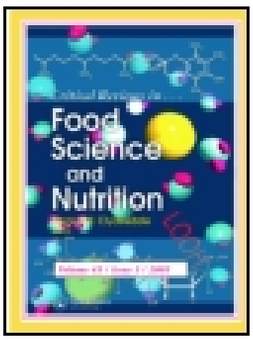

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REVIEW

Freezing as a solution to preserve the quality of dairy products: the case of milk, curds and cheese

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ABSTRACT

When thinking of the freezing process in dairy, products consumed in frozen state, such as ice creams come to mind. However, freezing is also considered a viable solutions for many other dairy products, due to increasing interest to reduce food waste and to create more robust supply chains. Freezing is a solution to production seasonality, or to extend the market reach for high-value products with otherwise short shelf life. This review focuses on the physical and chemical changes occurring during freezing of milk, curds and cheeses, critical to maintaining quality of the final product. However, freezing is energy consuming, and therefore the process needs to be optimized to maintain product's quality and reduce its environmental footprint. Furthermore, the processing steps leading to the freezing stage may require some changes compared to traditional, fresh products. Unwanted reactions occur at low water activity, and during modifications such as ice crystals growth and recrystallization. These events cause major physical destabilizations of the proteins due to cryoconcentration, including modification of the colloidal-soluble equilibrium. The presence of residual proteases and lipases also cause important modifications to the texture and flavor of the frozen dairy product.

KEYWORDS

Cheese; curd; dairy processing; freezing; frozen storage

Introduction

Freezing is one of the most popular and common methods of food preservation. During freezing low temperatures are applied to the product to transform liquid water into ice crystals through removing the latent heat of crystallization. The product undergoes a phase transformation, from a liquid, colloidal suspension (in the case of milk and milk beverages) or a gel network (in the case of cheese), to a mixed phase containing ice crystals and a supersaturated solution. Depending on the composition of the serum, there will be a significant decrease in water mobility and mass transfer kinetics, leading to an amorphous or glassy state.

During frozen storage, the growth of microorganisms is stopped or delayed. Depending on freezing rate, microbial death or damage will also occur to the cells. The kinetics of chemical and enzymatic reactions such as proteolysis and oxidation, as well as physical changes, such as mass transfers (i.e. recrystallization or phase separations) are minimized (Delgado and Sun 2001; Sun 2016), as a consequence of low water activity (a_w) and decreased molecular mobility. In general, the lower the freezing temperatures, the slower the kinetics of deteriorative reactions will be (Verdini and Rubiolo 2002).

However, freezing, thawing and storage need to be well controlled, as they can result in quality losses (Fu and Labuza 1997). Several factors can affect the reaction kinetics, such as freeze-concentration effects, catalytic effect of ice crystals, a greater mobility of protons in ice than in water, a_w. Moreover, structural, textural, and rheological modifications occur, aligned to changes in sensory properties, during frozen storage or after thawing.

A significant amount of research has been carried out to study how to control ice-crystal size and growth, during freezing or storage. The formation of the ice crystals, and the control of nucleation and growth have been extensively reviewed (Cook and Hartel 2010), as it is critical to the quality of frozen dairy products such as ice cream and frozen desserts. The ice crystal growth affects sensory properties, but also causes cryo-concentration effects with consequences to the protein physico-chemical stability. The changes occurring to other dairy products, such as milk, curd and various cheeses, during freezing and storage, have been less studied. This area is of growing interest, as freezing improves the presence of high-quality foods in markets with limited access to fresh specialty dairy products (Tejada et al. 2002; Bertola et al. 1996a). Freezing also allows for innovation in the field of ingredients and semi-finished products, as a way to transport raw materials to further destinations where they could then be further processed in a fresh product (Picon et al. 2013; Vélez et al. 2015).

Considering the growing global interest for more sustainable supply chains, even with the high energy consumption

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of the process (Baldwin 2009), freezing is considered a good solution due to greater storability, convenience and decrease in waste (Pollack 2001). In some cases, due to the decreased waste in the household or in food service, frozen foods have shown to have a lower carbon footprint impact when compared with fresh, refrigerated products (Martindale 2014). Moreover, freezing is the solution to seasonality challenges (Anaya Stucchi and Pollitt 2019) and to the increased global demand for highly perishable traditional cheeses (e.g. high moisture cheese), by reducing the costs of transport (maritime vs air) (Estrada-Flores 2016). Freezing decreases waste from expired product, or transport delays and can ensure a well-controlled, transparent, supply chain, and consistent quality. For example, overseas export of Mozzarella cheese manufactured in Italy increased by 16.4% from 2016 to 2017, with Japan, South Korea and USA that imported more than 30% of the total amount of exported product (CLAL 2020).

As the process of freezing is energy-consuming, the correct prediction of cooling and freezing times is important to achieve better energy efficiencies, as well as optimal quality parameters for the final product. Thanks to technology improvements and the study of processes by computational methods (Delgado and Sun 2001), new and more efficient processes have been developed (Cheng et al. 2017; Evans 2016), increasing both the sustainability and quality of frozen foods (Hall and Howe 2012). Moreover, optimization of supply chain (batch sizes, transportation temperatures and storage times and digitalization) can further contribute to lower the environmental footprint of frozen food products (Zanoni and Zavanella 2012). A decrease of the environmental footprint of frozen products may be obtainable by an improvement of storability and the consequent reduction of the number of products at the end of shelf life to be withdrawn from the market (Fisher and Whittaker 2018); moreover, the increased storability of the frozen product gives also the possibility to use slower and more energy-efficient transport (e.g. maritime transport if compared to air transport) (Alvarenga et al. 2013; Alinovi et al. 2020a). This can further improve the sustainability of the whole supply chain.

