increased cheese yield, as a result of improved fat and moisture retention in the cheese curd [60, 85]. Despite enzymatic modification of the fat globule membrane, the fat globules retained their original size and appearance [85]. Lysophospholipids were retained in the curds in higher amounts compared to native phospholipids, possibly because of interaction with casein and subsequent incorporation into the cheese matrix. It was proposed by Lilbaek et al. [85] that the observed improvement in yield results from improved emulsification and water-holding capacity as a consequence of the presence of lysophospholipids in the curd. No significant differences in cheese microstructure or functionality (melt, stretch, browning) were observed between control cheeses and those treated with phospholipase, and there was no significant effect of phospholipase treatment of cheesemilk on sensory attributes of the cheese or downstream processing of the whey [60].

The potential for further yield improvements by combining use of phospholipase with enrichment of cheesemilk with buttermilk phospholipids to increase the amount of lysophospholipids in the curd was suggested by Lilbaek et al. [85]. Enrichment of cheesemilk with phospholipids from buttermilk or soy milk increased cheese yield and improved the texture of low-fat cheese [30, 45, 97, 99]. Enhanced fat retention has also been reported in full-fat Colby cheese manufactured with added soy lecithin [58].

9.2. Applications of transglutaminase in cheese-making

The enzyme transglutaminase (TGase; EC 2.3.2.13) catalyzes the formation of an intermolecular covalent isopeptide-bond between protein molecules via an acyl-transfer reaction between the ε-amino group of a lysine residue and the γ-carboximide groups of a glutamine residue [73]. The caseins are excellent substrates for TGase-induced cross-linking, because of their unfolded native structure.

For micellar casein, the order of susceptibility to TGase-induced cross-linking is κ-CN > β-CN > α_s-CN, which is predominantly related to the easy of accessibility of the caseins for the enzyme [131]. Light-scattering measurements have shown that, in unconcentrated milk, cross-linking of micellar caseins is exclusively intra-micellar and not inter-micellar [64]. This intra-micellar cross-linking stabilizes the micelle against disintegration under unfavourable conditions [64, 131] and also has major implications for the hairy brush, consisting predominantly of κ-CN, which provides colloidal stability to the micelle [64].

Pre-treatment of milk with transglutaminase increases RCT considerably [63, 88, 111], and small-deformation oscillatory rheology shows similar trends. When most κ-CN is cross-linked, no rennet-induced flocculation of micelles is observed at all [63]. Cross-linking of caseins in the micellar core is unlikely to be the cause of TGase-induced increases in RCT, since internally cross-linked *para*-casein micelles, prepared by TGase treatment of casein micelles following cold-renneting, do form a firm coagulum on warming to 30 °C [111]. Lorenzen [88] and O'Sullivan et al. [111] observed that the amount of CMP released during renneting decreases with increasing degree of cross-linking and concluded therefrom that rennet-induced increases in RCT are due to a reduction in the rate of enzymatic hydrolysis of κ-CN. However, decreases in the rate of release of CMP do not necessarily indicate that κ-CN is not hydrolysed, since it can remain attached to the micelles following hydrolysis by chymosin [63]. This point was further highlighted by in-situ diffusing wave spectroscopy measurements during renneting by Huppertz and De Kruif [63] which strongly indicated that the time point at which inter-micellar repulsion is reduced sufficiently to induce micellar flocculation is only increased slightly by TGase-treatment, and that it is the rate of aggregation of these renneted micelles that is reduced.

Studies on the properties of cheese curd or ripening of cheese prepared from milk pre-treated with TGase are, to our knowledge, not available at the moment. However, based on the aforementioned impaired coagulation properties of milk following TGase treatment, it is unlikely that many beneficial effects may be expected in cheese-making.

10. CONCLUSIONS

Where centuries ago, cheese was essentially prepared from unprocessed milk, cheese-making nowadays can involve a complex sequence of milk pre-treatments, e.g., membrane filtration, (partial) homogenization and heat treatment. These treatment steps are performed to reach required or desirable characteristics of the milk prior to cheese-making. As outlined in this review, such treatment in general do not only induce the desired change, but are generally accompanied by other changes, which are often less desirable and require adjustment of operations and parameters of the cheese-making process. The recent developments in this area that are described highlight the importance of understanding the manner in which the changes induced by particular pre-treatments of milk will lead to a successful application thereof, and ultimately allow cheese-makers to achieve their goals, e.g., increased yield, consistency, throughput, controlled or accelerated ripening or simply optimal control of food safety.

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