ABSTRACT

Scientific interest in cheese crystals extends back more than a century. However, starting around the 1970s, industry interest, and interest on the part of cheese scientists, grew dramatically as changes in cheesemaking technology and market changes caused the presence of crystals in the marketplace to increase; advanced analytical capabilities enabled new crystalline species to be identified, their origins and causative factors to be elucidated, and their contributions to cheese texture to be better understood. It is now evident that a host of organic- and inorganic-based crystals occur in natural cheeses. Some crystals form preferentially at the surface of rindless or rinded cheeses, others in the irregular openings or spherical eyes that occur within the body of some cheeses, and still others embedded within the cheese matrix. It is also evident that crystals may profoundly influence cheese texture, both as a direct consequence of their abundance, size, shape, and hardness, and as an indirect result of cascading physiochemical events initiated by crystal formation. Consumer response to increased incidence of crystals in the marketplace has been mixed. On the one hand, surface crystals of calcium lactate pentahydrate on Cheddar cheese came to be viewed quite negatively in some markets, often being mistaken for mold growth and spoilage. This triggered industry concern and led to considerable research to determine the underlying causes and to develop strategies to limit or prevent calcium lactate pentahydrate formation. On the one hand, surface crystals of calcium lactate pentahydrate on Cheddar cheese came to be viewed quite negatively in some markets, often being mistaken for mold growth and spoilage. This triggered industry concern and led to considerable research to determine the underlying causes and to develop strategies to limit or prevent calcium lactate pentahydrate formation. At the same time, other forms of crystallization increasingly came to be viewed as positive features in the growing market dedicated to artisanal and traditional cheeses, giving rise to a bifurcated consumer response to cheese crystals that is evident today. Traditional artisanal cheesemakers perhaps have the most to gain from advances in cheese-crystal research. Traditional artisanal cheeses rely heavily on stories that are woven around their identity to create uniqueness and add value. A challenge and opportunity for these cheesemakers in the United States and globally will be to translate the fascinating science of their cheese crystals into engaging narratives that capture the imagination, add value to their cheese, and enhance the enjoyment of their cheese by consumers.

Key words: X-ray diffraction, texture, quality

INTRODUCTION

Presence of crystals in cheese has been the object of scientific curiosity and investigation since the early 20th century (Babcock and Russell, 1901; Babcock et al., 1902). By the latter decades of the 20th century, however, the study of cheese crystals took on added importance as new and more powerful analytical capabilities enabled new crystalline species in natural cheeses to be identified, their origins and causative factors to be elucidated, and their contributions to cheese texture to be better understood. Crystals also occur in pasteurized processed cheese, where they are generally considered defects. They are usually associated with emulsifying salts that are added during product formulation, which lead to the formation of various forms of calcium phosphate, disodium phosphate dodecahydrate, monoclinic calcium pyrophosphate dihydrate, and calcium citrate. Other crystals in processed cheese may originate as preformed crystals present in the ingredient cheeses, which carry over into the final pasteurized processed product, or from lactose present in dairy ingredients. Crystals in pasteurized processed cheese will not be covered in this review; the reader is directed to other reference works for comprehensive discussions on this topic (Guinee, 2007; Milani, 2009; Lucey et al., 2011; Guinee, 2017).

The purpose of this review is to address advances in cheese-crystal science that have occurred over the past few decades in parallel with cheesemaking and market changes that have increased the presence of cheese crystals in the marketplace. The diversity of crystals found in natural cheeses, factors that affect their formation,
the effect of crystals and the crystallization process on texture and sensory properties, and relevant historical context will be emphasized.

**EARLY CHEESE-CRYSTAL RESEARCH**

References to cheese crystals in the dairy literature extend back to the start of the 20th century when Babcock and Russell (1901) noted that Cheddar cheese cured at low temperatures (<10°C) developed “white specks.” Looking at these experiments through the lens of modern science, it is likely that they were observing the first widespread occurrence of calcium lactate pentahydrate (CLP) crystals. Around the same time, Dox (1911) reported the first occurrence of crystals of the AA Tyr in Roquefort cheese. By the 1920s, microscopy techniques began to be used to study cheese crystals (Laxa, 1926, 1927). Crystals of Leu and Tyr (both AA) and lactate of lime (probably CLP) were noted across many different cheese varieties. By the 1930s, researchers began to apply powder X-ray diffractometry (PXRD) to study Cheddar cheese, and in the process generated reference patterns (d-values) for crystals of calcium phosphate and CLP, which enabled white specks in Cheddar cheese to be definitively identified for the first time (Tuckey et al. 1938a,b,c).

During the last 3 decades of the 20th century, developments in the Cheddar cheese industry sharply elevated industry concern over, and research interest in, cheese crystals, specifically CLP crystals. Around that time, Cheddar cheesemakers began to standardize their milk with nonfat dry milk to smooth out changes in plant production caused by seasonal variation in milk composition, as well as to improve consistency of cheese composition and increase total weight of cheese produced per vat (Freeman et al., 1970; Barbano, 2000). The considerable economic advantage of nonfat dry milk standardization was immediately evident, and the practice soon became widespread (Papadatos et al., 2002). However, nonfat dry milk contains around 51% lactose, which meant that nonfat dry milk fortified cheese milk contained higher lactose in the serum phase, resulting in greater carryover of residual lactose to the finished cheese. This, in turn, elevated the risk of CLP crystal formation. Increased incidence of CLP crystals in Cheddar cheese soon sparked industry concern and prompted considerable research into the causes of CLP crystals and their prevention, as discussed in detail later in this review. Even as surface crystals of CLP came to be viewed quite negatively in some consumer markets, often being mistaken for mold growth and spoilage, other forms of crystals were also gaining recognition as positive features in other markets dedicated to traditional artisanal cheeses, giving rise to a bifurcated consumer response to cheese crystals that is evident today.

**CLASSIFICATION OF CHEESE CRYSTALS**

Crystals in cheese fall into 2 broad categories, namely those that are organic, meaning that they contain carbon and are of direct biological origin, and those that are inorganic, or mineral-based, but which may also include species derived from carbon dioxide (carbonates). Using this as the primary criterion for differentiation, cheese crystals can be further grouped into the assemblages presented in Figure 1.