Freezing process conditions deeply influence the final quality of the product, as the growth of ice crystals and formation of phase separated systems may result in significant damages to the matrix. Ice crystal growth (due for example, to Ostwald ripening), dehydration, residual activity of proteases and lipases can cause these changes. For example, during freezing, bulk water tends to freeze faster than water entrapped by biopolymers (proteins, polysaccharides). This can generate concentration gradients that result in the acceleration of reactions, protein aggregation or unwanted micro-phase separations (Fennema, Powrie, and Marth 1973). These reactions can be detrimental to the quality of products. Furthermore, the freezing–thawing conditions also need to be controlled, and in some cases, formulations need to be modified to control the reactions that may occur in the unfrozen phase (Diefes, Rizvi, and Bartsch 1993; James, Purnell, and James 2015). The time in this phase transition zone, at temperatures around −1 °C to −6/-8 °C (Diefes, Rizvi, and Bartsch 1993; Fikiin 2008), must also be minimized to optimize ice crystals nucleation at the expenses of their growth (Kiani and Sun 2011).

Storage temperatures also play a crucial role in maintaining quality. The lowest the temperature conditions, the lowest will be the amount of unfrozen water available for chemical, enzymatic reactions and growth of ice crystals. In other words, the properties of the components which were preserved by applying a fast-freezing process can be lost under deteriorative storage conditions (Alvarenga et al. 2013).

For cheeses, the preservation of quality by freezing will depend on the matrix. High moisture cheeses will rapidly over-ripen during refrigerated storage, shortening their shelf life; in these cases, freezing can be a help to preserve cheese characteristics. During freezing, enzymatically-driven reactions and microbial growth are significantly decreased (Alinovi et al. 2020c; Casla et al. 1995). However, the methods of freezing must be optimized for these products (Alvarenga et al. 2013), as different freezing or frozen storage temperatures, heat flow coefficients, product's size and geometry can strongly influence the freezing time and the resulting quality loss (Alinovi and Mucchetti 2020a; Zanoni and Zavanella 2012).

In sum, freezing needs to be a well-designed process, with controlled and optimized storage conditions across the entire supply chain, so that no major changes in product quality will be observed during shelf life. The continuous process of freezing for ice cream type products has been optimized over the years, and it is well understood (Cook and Hartel 2010). It involves the use of scrape heat exchangers which agitate, whip, destabilize the fat and scrape the ice crystals from the surface of the barrels. This process controls the nucleation process, and after this stage, the product is further cooled to harden, with an increase in the ice crystal sizes (Cook and Hartel 2010). In this review, we will focus on describing the effect of freezing and its possible application in milk, cheese and curd products.

Microbial ecology and quality

As a consequence of the increase of solutes concentration, the subzero temperatures and the very limited molecular mobility, the microbial growth in frozen dairy matrices is generally halted. Moreover, both the freezing process and the storage at subzero temperatures may increase the mortality of microorganisms, because of the mechanical damage caused by the intracellular and extracellular ice crystal formation to the microbial membranes, to dehydration of the cells caused by water pressure differences and the presence of osmotic gradients (El-Kest and Marth 1992; Smith et al. 2011).

Coliforms show high mortality during freezing (Katsiari, Voutsinas, and Kondyli 2002; Voutsinas et al. 1995a; Voutsinas et al. 1995b). Also in the case of other mammary pathogens in sheep milk, freezing reduced the count of *Staphylococcus aureus*, *Mannheimia haemolytica*, and *Escherichia coli*, even in the presence of cryoprotectants such as glycerol (Smith et al. 2011). De Garnica et al. observed a

reduction of mammary pathogens of between 0.5 and 2 log cycles as a consequence e of freezing at −20 °C (de Garnica, Santos, and Gonzalo 2011). Balthazar et al. observed an increase (0.5–2 log cfu/mL) of coliforms and other microorganism (with the exception of lactobacilli), indicating that the frozen storage at −20 °C until 30 days of storage was not beneficial to the microbiological quality of sheep milk; conversely, after 90 days the viable counts decreased (1.3–2.8 log cfu/mL) (Balthazar et al. 2019). Despite of the count reduction, mastitis pathogens are able to survive for several weeks in milks frozen at −20 °C and −196 °C (Petzer et al. 2012).

On the contrary, micrococci, staphylococci and streptococci are more resistant to freezing and frozen storage. Generally, gram-negative are more sensitive to freezing than the gram-positive bacteria, cultures in the log-phase more than in the stationary phase, and vegetative cells more than bacterial spores (Speck and Ray 1977). Wendorff (2001) determined that higher storage temperatures (-15 °C vs −27 °C) lead to a higher microbial mortality because of the damage caused by larger ice crystals.

Two different strains of *Listeria monocytogenes,* Scott A and California showed a survival rate of 95% and 40–50% of the initial inoculum after storage at −18 °C and −38 °C for 7.5 months inoculated with both (Papageorgiou, Bori, and Mantis 1997). However, in the case of *Listeria monocytogenes,* greater stability of Scott A was noted compared to other strains in milk (Gianfranceschi and Aureli 1996). Milk components can act as cryoprotectants. The addition of fat, lactose and caseins to phosphate buffer significantly reduces death and injuries during frozen storage at −18 °C of *L. monocytogenes* strains Scott A, V7 and California, with caseins being the most protective (El-Kest and Marth 1991a; El-Kest and Marth 1991b). Similarly, only one log reduction has been reported for *Staphylococcus aureus* in whole milk after several months of frozen storage (B. M. Lund 2000). The milk matrix also has an effect on mortality, as freezing of cheese curds seemed to be more effective with *L. monocytogenes*, compared to milk freezing. *L. monocytogenes* strains Scott A and California are less freezing tolerant in cheese curd, because of the lower pH compared to milk. There are differences in the distribution within the cheese; a higher survival rate was observed in the outer part of the cheese curd than in the center, because of the difference in the size of the ice crystals, with the smaller ice crystals forming on the surface compared to the center, due to a freezing rate gradient (Papageorgiou, Bori, and Mantis 1997).

Higher freezing rates are generally associated to a lower damage inflicted to microbial cells, because of the formation of a larger number of smaller nuclei, and limited ice crystal growth (Wang et al. 2019). Moreover, also the thawing temperatures and conditions and the storage after thawing are important in defining the microbial viability in the product, as during thawing psychrotolerant and psychrophilic are able to grow; by thawing 1-month frozen stored sheep milk, Tribst, Falcade, and de Oliveira (2019) observed that higher thawing temperatures (25 °C vs 7 °C), volumes of packaged product (5 L vs 1 L) and the presence of refrigerated storage post-thawing, caused an slight increase (<1.5 log) in the total and psychrotolerant bacterial count. Moreover, during thawing changes in electrolyte concentrations or water recrystallization during thawing can also lower microbial viability (Fernandez Murga, De Ruiz Holgado, and De Valdez 1998).

Despite a reduction in microbial counts is generally expected with freezing and storage at subfreezing temperatures, these processes and storage conditions are not a guarantee for microbiological safety (Katsiari, Voutsinas, and Kondyli 2002), especially when a long thawing time is required (Tribst, Falcade, and de Oliveira 2019). Also, freezing/thawing processes may cause sublethal damages to pathogen cells (Speck and Ray 1977), that can result in the presence of reversibly injured cells that can be able to recover in favorable conditions (Fernandez Murga, De Ruiz Holgado, and De Valdez 1998). Sierra et al. (2009) observed only a slight reduction in total microbial count (about 1 log) of frozen goat's milk stored for 24 h if compared to refrigerated milk.

The viability of LAB cultures and probiotics as a function of freezing and frozen storage has been largely studied both for the industrial production of frozen or freeze-dried starters or for the utilization in frozen yogurts and ice creams. Use of rotary freezers for quickly freezing of LAB cultures in form of small individual spheres followed by packaging is a common practice of many starter manufacturers. LAB freezing-resistance has been shown to be species and strain specific (Fernandez Murga, De Ruiz Holgado, and De Valdez 1998). A high probiotics and LAB viability can be guaranteed by the utilization of cryoprotectants, by the adoption of microencapsulation techniques (Cruz et al. 2009; Wang et al. 2019), or by the addition of prebiotics (Balthazar et al. 2018; Ayar et al. 2018; Pandiyan et al. 2012). In particular, a mixture of trehalose, sucrose, glycerol and skimmed milk demonstrated to enhance significantly the survival rate of *Lactobacillus plantarum*, *Lactobacillus casei* at levels higher than 90% (Wang et al. 2019). Also the production of exopolysaccharides from LAB species has been suggested to enhance cryotolerance during freezing processes or frozen storage (Monnet, Béal, and Corrieu 2003; O'Brien et al. 2016).

Freezing can also be an important factor conditioning cheese ripening. This may be of particular importance in the case of semi ripened cheese, transported frozen, which may develop different ripening characteristics once thawed, compared to the original one. This is also very important when the product is further processed. When frozen-stored curds were mixed with fresh ones, little counts of lactic acid bacteria (LAB), gram negative, mesophilic, coliforms and staphylococci bacterial counts were observed (Campos et al. 2011; Alonso et al. 2011; Picon, Gaya, et al. 2010). However, differences were found in the *Lactobacillus* population and in particular of *Lactobacillus plantarum* during ripening. Because of the complex set of peptidases that contribute to the formation of volatile compounds and flavors, the presence of a modified microbial flora throughout the ripening will promote differences in flavor and taste of the final cheese. Moreover, a higher degree of proteolysis, aminopeptidase and esterase activity is