Inorganic cheese crystals are subdivided based on whether they include the minerals calcium or magnesium. Calcium-based crystals occur as calcium phosphate and calcium carbonate. The former consist of brushite (Conochie and Sutherland, 1965; Tansman et al., 2017b,c) and possibly other forms of calcium phosphate such as tricalcium phosphate, which are suspected in cheese but have not been confirmed definitively (Le Graet et al., 1983; Karahadian and Lindsay, 1987; Gaucheron et al., 1999). Calcium carbonate crystals that have been definitively identified in cheese include calcite (Tansman et al., 2017c; Polowsky et al., 2018b) and Ikaite (Tansman et al., 2017a;c; Polowsky et al., 2018a,b). Only 1 magnesium-based crystal, struvite, has been definitively shown to occur in cheese (Tansman et al., 2017a,c; Polowsky et al., 2018b), although other forms of magnesium phosphate and carbonate are suspected (Gaucheron et al., 1999; Le Graet et al., 1986).

Organic crystals in cheese derive from 3 different organic compounds: AA, lactate, and citrate. Calcium citrate and magnesium citrate crystals are suspected in natural cheeses, but await further work to establish their presence with certainty (Morris et al., 1988; Gaucheron et al., 1999). Crystalline forms of the AA L(+) Tyr and L(+) Leu occur in certain hard aged cheeses (Tansman et al., 2015). Crystals of the AA Cys and Ile have been reported in extremely long-aged Cheddar cheeses, but further work is needed to determine whether they occur in cheeses more typical of the marketplace (Harper et al., 1953). Finally, both the L(−) and D(−) isomers of lactate combine with calcium to produce 2 different isomeric forms of CLP in natural cheeses, one form that contains 2 L(+)lactate molecules, and the other that exists as a racemic mixture of 1 lactate molecule in the D(−)-form and a second in the L(+) form (Johnson et al., 1990a,b; Chou et al., 2003; Tansman et al., 2014). The formation of D(−)-L(+) CLP crystals does not require equal concentrations of D(−)- and L(+)lactate in the cheese; indeed, crystals are able to form when D(−)-lactate comprises as little as 25% of the total...
cheese-lactate content (Johnson et al., 1990a). The CLP crystals that consist exclusively of D(−)-lactate have not been reported in cheese.

**SPATIAL DISTRIBUTION OF CHEESE CRYSTALS**

Crystals may form preferentially at the surface of cheese (including on some cheeses that are rindless and others that possess a rind) or in the body of the cheese. In the body of the cheese, crystals may be embedded tightly within the curd matrix or in open regions within the body of the cheese (including irregular mechanical crevices and spherical eyes), depending the variety of cheese and type of crystal.

**Crystals That Form at the Surface of Rindless Cheese**

Crystals of CLP that form at the surface of Cheddar (and Colby) cheese blocks, as well as at the surface of consumer portions that have been cut from larger blocks and repackaged in barrier film, are probably the most commonly occurring surface crystals on ripened rindless cheese varieties (Figure 2). At the microscopic level (i.e., scanning electron microscopy), CLP crystals appear as aggregates of needlelike crystals (Bottazzi et al., 1982; Washam et al., 1982; Washam et al., 1985). At the macro level (i.e., visible to the naked eye), white CLP crystalline elements may appear as circular pinpoint crystal regions or as irregular crystal patches that develop when multiple adjacent circular crystals grow in diameter over time, communicate, and fuse together into larger irregularly aggregated regions (Agarwal et al., 2005; Rajbhandari and Kindstedt, 2008). Surface crystals of CLP are generally soft, but the apparent structure and density of the crystalline mass may vary for reasons that are not well understood (Rajbhandari and Kindstedt, 2005b). Furthermore, surface crystals grow 3-dimensionally and are capable of pushing up on the packaging film as they grow vertically, eventually imparting a bumpy feel to the wrapped surface.

Serum separation (i.e., sweating or weeping), which leads to the accumulation of cheese serum between the cheese surface and packaging film, increases the risk of CLP crystallization incidence and intensity because free serum provides a medium through which calcium and lactate ions diffuse unimpeded with the potential to fuel explosive crystal growth (Johnson, 2014). This risk may be further elevated when serum separation is caused by low cheese pH that results from high lactate levels in the cheese, which in turn shifts calcium from the casein-associated state to the serum phase (Swearingen et al., 2004). Thus, the direct effect of serum separation on CLP crystallization may be confounded with indirect effects that are related to the factors that cause serum separation, such as low cheese pH or high storage temperature (Swearingen et al., 2004; Rajbhandari et al., 2013).

Several researchers investigated the chemical composition of macro CLP crystals that were carefully
scraped from the surface of Cheddar cheese or picked from within the cheese body (McDowall and McDowell, 1939; Farrer and Hollberg, 1960; Dybing et al., 1988; Rajbhandari and Kindstedt, 2005a). In these studies, the crystalline mass consisted of around 60 to 85% CLP and 10 to 20% free water, and the balance of the collected material comprised largely of free lactate, protein, fat, and NaCl. The non-CLP matter within the crystalline mass probably included cheese residue that adhered to the crystals, but also soluble components from the cheese that were dissolved in the free serum occluded within the crystalline mass (Washam et al., 1985; Johnson, 2014).

During the 1970s and early 1980s, the first systematic research aimed at preventing CLP crystal formation by reducing residual lactose content in the final cheese was reported (Pearce et al., 1973; Sutherland and Jameson, 1981). Around the same time, the role of nonstarter bacteria in the production of the much less soluble D(-)L(+)-form of CLP crystals in Cheddar became the focus of much research. By that time, it was well established that the traditional mesophilic Lactococcus lactis starter cultures used in Cheddar cheesemaking produce only the more soluble L(+)-form of lactate through lactose fermentation (Lawrence et al., 1976). However, while studying the effects of salt content on starter inhibition and residual lactose fermentation during Cheddar cheese ripening, Turner and Thomas (1980) isolated cheese nonstarter bacteria (predominantly Pediococci) that produced both the D(-) and L(+)-forms of lactate from lactose and, more importantly, were also able to convert L(+)-lactate to D(-)-lactate. Furthermore, they noted that when nonstarter bacteria grew to high populations during ripening, levels of D(-)-lactate in Cheddar cheese increased and L(+)-lactate decreased until they reached similar concentrations. These findings were rendered particularly interesting by the authors’ claim that crystals consisting of a racemic mixture of D(-)l(+)-CLP had recently been identified on commercial Cheddar cheeses (though the authors presented no data to support this claim), suggesting that nonstarter bacteria may play a role in D(-)l(+)-CLP formation.