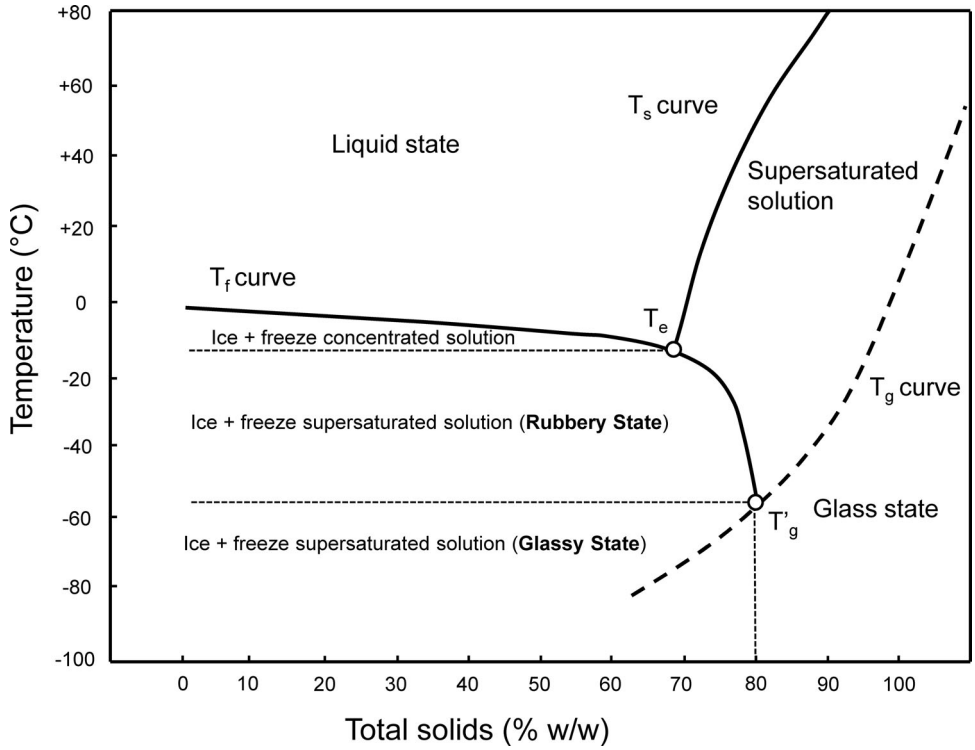

Figure 1. State diagram of an aqueous solution at constant pressure. T_f, T_s delineate the freezing point and solubility curves as a function of total solids. T_g is the glass transition curve, again as a function of solids concentration. T_e is the eutectic point, T_g' is the maximal glass transition temperature of the supersaturated solution. Dashed lines indicate metastable equilibriums.

found in frozen curd, and in the cheese made with frozen stored curd, due to the presence of enzymes leaked during membranes rupture, and not inactivated by freezing (Picon, Alonso, et al. 2010). This may lead to a less balanced flavor intensity and bitterness in ripened cheese (Alonso et al. 2013).

The freezing process

In physical and chemical terms, freezing is a phase change process, which has been extensively reviewed in literature (Alvarenga et al. 2013; Zhao and Takhar 2017). As the temperature is lowered below the freezing point of the system, liquid water starts to nucleate, and ice crystals form (Figure 1). If the temperature during freezing is sufficiently low, transitions from a liquid to a rubbery state of the matrix, to a vitreous, glassy state occur (Zhao and Takhar 2017). This second phase transition is related to the solidification of water that induces freezing-concentration of salts, sugars and organic acids that significantly increase viscosity of the unfrozen matrix, leading to arrested molecular motion.

Several processes have been utilized to freeze cheese and dairy products such as conventional air-still freezing (Simov and Ivanov 2005), air-blast freezing (Diefes, Rizvi, and Bartsch 1993), spiral or tunnel freezing, plate freezing, deep immersion freezing in brine solutions (Ribero, Rubiolo, and Zorrilla 2007; Ribero, Rubiolo, and Zorrilla 2009) or in cryogenic liquids (Bertola et al. 1996a).

Air-still freezing is the most widely practiced freezing process, at all scales, but very challenging to optimize, as time-to-freeze can be very long, with variations between sizes and geometries of the product, differences depending on the location and the type of packaging set up, all parameters with an obvious effect on the final quality of the product.

In cheese, for example, temperature curves can be different depending on the size of the product, and the cooling is faster on the surface than in the central part of the cheese, especially for large size products. The portion of unfrozen water would be more abundant in the center than at the edges of the cheese, causing diffusion of water molecules from the center to the edges, resulting in overall drying of the product and increased solute concentration in the unfrozen portion of the product (Norwig 1984; Alinovi and Mucchetti 2020b). Moisture, solutes and temperature gradients can affect the biochemical changes occurring to the product and their kinetics: protein dehydration in cheese matrix is a consequence of water migration phenomena that take place at a microscale level (Bunker 2016; Reid and Yan 2004) (Figure 2); enzymes distribution and subsequent activity can be conditioned by water migration, and can contribute to the softening of fresh cheeses when proteases are involved (Alinovi, Rinaldi, et al. 2018); microbial death and cellular lysis are also affected (Papageorgiou, Bori, and Mantis 1997).

Macroscopic surface dehydration also needs to be avoided, and glazes are not generally used for dairy products, unlike in the case of other food categories (Popelka et al. 2012); hence, packaging materials that provide barrier to water evaporation may be needed to provide further protection during freezing. Macroscopic, surface dehydration causes skin burn defects, and with oxygen contact, promotes oxidation reactions and sensory defects. Surface dehydration is usually a problem in air-blast freezing processes (Norwig

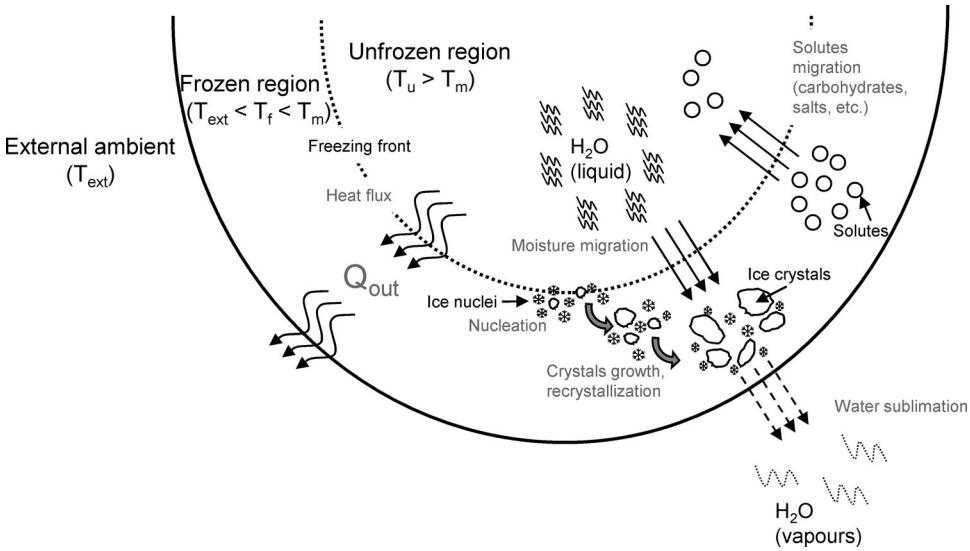

Figure 2. Two-dimensional, schematic representation of the freezing process of curd and cheese, indicating heat and mass flows across the product's freezing front. T_{ext} is the temperature of the external ambient, T_m is the melting point temperature of the product, T_f is the temperature of the frozen region of the product, T_u is the temperature of the unfrozen region.

1984). Immersion freezing into brine solutions is sometimes used as an alternative to air freezing, but this can also have problems related to brine concentration changes, mass transfer reactions (Fikiin 2008). An evolution of this method is the hydrofluidisation freezing, that combines the use of immersion brines with the application of nozzles to obtain high turbulence flow in these high viscosity brines (Fikiin 2008).

Air-blast freezing and plate freezing are batch or semi-continuous processes that may result in significant thermal gradients (Campos et al. 2011). On the contrary, continuous freezers suited for products with a well-defined size allow for process optimization. Tunnel freezers or spiral freezers represent a continuous application of air-blast freezing and can be optimized for different kind of products with different sizes, by modulating the dwell speed (George 1997). To maintain the product unit separated, fluidized bed systems or immersion systems are used. This ensures that each unit is individually frozen and minimizes freezing time. In these systems, the high heat transfer coefficient of the process is obtained by using freezing media such as liquid nitrogen, liquid nitrogen vapors or low-temperature air, flowed at high velocity ($2-6$ m/s). The freezing medium is applied via an insulated tunnel or in immersion vessels, while keeping the product in motion, thus avoiding the formation of clumps and improving the heat transfer during the process.

Fluidized bed freezing technology, is widely applied in foods, including dairy products, to obtain Individual Quick-Frozen (IQF) Mozzarella cheese and other small size high moisture dairy products, including shredded cheese. The main limitation concerning fluidized bed freezing is the dimensions and weight of the product, that has to be small enough to become suspended by the air flow (George 1997).

The cost of cryogenic-IQF is higher than conventional freezing due to the use of cryogenic liquids (Fikiin 2008), but the product quality is superior to conventional methods (Khadatkar, Kumar, and Pattanayak 2004). IQF techniques are commonly used to freeze whole fruits of small, uniform size and not prone to damage as a consequence of the high mixing velocities (Chaves and Zaritzky 2018).

Direct immersion in cryogenic fluids or application of fluidized beds can cause cracking and shattering of the surface of the products (Reid and Yan 2004), precooling is necessary to prevent such defects. A possible limitation to the diffusion and applicability of cryogenic fluids is given by the high cost of production, due to the use of nitrogen production process is energy intensive (Pearson 2008) but can be a solution for high moisture, fresh products such as HM Mozzarella cheese and curd to better preserve the structure and juiciness (Zambrini and Bernardi 2017).

Freezing processes have been tested to optimize energy use and limit undesirable mass and moisture gradients in the product and to increase the freezing rate; for example systems could be combined to apply a cryogenic pre-stage to freeze the outer layer and a mechanical final stage to freeze the core (George 1997); magnetically and electrically disturbed freezing, microwave assisted freezing, high-pressure freezing, ultrasonic freezing, have been suggested to promote supercooling or to reduce freezing times (Cheng et al. 2017; Fikiin 2008; James, Purnell, and James 2015; Johnston 2000). Magnetically, electrically and microwave assisted freezing techniques rely on the ability of magnetic or electric fields and microwaves to modify the electric dipole moment of water and its distribution into the product; this phenomenon cause the suppression of ice formation and enhance the supercooling effect (Cheng et al. 2017). Ultrasonic freezing involves the formation of cavitation bubbles, which favor the formation of small ice nuclei and the fragmentation of big ice crystals (James, Purnell, and James 2015). This technique combines immersion freezing with high-intensity ultrasound (usually 20– 100 kHz), and it has been reported to increase the freezing rates and the convective heat flow coefficient, and to inhibit the residual activity of enzymes in a large number of food applications (Cheng et al. 2017; Kiani et al. 2011). However, no reports are available on the

effect of ultrasonic freezing in dairy products. On the other hand, high-pressure freezing has been tested on cheese, and showed the formation of a plasticized texture in Mozzarella and Cheddar cheeses (Johnston 2000); this texture change was attributed to the effect of high pressure on the supramolecular structure of proteins (Huppertz, Fox, and Kelly 2004).