Follow up studies by Thomas and Pearce (1981) confirmed the presence of D(-)-lactate in Cheddar cheeses sampled repeatedly from a large manufacturing plant in New Zealand. These authors also reported that D(-)-lactate was present in Cheddar cheese sampled from other New Zealand cheese plants. Around the same time, D(-)-lactate was also reported in Cheddar cheese produced in Australia (Tinson et al., 1982), as well as in cheeses imported from the United States (Thomas and Crow, 1983). Thus, evidence was mounting that nonstarter bacteria capable of producing D(-)-lactate are widely distributed and common adventitious contaminants of Cheddar cheese. These findings clearly had significant commercial implications, pending corroboration of the earlier claim of Turner and Thomas (1980) that D(-)l(+)-CLP crystallization occurs in Cheddar cheese.

Further studies by Thomas and Crow (1983) confirmed that nonstarter pediococci and lactobacilli isolated from commercial Cheddar cheese possessed the
ability to convert L(+)- to D(−)-lactate via 2 racemizing stereospecific lactate dehydrogenases. Furthermore, they demonstrated that the isolated racemase-positive nonstarter pediococci were able to proliferate in Cheddar cheese during ripening and convert L(+) to D(−)-lactate, with additional D(−)-lactate produced through residual lactose fermentation in cases where the starter bacteria failed to quickly ferment residual lactose in the cheese. These authors also reported that studies had recently determined that D(−)l(+)CLP is less soluble than the L(+)form of CLP, although they referenced unpublished data support the claim. Thus, evidence continued to mount that (1) nonstarter bacteria in Cheddar cheese are capable of producing high levels of D(−)-lactate during ripening, (2) these nonstarter bacteria are evidently widely distributed and normal inhabitants of Cheddar cheese, (3) D(−)-lactate, when present in Cheddar cheese, may give rise to crystals of D(−)l(+)CLP (though the occurrence of such crystals in Cheddar cheese remained to be confirmed), and (4) crystals of D(−)l(+)CLP are less soluble than those of L(+)CLP, and thus may be more troublesome in commercial practice [though again, the lower solubility of D(−)l(+)CLP needed to be confirmed].

Severn et al. (1986) were the first to present definitive evidence that crystalline deposits scraped from the surface of Cheddar cheese consisted of D(−)l(+)CLP crystals. Soon thereafter, Johnson et al. (1990a) established a direct link between the growth of a nonstarter racemase-positive lactobacillus strain [originally isolated from Cheddar cheese displaying D(−)l(+)CLP surface crystals] in Cheddar cheese, the concomitant production of a racemic mixture of L(+)D(−)-lactate in the cheese, and the development surface crystals of D(−)l(+)CLP. These watershed advances were then followed by studies to identify sources of nonstarter racemizing bacteria such as Lactobacillus curvatus and Pediococcus acidilactici in cheese plant environments, especially biofilms that form on equipment surfaces (Wong, 1998; Somers et al., 2001; Agarwal et al., 2006a). This work demonstrated clearly that biofilms may serve as persistent reservoirs of racemizing nonstarter bacteria that can lead to D(−)l(+)CLP crystallization in Cheddar cheese, thus highlighting the need for more effective cleaning and sanitizing regimens for equipment lines, especially in complex high capacity cheese plants that operate almost continuously.

Confirmation of the lower solubility of D(−)l(+)CLP also appeared in the literature around this time (Cao et al., 2001; Johnson, 2004; Kubantseva et al., 2004), further corroborating the critical role that racemizing nonstarter bacteria play in elevating the risk of CLP crystallization incidence and intensity by producing D(−)l(+)CLP crystals of lower solubility. The CLP crystals in the L(+)form can also occur in Cheddar cheese that contains little or no D(−)-lactate when manufacturing conditions such as protein fortification of cheese milk, use of salt tolerant starter cultures, and low salt-in-moisture content in the cheese result in the accumulation of higher levels of L(+)lactate combined with lower cheese pH, which in turn shifts calcium in the casein-associated state to the serum phase, producing higher soluble calcium (Agarwal et al., 2008). The combination of higher L(+)lactate, lower pH, and higher soluble calcium creates conditions favorable for supersaturation of calcium lactate in the cheese serum and elevates the potential for L(+)CLP crystallization, despite its higher solubility (Johnson, 2004; Swearingen et al., 2004; Agarwal et al., 2005, 2006b, 2008).

Crystallization of L(+)CLP may also occur at the surface of consumer cuts of naturally smoked Cheddar cheese with little or no D(−)-lactate. Natural smoking causes dehydration and an acidic environment at the cheese surface, which elevates levels of L(+)lactate and calcium ions in the serum phase. (Rajbhandari and Kindstedt, 2005a; Rajbhandari et al., 2009).

As research on CLP crystals progressed from the early 1990s onwards, it also became increasingly evident that other confounding factors related to the cut-and-wrap operation of cheese destined for retail markets also affected CLP crystallization in complex and confounding ways. Decades earlier, Farrer and Hollberg (1960) had reported a high incidence of CLP crystallization on consumer cuts of Cheddar cheese wrapped in flexible packaging material. Now, systematic studies on the effects of cutting, wrapping, and storage conditions on CLP crystallization appear in the literature. Johnson et al. (1990a) first reported that much greater surface crystallization occurred on consumer-sized cheese samples that were wrapped loosely (i.e., sealed without vacuum treatment) than on samples that were vacuum packed tightly.