In the case of milk or concentrated milk, freezing may be applied after packaging in sealed bags, as this would reduce oxidation. Several freezing techniques can be used in this case, such as tunnel, spiral freezers or air-blast freezers. Batch processes, such as multiplate and plate freezing, have been reported, to obtain frozen blocks of various dimensions (Antifantakis et al. 1980); however, volumes should be reduced as much as possible (e.g. volumes smaller than 1–5 L with 2 cm thickness) to decrease freezing times and ice crystal growth (Antifantakis et al. 1980; Haenlein and Wendorff 2006; Tribst, Falcade, and de Oliveira 2019). For example, a tray-multiplate freezing system that can produce small frozen milk blocks of sufficiently homogeneous characteristics and high stability has been developed (Zevlakis 2004); this system combines a supercooling phase of the liquid to produce small crystal sizes and a fast heating of the solid layer between the tray surface and the frozen product to allow the detachment of the frozen segments. For continuous freezing of fluid or concentrated milk, drum contact freezers equipped with scraped blades and twin-screw extrusion devices have been suggested to obtain thin flakes of frozen product. These systems can be used prior to packaging (Addeo et al. 1992; Groux, Fayard, and Jimenez-Laguna 2001; Pirisi et al. 1995). These processes minimize freezing times, and control ice crystals dimensions. In particular, a twin-screw extrusion device can minimize freezing times due to the supercooling caused by the increase in pressure in the extruder, with a rapid and homogeneous freezing as the pressure of the low-temperature product drops down (Groux, Fayard, and Jimenez-Laguna 2001).

Physical and chemical changes of milk

The effect of freezing on milk quality has been studied for decades (Pazzola et al. 2013; Muir 1984). For non-bovine species (sheep, goat, buffalo), seasonality issues caused by reproductive performances and/or the lack of pastures during some periods of the year are mitigated using freezing of bulk milk (Pazzola et al. 2013). In particular, the freezing process has found its application for milk intended to be further processed (e.g. fresh cheese). Freezing can be advantageous to better control the fluctuations in milk supply and to produce cheese all year long, reducing the impact of fixed expenses and increasing market availability (Tribst et al. 2020; Tribst, Falcade, and de Oliveira 2019).

Milk can be frozen as is or after concentration processes, such as ultrafiltration (UF), reverse osmosis or evaporation. Using concentrates would reduce freezing, storage and transportation costs (Haenlein and Wendorff 2006). It has been suggested that, milk should be skimmed, as freezing can lead to fat globules rupture, increasing the rate of lipolysis and fat oxidation during frozen storage (Antifantakis et al. 1980; Voutsinas et al. 1995b), with consequences to the processability of this milk into fresh cheeses at destination. Moreover, fat globule breakage results in destabilization of emulsion, coalescence of globules after thawing and creaming separation favored by fatty acid crystallization and the activity of agglutinins (Tribst et al. 2020; Tribst, Falcade, and de Oliveira 2019).

Enzymatic and chemical kinetics are directly proportional to the storage temperature, and changes in composition (Needs 1992; Wendorff 2001). Storage of frozen milk can result in off-flavors, due to reactions such as oxidations, lipolysis and proteolysis. Lipid oxidation has to be controlled. In sheep's milk, if not controlled, the peroxides number and the free fatty acids content can significantly increase by 7 to 90 times and 2.5 times, respectively, after only 5 months of frozen storage at −20/-30 °C (Antifantakis et al. 1980). The presence of oxygen, light and metals, especially copper and iron, that can be found in traces (Coni, Bocca, and Caroli 1999), promote the rate of oxidation. Oxidation can be reduced using oxygen impermeable (Voutsinas et al. 1995b) and light barrier packaging materials (e.g. polyethylene-aluminum foils) and by the application of thermal treatment prior to freezing, to cause inactivation of indigenous lipase enzymes (Katsiari, Voutsinas, and Kondyli 2002). It has been shown that frozen storage induces a significant deactivation of lipase enzymes after 6 months at −12 °C, −20 °C and −27 °C, as the residual activity decreases to 2%, 11% and 24% of the initial activity values, respectively (Needs 1992).

Lipid oxidation causes a decrease in lipophilic vitamins content. While milk after short-term frozen storage does not show a significant reduction in α-tocopherol content, this is not the case for UHT treated milk, where long-term frozen storage at −20 °C for 6–8 months shows significant losses (as high as 21%) (Vidal-Valverde, Ruiz, and Medrano 1993). Non enzymatic oxidative reactions, such as Maillard reactions also create many precursors for oxidative reactions causing crosslinked amino acid products, with higher incidence in lactose free UHT milk (Jansson et al. 2014).

The residual activity of both exogenous and indigenous proteases such as cathepsin D and plasmin can cause significant quality challenges. Plasmin is an indigenous protease that can hydrolyze Lys-X peptide bonds of β-casein and is mainly active in the hydrolysis of β-casein, despite cleavage sites can also be found in α_{s1}- and α_{s2}-caseins (Kelly and McSweeney 2003). It has been shown that plasmin is active at temperatures < 5 °C, and an increase in solubilization of the caseins from the micellar phase can facilitate their hydrolysis (Ismail and Nielsen 2010). Residual microbial enzymes have a serious impact on the physical stability and overall quality of frozen dairy products. It is well known that during freezing of milk, it is important to control the microbiological quality and the storage time in the raw bulk tanks (Portmann 1970). High microbial counts of psychrotrophic bacteria before processing will result in higher concentrations of exogenous proteases and lipases, some of them also heat resistant (Chavan et al. 2011; Tribst, Falcade,

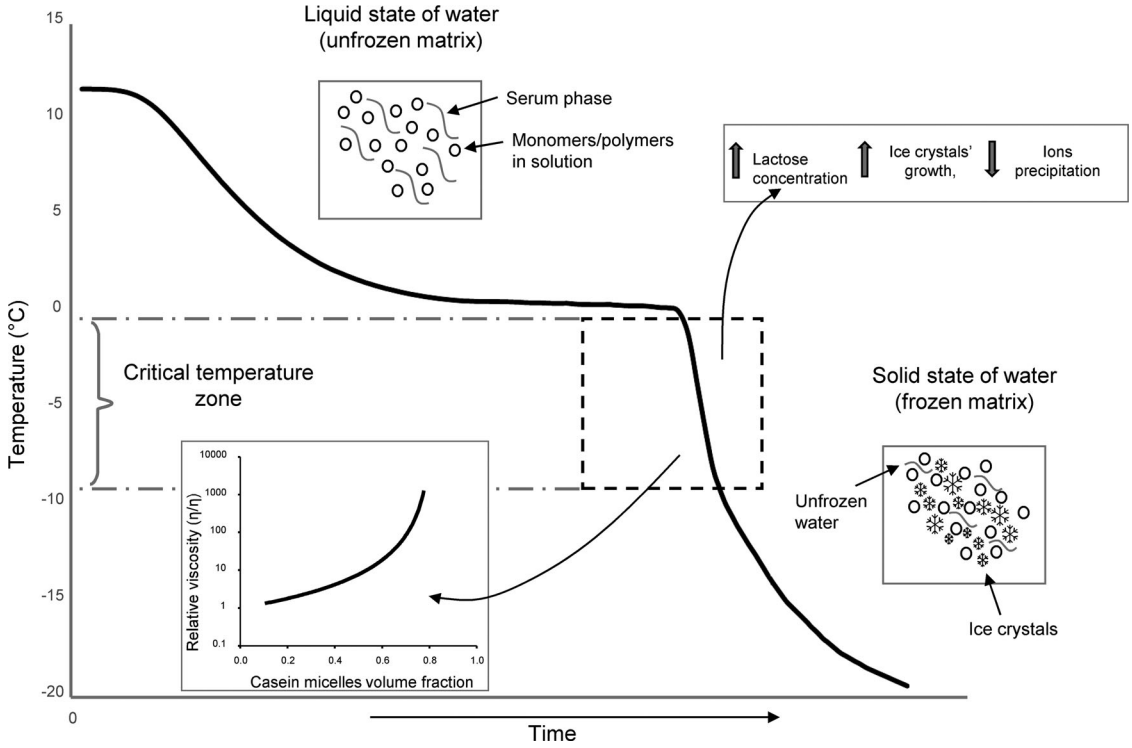

Figure 3. Schematic representation of temperature time freezing curve and physical modifications that occur during phase change.

and de Oliveira 2019). Lipolytic and proteolytic reactions will continue to occur during freezing, and the freeze-induced concentration of solutes may increase reactions kinetics, before the supersaturated phase reaches a vitreous state.

In addition to chemical and enzymatic reactions such as oxidation and hydrolysis, during freezing a number of physical changes occur to the milk matrix, as illustrated in Figure 3. As the freezable water progressively transforms into ice crystals, the unfrozen solution becomes more and more concentrated, suppressing the phase transition temperature. Freezing can improve the opportunity for enzymatic reactions to occur, as macromolecules are forced closer together as a result of the increased concentration of the unfrozen phase; this will also increase the concentration of enzymes inhibitors (Ashie, Simpson, and Smith 1996). The formation of ice crystals (and fat crystals) and their growth will cause physical damage to the milk fat globule membrane which promote clumping and ultimately partial coalescence (Tribst et al. 2020; Zhang et al. 2006). The process of homogenization can reduce the destabilization if applied before freezing, or it can be used after thawing to mitigate destabilization (Tribst et al. 2020). However, if the milk is used for cheesemaking, this practice may not be desired.