A follow up study by Johnson et al. (1990b) compared surface crystallization on Cheddar samples packaged by 2 different widely used packaging technologies: vacuum packaging and packaging with carbon dioxide gas flush. All of the cheese samples in this study had the potential to form CLP crystals based on their total and D(−)-lactate contents, but those that were packaged with carbon dioxide gas flush developed crystals earlier, and the crystals intensified more rapidly, than samples that were vacuum packaged tightly; indeed, crystal development was substantially delayed or prevented completely in vacuum packaged samples. The authors also noted that the packaging film of gas-flushed packed cheeses became looser over time and that crystal development almost always occurred in regions where the plastic film had lost contact with cheese surface. Furthermore, they
reported that bleaching of the cheese surface preceded the loosening of the packaging film and CLP formation. Thus, a sequence of events in gas-flushed packaged samples, initiated by surface bleaching and followed by loosening of the packaging film, seemed to predispose the cheese surface to CLP crystallization.

A more complete understanding of the role of gas-flushed packaging emerged when Agarwal et al. (2005) compared tightly vacuum-packed Cheddar cheese samples with samples packed loosely under 3 different gas-flush treatments, namely flushing with pure carbon dioxide, a 50:50 mixture of carbon dioxide and nitrogen, or pure nitrogen gas. When carbon dioxide was used for gas flushing (either alone or in combination with nitrogen), carbon dioxide gas that remained in the headspace above the cheese after sealing eventually dissolved into the serum phase at the cheese surface, causing a localized decrease in pH due to the formation of carbonic acid. The resulting decrease in surface pH may explain the perplexing observations noted by Johnson et al. (1990b). For example, localized pH decline may have caused the observed bleaching effect and may also have led to localized curd contraction and serum separation, which in turn may have caused the packaging film to loosen, all of which preceded the formation of CLP crystals. Thus, gas flushing with carbon dioxide may increase the risk of CLP crystallization in some cases through localized decreases in surface pH that shift casein-associated calcium into the serum phase, and through curd contraction, serum separation, and subsequent loosening of the packaging film.

Agarwal et al. (2005) also confirmed the earlier observations of Johnson et al. (1990a,b) that tightness of the packaging film to the cheese surface profoundly influences the incidence and intensity of CLP crystallization in Cheddar cheese. They reported that samples that were vacuum packaged tightly displayed little or no surface crystallization, even when cheeses contained a racemic mixture of D(−)- and L(+)lactate. In contrast, all loosely packaged gas-flushed samples developed intense CLP crystallization, even those that were flushed with inert nitrogen (which had no effect on cheese surface pH), thus implicating the looseness of the wrap as the causative factor.

Furthermore, during postpackaging storage, 2 confounding factors combine with package tightness to strongly influence CLP surface crystallization on Cheddar cheese: roughness of the cheese surface and storage temperature. Rajbhandari and Kindstedt (2014) demonstrated that cheese samples that were etched to create rough surfaces developed much greater CLP crystal coverage than samples with smooth surfaces when both were packaged loosely and stored at 4°C. In contrast, very tight packaging strongly inhibited crystal formation, even on samples with rough surfaces, presumably by pressing down upon and smoothing out surface irregularities that may serve as nucleation sites for CLP crystals (Johnson, 2004). Storage temperature also plays a complicated role in CLP crystallization. Lower temperature generally results in a greater risk of CLP crystallization due to lower CLP solubility (Pearce et al., 1973; Johnson et al., 1990a,b; Chou et al., 2003). However, higher storage temperature can also lead to increased crystallization when it causes serum separation at the cheese surface or stimulates the growth of racemizing nonstarter bacteria that, in turn, convert L(+) to D(−)-lactate (Agarwal et al., 2005; Rajbhandari et al., 2013).

In summary, minimizing CLP crystallization in Cheddar cheese requires careful control over both cheese manufacturing conditions and conditions used during ripening, packaging, and postpackaging storage. As packaging technologies continue to evolve and advance, there is an ongoing need to evaluate the influence of new packaging approaches on CLP surface crystallization in Cheddar cheese. There is also a need to investigate CLP surface crystallization in other cheeses such as Gouda, which are susceptible to CLP formation, but which have not been systematically reported on in the literature.

An alternative approach to preventing CLP crystallization is to use sodium gluconate, a generally recognized as safe ingredient, to increase the solubility of calcium lactate through formation of calcium-lactate-gluconate complexes or calcium chelation (Phadungath and Metzger, 2011; Johnson, 2014). Sodium gluconate used in Cheddar cheesemaking to limit CLP crystals may be applied to the curd during the salting step at rates of around 0.03% to 0.06% of milk weight, evidently with good crystal-inhibiting efficacy (Nutricepts Inc., 2018). Sodium gluconate is not a traditional Cheddar cheese ingredient and is not included in the US Food and Drug Administration Standard of Identity for Cheddar cheese (US Food and Drug Administration, 2019). However, it may qualify as a processing aid, and thus be exempt from ingredient restrictions and labeling requirements. Potential disadvantages of this approach to limit CLP crystallization is that some markets are very sensitive to the use of nontraditional ingredients in cheese and to issues of transparency in labeling.

Finally, recent reports of extensive crystallization of leucine at the surface of rindless Parmesan-style cheese have confirmed that other varieties of rindless cheese in addition to Cheddar may be susceptible to surface crystallization by other crystal species (Johnson, 2014; Tansman et al., 2015).
**Crystals That Form at the Surface of Rinded Cheese**

**White Mold-Ripened (Bloomy-Rind) Cheese.** The migration of calcium and phosphorus from the center to the surface of white mold-ripened cheeses (also called bloomy-rind cheeses) became the focus of much research and industry interest beginning around the 1960s (Jakubowski and Reps, 1966; Metche and Fanni, 1978). In a landmark study, Le Graet et al. (1983) demonstrated that the outward migration of calcium and phosphate ions in traditional Camembert cheese was caused by the high surface pH that developed during ripening. In traditional Camembert, surface pH rises to near neutrality due to the fermentation of lactate by yeasts, Geotrichum candidum and Penicillium camemberti molds, and the production of ammonia by P. camemberti (Spinnler, 2017). However, Le Graet et al. (1983) observed a similar outward movement of calcium and phosphate ions when the pH at the cheese surface was increased in the absence of surface flora by storing cheese in an ammoniated atmosphere. High surface pH, in turn, caused calcium phosphate to crystallize out of solution in the rind, thereby creating gradients within the serum phase of the cheese that acted similar to a pump to move more calcium and phosphate to the surface to fuel further crystallization. The authors speculated that calcium phosphate crystallization took the form of tricalcium phosphate, based on the measured ratio of calcium to phosphorus at the cheese surface. Very importantly, they also demonstrated that the extent of demineralization in the body of the cheese increased with increasing pH at the surface. Thus, controlling the rise in surface pH, by whatever means, seemed to be a critical factor in controlling the extent of calcium phosphate migration, surface crystallization, and demineralization of the cheese body. Furthermore, around the same time, Vassal et al. (1986) reported that the rise in surface pH appeared to be a major factor driving the process of radial softening that characterizes white mold-ripened cheeses during ripening.

Karahadian and Lindsay (1987) soon confirmed that pH rise at the surface of white mold-ripened cheese is the driving force for the migration of calcium phosphate to the surface and its precipitation in the rind. They, too, proposed that calcium phosphate crystallized in the form of tricalcium phosphate based on the ratio of calcium to phosphorus in the rind. They also proposed a direct linkage between the rise in surface pH, the progressive demineralization of the cheese body through this mechanism, and the radial softening and liquefaction of cheese texture that characteristically occurs in traditional white mold-ripened cheese during ripening. Specifically, they proposed that the progressive demineralization of the cheese body, in concert with the radial increase in cheese pH, promotes increased solubilization of casein, which in turn drives the process of radial softening and ultimate liquefaction of the cheese body. Based on this model, the authors postulated that it should be possible to modulate texture development in Camembert cheese by modulating pH rise during ripening through control over lactate fermentation and ammonia production, and through manipulation of cheese composition.

These insights were soon brought to fruition with the commercialization of a new generation of so-called “stabilized” white mold-ripened cheeses with much longer shelf lives (Spinnler, 2017). In the production of stabilized cheeses, the traditional L. lactis mesophilic starter culture is replaced with thermophilic Streptococcus thermophilus. This has the effect of slowing down acidification during cheesemaking, enabling the production of Camembert-like cheese with much higher mineral content, buffering capacity, and initial pH than the traditional version. Incorporation of a curd-washing step may also be applied to reduce residual lactose content to further modulate cheese pH. During ripening, high cheese-mineral content and buffering capacity substantially lessen the rise in pH mediated by the surface flora compared with the traditional cheese, resulting in substantially less outward migration of calcium and phosphate ions, and less calcium phosphate precipitation in the rind (Tansman et al., 2017b). This modulates the softening of the body over time and may prevent liquefaction altogether.

Characterization of the crystalline species that precipitate at the surface of white mold-ripened cheese has been subject of several studies. Brooker (1987) applied a suite of microscopy techniques, including transmission electron microscopy, scanning electron microscopy coupled with X-ray spectrometry, and polarized light and bright field light microscopy coupled with phosphate-specific staining, to examine the crystalline species in white mold-ripened cheeses. Taken in total, these microscopic data confirmed the presence of vast numbers of crystals tentatively identified as a form of calcium phosphate at the surface of several white mold-ripened cheeses. The author also proposed that proteolysis in the rind mediated by extracellular proteases produced by the surface mold may play a role in favoring calcium phosphate crystallization by reducing the level of intact casein and large peptides that may serve as local sources of crystal inhibition.

Subsequent studies of traditional Camembert cheese (Le Graet and Brule, 1988), and of a model Camembert-like cheese stored in an ammoniated atmosphere (Gaucheron et al., 1999), demonstrated that magnesium and citrate also migrate outwards and accumulate in the rind during ripening. Gaucheron et al. (1999)
proposed that magnesium phosphate, magnesium citrate, and calcium citrate crystallize in the rind in parallel with calcium phosphate based on their solubilities; however, no direct evidence of the occurrence of these crystals was presented. More recently, Tansman et al. (2017b) also observed the outward migration of magnesium along with calcium and phosphorus, and their concomitant accumulation in the rind of stabilized white mold-ripened cheese. In this study, crystals of brushite, which were clearly visible by polarized light microscopy and definitively identified by PXRD, increased in number and size in the rind during ripening (Figure 3). However, no crystalline species other than brushite were detected in this study.

Further work is needed to confirm the crystalline forms in which magnesium (and possibly citrate) accumulate in the rind of white mold-ripened cheese. More importantly, additional studies are needed to definitively identify the crystalline forms in which calcium phosphate accumulate in the rind of traditional bloomy-rind cheeses such as Camembert. Although brushite appears to be the sole form present in the much lower pH stabilized–type cheese (Tansman et al., 2017b), it is possible that species of crystalline calcium phosphate shift to other forms such as tricalcium phosphate at the much higher pH levels that develop at the surface of the traditional cheese. Application of PXRD to study crystal development in traditional Camembert will likely shed important light on any such pH-induced progression of crystal species.

**Smear-Ripened (Washed-Rind) Cheese.** Soft smear-ripened cheeses (also called washed-rind cheeses) share some striking commonalities with soft white mold-ripened cheeses. They are high in moisture, they develop complex surface microflora during ripening (which mediate a dramatic rise in surface pH), and they undergo characteristic radial softening and eventual liquefaction as ripening progresses. There are also noteworthy differences. They are produced under conditions of slow acidification that result in cheese with higher mineral retention and buffering capacity. In this respect, they are more similar to the stabilized version of soft white mold-ripened cheese. Also, the surface microflora are dominated by yeasts, so-called coryneform bacteria, and *Geotrichum candidum* in some cheeses (Bockelmann, 2014). Surface smear microflora tend to be more proteolytic, produce more ammonia, and to raise the surface pH to even higher levels than the surface microflora of traditional white mold-ripened cheeses.

Building upon the previous discussion of surface crystallization in white mold-ripened cheeses, it is not unreasonable to predict that the high rind pH of smear-ripened cheese may similarly trigger localized crystallization of calcium phosphate, thereby creating gradients within the cheese serum phase that act similar to a pump to move more calcium and phosphate to the surface to fuel further crystallization. Because smear-ripened cheeses are highly mineralized, heavy surface crystallization of calcium phosphate would presumably be necessary to demineralize and deplete the buffering capacity of the cheese body sufficiently to account for the extreme rise in cheese pH and rapid radial softening and liquefaction of the cheese body.

This scenario seemed to gain plausibility when Tansman et al. (2015) reported the presence of high concentrations of calcium and phosphorus in smear material that had been collected from the surface of a soft smear-ripened cheese. However, these authors also observed only 1 X-ray diffraction pattern in the smears, which matched that of ikaite (calcium carbonate hexahydrate), indicating the sole occurrence of ikaite crys-
tals. This result was quite unexpected for 2 reasons. First, ikaite is very rarely found in nature; it occurs in harsh cold-water environments such as in Arctic and Antarctic ice cores (Rysgaard et al., 2012). Second, because ikaite does not contain phosphorus, it could not account for the accumulation of high phosphorus levels in the rind. However, analyses by single crystal X-ray diffractometry of individual crystals harvested from a cheese smear confirmed the presence of ikaite plus struvite, a phosphate-containing crystal (magnesium ammonium phosphate hexahydrate), in the smear (Tansman et al., 2017a). Thus, crystal development in smear-ripened cheese seemed to follow a different track from that observed in white mold-ripened cheese.

Tansman et al. (2017c) then investigated mineral migration and crystal formation in a smear-ripened cheese throughout 10 wk of aging. By wk 3 after production, rind pH increased to near neutrality, and calcium, phosphorus, and magnesium ions commenced migration to the surface, where they accumulated in the smear. Concomitantly, crystals of brushite and calcite were detected in the smear by PXRD. By wk 5, however, as the outward flow of minerals continued, brushite crystals in the smear were replaced by ikaite crystals, which then persisted along with calcite throughout the remainder of the study. By wk 10, struvite crystals appeared prominently in the X-ray diffraction spectra of the smears, along with calcite and ikaite. Crystals of ikaite and struvite were readily visible and identifiable using polarized light microscopy, attaining lengths greater than 250 μm in some crystals over the 10-wk aging study. Ovoid entities of approximately the same size and shape as the ikaite crystals have also been observed in cheese smears by scanning electron microscopy (Figure 4).

In summary, although surface crystals of calcium phosphate (brushite) appeared very early in ripening, they were superseded by carbonate (calcite and ikaite)- and ammonium (struvite)-based crystals that became the principal drivers of outward migration of calcium, phosphate, and magnesium and their accumulation in the high pH smear. Thus, it seems likely that carbon dioxide and ammonia in the atmosphere of the ripening room partitioned into the serum phase of the smear at the cheese surface, thereby generating carbonate and ammonium ions and subsequently triggering crystallization of calcite, ikaite, and struvite. A proposed model to describe this process is presented in Figure 5. If this sequence is confirmed, it may be possible to attenuate the rise in surface pH, surface crystallization of ikaite and struvite, and demineralization of the cheese body by limiting atmospheric carbon dioxide and ammonia levels in the ripening room. This approach could conceivably be used to modulate radial softening and liquefaction in smear-ripened cheese.

The large sizes of the crystals reported by Tansman et al. (2017c) seemed likely to exceed the sensory threshold and impart a direct textural perception, in view of the demonstrated sensory effect of crystals in other foods, such as ice crystals and grittiness in ice cream (Goff and Hartel, 2013) and lactose crystals and sandiness in dulce de leche (Hough et al., 1990). Informal reports from the industry also suggest that a gritty or sandy mouthfeel was a common characteristic associated with the rinds of smear-ripened cheeses (Tansman et al., 2017a). Polowsky et al. (2018b) subsequently applied a novel polarized light microscopy technique (Polowsky et al., 2018a; Figure 6) to characterize the relationship between crystal size and grittiness perception in smears from commercial soft smear-ripened cheeses. This work demonstrated that crystals varied widely in length and area as measured by image analysis of polarized light micrographs (Figure 7), confirming the observation of Tansman et al. (2017c) that smear crystals may attain very large sizes during ripening. Furthermore, 17 of the 24 smear samples exceeded the sensory threshold of the trained panel for grittiness, which occurred at crystal length of approximately 66 μm (Figure 7), indicating that grittiness is a common characteristic in commercial soft smear-ripened cheeses. Thus, surface crystallization in soft smear-ripened cheeses influences cheese texture both indirectly and directly (indirectly through demineralization, which leads to radial softening and liquefaction, and directly through attainment of crystals of sufficient size to be perceived as gritty).
A parallel process of surface crystallization evidently also occurs in the high pH surface smears that develop on semihard and hard washed-rind cheeses such as Beaufort and Appenzeller. Le Graet et al. (1986), for example, reported the migration of calcium and magnesium (but not phosphorus) from the body to the surface of Beaufort cheese, where they accumulated in the high pH smear of the outer rind, during ripening. The authors postulated that migration was driven by the precipitation of calcium and magnesium carbonates or hydroxides that became insoluble at the high pH. More than 3 decades later, Polowsky et al. (2018c) confirmed the presence of the calcium-containing carbonates of ikaité and calcite, as well as magnesium-based struvite in the outer rind of Appenzeller cheese.

**Crystals That Form Within the Body of the Cheese**

**Embedded in the Body of the Cheese.** At least 5 different crystals (calcium phosphate, CLP, calcium carbonate, Tyr, and Leu) have been observed embedded within the cheese body, in contrast to forming in open spaces (mechanical openings, eyes) within the cheese body. In Cheddar cheese, microcrystals (i.e., not visible to the naked eye) of calcium phosphate are widely distributed throughout the body of cheese, but also tend to concentrate along the fusion lines of curd particle junctions, especially in cheeses that display seaminess defect (Conochie and Sutherland, 1965; Brooker et al., 1975; Brooker, 1987; Morris et al., 1988). Using X-ray diffractometry, Conochie and Sutherland (1965) identified the precise form of calcium phosphate crystals in Cheddar cheese as brushite. Crystals of calcium phosphate, including both macro- (visible to the naked eye) and microcrystals, have also been reported in the body of Parmigiano Reggiano, Grana Padano, Perorino Sardo, Roquefort, Sbrinz, Swiss, Tilsit, Trappist, and Manchego cheeses (Swiatek and Jaworski, 1959; Bottazzi et al., 1982; D’Incecco et al., 2016, 2020; Polowsky et al., 2018c). In Pecorino Sardo, Manchego, and Sbrinz, the precise form of calcium phosphate crystals was confirmed by PXRD to be brushite (Polowsky et al., 2018c; Figure 8).

More than 1 factor may be involved in creating microenvironments within the cheese body that favor nucleation and growth of calcium phosphate crystals. For crystals that form in Cheddar along the fusion lines of curd particle junctions, the high salt concentrations that persist at the surface of curd particles after salting may draw water containing calcium and phosphate ions to the particle surface, which forms a film of water rich in calcium and phosphate ions between the unfused curd particles after pressing. Continued migration of sodium chloride into the curd particles displaces more calcium and phosphate ions, which diffuse into the boundary film, eventually exceeding the solubility of brushite and causing crystallization (Conochie and Sutherland, 1965; Brooker et al., 1975). In contrast, crystals of calcium phosphate that are uniformly distributed throughout the cheese body may be associated with microbial colonies that die and lyse, possibly serving as nucleation sites for crystal formation or causing localized rise in pH that triggers crystallization thereafter (Kalab, 1980; Bottazzi et al., 1982; Morris et al., 1988).

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**Figure 5.** Proposed model for the formation of calcite, ikaité, and struvite in the surface smear of smear-ripened cheese during ripening.
Figure 6. Polarized light micrographs captured via the smear scraping method from 4 different smear ripened cheeses (A, B, C, and D). Examples of ikaite crystals labeled with solid arrows. Examples of struvite crystals labeled with open arrows. Scale bars represent 250 μm. Viewed under quartz filter. From Polowsky et al., 2018a. Used with permission.

Figure 7. Mean crystal area and length measurements, and sensory panel threshold for grittiness, for crystals harvested from the smears of 24 smear ripened cheeses produced in the United States and European Union. Error bars show 1 SD from mean. From Polowsky et al., 2018b. Used with permission.
Both micro- and macrocrystals of CLP also may occur as embedded entities in the body of cheese. Microcrystals have been reported in Cheddar (Brooker et al., 1975) and Grana cheeses (Bottazzi et al., 1982); very small macrocrystals of d(+)-l(−)-CLP, combined with brushite, have also been observed distributed widely in the body in Pecorino Sardo (Figure 8) and Manchego cheeses (Polowsky et al., 2018c). The co-identification of CLP and brushite in macrocrystals that were carefully picked out of the latter cheeses and analyzed by PXRD raises questions as to whether discrete crystals of CLP and brushite that were morphologically indistinguishable existed side-by-side in the cheese, or whether CLP cocrystallized on nucleation sites consisting of preexisting crystals of brushite, as hypothesized by Johnson (2014). Alternatively, it is possible that cheese matrix material containing brushite adhered to the isolated crystals and registered the brushite diffraction pattern. Pecorino Sardo and Manchego cheeses are both traditionally produced using ovine milk, which has a considerably higher casein content than bovine or caprine milk. This increased casein, and thus mineral, content could be a potential factor contributing to the presence of macrocrystals of calcium phosphate (i.e., brushite). However, further work is needed to confirm this supposition.

The occurrence of large hard internal crystals of Tyr that impart perceptible crunchiness to Grana cheeses such as Parmigiano Reggiano and Grana Padano is well established (Bianchi et al., 1974; Tansman et al., 2015; D’Incecco et al., 2016, 2020). Indeed, the abun-

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**Figure 8.** Sample of Pecorino Sardo cheese displaying white crystals embedded in the body of the cheese (A). Magnified view of embedded crystals by phase contrast microscopy (B).

**Figure 9.** Large interior crystals collected from the body of a commercial Cheddar cheese that was aged for 2 yr before distribution for retail sale. The scale is in millimeters. From Tansman et al., 2015. Used with permission.
The abundant presence and sensory perception of Tyr crystals are considered fundamental aspects of the identity and quality of traditional Grana cheeses such as Parmigiano Reggiano (Zannoni et al., 1994; Noël et al., 1996; Zannoni and Pigoni, 1997). The formation of Tyr crystals appears to take place in localized environments within the cheese matrix where Tyr accumulates to higher concentrations. This probably occurs in close proximity to bacterial colonies that contain viable and lysed cells that are responsible for intense proteolysis and localized accumulation of Tyr and other free AA (Bianchi et al., 1974; D’Incecco et al., 2016). Lactobacillus helveticus, the principal bacterium in traditional starter cultures used in Grana cheese production, has very active peptidase activity capable of producing high levels of free Tyr during ripening, which may play a role in Tyr crystallization (Johnson, 2014). Tyrosine crystals have also been reported in Gouda (Tansman et al., 2015), Norvegia (Norwegian Gouda; Framstad, 1977), Emmental (Blanc et al., 1979), and Sbrinz and Comté cheeses (Polowsky et al., 2018c).

Crystals of Leu have also been observed enclosed within peculiar spherical structures referred to as spots or pearls within the body of Parmigiano Reggiano, Grana Padano, and Gouda cheeses, (Bianchi et al., 1974; Tansman et al., 2015; D’Incecco et al., 2016). D’Incecco et al. (2016) reported that spots contained disproportionately high concentrations of 6 hydrophobic free AA (Leu, Ile, Met, Val, Phe, and Tyr), which evidently remained localized near the place where they were produced as a result of proteolysis. In contrast, water and polar free AA evidently diffused outwards, leaving behind a lower moisture region in the spot enriched with the hydrophobic AA, including Leu. The same authors also reported the presence of microcrystals of calcium carbonate within the body of Parmigiano Reggiano and Grana Padano cheeses (D’Incecco et al., 2016).

In Irregular Mechanical Openings. Irregular openings within the body of a cheese can serve as local environments that favor crystallization. For example, large (up to 5 mm in length), dense crystals consisting of d(-)-L(+)-CLP have been reported in commercial long-aged Cheddar cheese (Tansman et al., 2015; Figure 9). Unlike surface crystals of CLP, large internal crystals impart a perceptible crunchiness.

**Figure 10.** Sample of Roquefort cheese displaying numerous white crystals lining the walls of large interior mechanical openings in the body of the cheese.

**Figure 11.** Sample of Emmental cheese displaying numerous white crystals lining the walls of an internal eye (see arrow) in the body of the cheese (A). Magnified view of a crystal region by stereophase contrast microscopy (B).
to the cheese. Internal d(−)l(+)‐CLP crystals seem to be particularly common in long‐aged Cheddar cheese made from unpasteurized milk and are likely associated with nonstarter racemizing bacteria from the raw milk that proliferate in the aging cheese. These crystals also appear to form preferentially in small mechanical openings within the cheese body. Cheddar cheese made from unpasteurized milk may contain higher populations of nonstarter lactobacilli that include species that produce gas (which promotes slits and openness), in addition to species that racemize l(+)‐ to d(−)‐lactate (Martley and Crow, 1996). This combination of nonstarter bacteria likely render Cheddar cheese made from unpasteurized milk more vulnerable to formation of internal d(−) L(+)‐CLP crystals than cheese made from pasteurized milk, which generally contains much lower populations of nonstarter bacteria.

In the making of blue mold‐ripened cheeses, an open cheese structure is necessary to support internal proliferation of the mold Penicillium roqueforti; therefore, gas producing cultures of Leuconostoc or citrate‐utilizing L. lactis are often used to promote and maintain open texture (Martley and Crow, 1996), which in turn creates local environments prone to crystallization. For example, it has long been recognized that small white specks, tentatively identified as crystals of Tyr and Leu, occur abundantly along the many internal cracks and crevices lined with mold within the body of Roquefort cheese (Dox, 1911). Using PXRD, Polowsky et al. (2018c) recently confirmed the presence of l(+)‐

![Figure 12](image1.png)

**Figure 12.** Visual appearance of a commercially produced Gouda cheese that was aged for 2 yr before retail distribution. Eyes within the cheese (solid arrow) were lined with crystals (A). Phase contrast photomicrograph (11.25× magnification) of crystals lining the surface of an eye (B). Two different crystal morphologies, one characterized by an open structure (solid arrows) and the other by a compact structure (dashed arrows), appear to be present. Adapted from Tansman et al., 2015. Used with permission.

![Figure 13](image2.png)

**Figure 13.** Sample of Mimolette cheese displaying white crystalline sheets lining the walls of an internal eye in the body of the cheese (A). Close up view of a crystalline sheet lining an internal eye (B).
Tyr and L(+)-Leu crystals, as well as brushite crystals, abundantly distributed throughout open areas within Roquefort cheese (Figure 10).

**In Eyes.** The spherical holes or eyes that characteristically form in many alpine (Swiss) and Dutch (Gouda) cheeses also serve as fertile ground for crystal formation. For example, crystals consisting primarily of Tyr, but also containing brushite, as determined by PXRD, have been observed lining the surface of eyes in Emmental cheese (Polowsky et al., 2018c; Figure 11). These crystals appeared as discrete spherical entities comprised of aggregated needlelike fibers. Tansman et al. (2015) reported the presence of crystals with 2 different apparent morphologies lining the surface of eyes in aged Gouda cheese; the crystals were identified as L(+)-Tyr and L(+)-Leu when analyzed by PXRD (Figure 12). Crystals of L(+)-Tyr and L(+)-Leu have also been observed lining the surface of internal eyes in aged Mimolette, a French cheese that is very similar to Gouda. However, in this case, crystallization occurred in the form of a continuous sheet (Polowsky et al., 2018c; Figure 13). The ability of Leu to crystallize in an unusual sheet configuration under specific conditions has been reported elsewhere and is of interest to the pharmaceutical industry because of the potential of crystallized Leu to serve as a coating material for drug delivery (Banno et al., 2004; Sou et al., 2013). Evidently, this unusual crystalline sheet form of Leu occurs naturally in the eyes of Mimolette (Figure 13), but not in those of Gouda (Figure 12), for unknown reasons. Therefore, future studies aimed at understanding the crystallization behavior of Leu in cheese eyes could potentially contribute valuable insight for pharmaceutical applications.
The formation of Tyr and Leu crystals can only occur in cheese if sufficient concentrations of the free AA are generated by proteolysis to exceed their solubilities. \textit{Lactobacillus helveticus}, the thermophilic lactic acid bacterium that is commonly used in starter cultures for highly cooked cheeses such as Emmental and Parmesan-type cheeses, is increasingly being used as an adjunct culture in other cheeses including Gouda-types (Düsterhöft et al., 2017). \textit{Lactobacillus helveticus} is strongly proteolytic and capable of forming high concentrations of free AA, and there is growing evidence of an association between \textit{L. helveticus} and incidence of Tyr and Leu crystals in cheese (Johnson, 2014). Thus, it is possible that new uses of highly proteolytic adjunct cultures such as \textit{L. helveticus} in cheesemaking could lead to a proliferation of AA-based crystals that have not been observed in traditional versions of the cheese.

CONCLUSIONS

The amount, type, and location of crystal formation have large effects on the physiochemical properties, sensorial properties, and overall consumer acceptance and indirect effects on ripening and textural changes. The advent of improved analytical techniques such as polarized light microscopy, PXRD, and single crystal X-ray diffractometry form a “toolbox” that will facilitate the future study of crystal identity and formation dynamics. The authors believe that an important next step is to communicate these findings to public audiences in engaging and novel ways. Cheese crystals are indeed more than curiosities. They can serve as powerful indicators of the unique natural phenomena occurring within various cheese varieties. The aforementioned bifurcation of consumer attitudes toward cheese crystals can be capitalized upon in the form of communicating how cheese crystals shed light on the fascinating world of dairy chemistry. Indeed, the “strange can be made familiar” with proper consumer education. For example, informational graphics can be created, distributed, displayed, and disseminated for public edification (Figure 14). With adequate background knowledge, consumers can make the connection between cheese crystals and the natural complexity of milk chemistry. Traditional artisanal cheeses, which rely heavily on “stories” that are woven around their identity to create uniqueness and add value, perhaps have the most to gain from this knowledge. An exciting opportunity for traditional artisanal cheesemakers in the United States and globally will be to translate the fascinating science of their cheese crystals into engaging narratives that capture the imagination, add value to their cheese, and enhance the enjoyment of their cheese by consumers.

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