The increase in concentration in the unfrozen phase promotes also a change in the colloidal properties of the protein suspension. In normal milk, the casein micelles occupy about 10% of the volume and their average distance is in the same order of magnitude of their diameter. During freezing, the volume fraction of the casein micelles continues to increase, causing an exponential growth of the relative viscosity, and an increase of the colloidal interactions (Figure 3) (Corredig et al. 2019). It has been previously discussed that casein micelles may change their structure during frozen storage, as water migrates into ice crystals, and the protein hydrophobic portions become exposed to the solvent. This causes protein-protein interactions and the formation of protein aggregates (Xiong 1997). Supersaturation of the unfrozen phase modifies the colloidal state and hydration of casein, resulting in salting out (Doan and Warren 1947), and a change in the micellar voluminosity. The presence of micellar aggregates was shown by electron microscopy (Saito, Niki, and Hashimoto 1963). The aggregation has also been confirmed by observations of sedimentation and precipitation of insoluble proteins (Koschak et al. 1981), particle size measurements and a decrease of functional and technological properties (Gaber et al. 2020), and a chalky mouthfeel (Nickerson 1964) in frozen milk. However, in all these studies the details on milk composition or its history were not reported in detail, and therefore, comparison between studies may be a challenge. Caseins precipitation may also be related to a change in ionic balance, as a consequence of the increasing ionic strength of the medium during freeze-concentration, causing precipitation of soluble calcium, a change in the buffering capacity and a decrease in pH of soluble phase (Kljajevic et al. 2016; Tribst, Falcade, and de Oliveira 2019; Van Den Berg 1961; Tribst et al. 2020).

In these studies the aggregation has also been attributed to the formation of calcium phosphate bridges with the caseins (Wells and Leeder 1963; Chen and Yamauchi 1971). Recently, Gaber et al. observed a decrease in the ionic calcium and phosphorous fraction of casein concentrates subjected to freezing and frozen storage. The same study demonstrated that the formation of freeze induced aggregates was influenced by the freezing rate, with a slower freezing rate favoring casein aggregation (Gaber et al. 2020).

Earlier studies also reported a decrease in concentration of soluble calcium and inorganic phosphate has been measured after 7 months frozen storage at −20 °C. This decrease was attributed to the formation of insoluble salts of calcium citrate and phosphate and the formation of calcium-phosphate-casein complexes, causing the precipitation of proteins (Chen and Yamauchi 1969a; Yamauchi and Yoneda 1977). This was also confirmed by Tribst et al. (2020) for sheep's milk destabilization following freezing and thawing. It has been reported that a slow rate of thawing or a resting period in refrigerated conditions after thawing, can improve the stability of bovine milk by allowing time to re-solubilize colloidal minerals (De La Fuente, Requena, and Juárez 1997; Tribst, Falcade, and de Oliveira 2019; Tribst et al. 2018).

Goat's and sheep's milk seem to be more stable than bovine milk after prolonged frozen storage, due to their higher buffering capacity compared to bovine milk (Katsiari, Voutsinas, and Kondyli 2002; Tribst, Falcade, and de Oliveira 2019; Voutsinas et al. 1995b; Voutsinas et al. 1995a; Wendorff 2001). A higher stability in these non-bovine milk also has consequences in products such as yogurt and cheese characterized by similar physical, sensory, microbiological and chemical characteristics to the ones obtained with fresh, refrigerated-stored sheep's milk (Katsiari, Voutsinas, and Kondyli 2002; Zhang et al. 2006).

In cow's milk, the supernatant fraction seems to have a higher amount of β-casein (Nakanishi and Takatoshi 1965) and κ-casein after long frozen storage time (180 days) (Chen and Yamauchi 1969b). This seems to be due to the increase in ionic strength during freezing that can cause some dissociation of the micelles and lower the binding of calcium ions to β-casein (Kljajevic et al. 2016).

It is important to point out that much of the work published in the past did not specify processing history conditions, nor used advanced physical or chemical tools to characterize the samples. Hence the structures formed during freezing remain to be fully elucidated. Furthermore, the literature is lacking detailed proteomic data to be able to give a complete understanding and distinguish between colloidal instability, ionic bridging and proteolysis. Nonetheless, there seems to be consensus on the fact that the ionic environment, and calcium specifically, plays and important role, because, in the absence of calcium or in the presence of chelating agents, the destabilization is reduced (Wells and Leeder 1963; El-Negoumy and Boyd 1965). Under the presence of chelating agents the colloidal structure of the casein micelles is also significantly affected.

As a consequence of freezing destabilization, cheesemaking and product final quality will be affected. For example, it has been reported that there is a significant decrease of sheep milk gelation abilities (lower curd firmness, higher renneting time), after freezing for 6 months (Pazzola et al. 2013). This may be caused by increased proteolysis. Other authors also showed changes in the functional properties, such as a lower retention of fat in the sheep's milk curd (Lin, Hsieh, and Su 1994), and a lower gel firmness and water holding capacity in sheep's milk yogurt (Tribst et al. 2018; Voutsinas et al. 1996a; Wendorff 2001), changes in the rennet-induced gelation of caprine, ovine and buffalo milk (Addeo et al. 1992; De La Fuente, Requena, and Juárez 1997; Kljajevic et al. 2016). In some cases, freezing may weaken the electrostatic interaction among casein micelles, resulting in less structured protein matrix, a lower gel contraction and less whey expulsion (Tribst et al. 2018).

Freezing may cause a significant change in cheese actual yield. This has been shown for cheese made with frozen sheep milk (Zhang et al. 2006). The reduction in yield was attributed to a reduced water holding capacity of the caseins, but also fat recovery, which can be lowered by a weaker casein matrix that can affect coagulum structure (Pazzola et al. 2013). A lower water content was also measured in the case of white brined cheese manufactured using frozen stored goat's milk (Kljajevic et al. 2017). In these studies, proteolysis was not tested. On the contrary, Pirisi et al. (1995) showed an increase of dry matter recovery and a higher cheese yield when frozen sheep milk has been used.

Lactose crystallization during freezing can also drive to instability. During storage, the β-lactose form is converted into the less soluble α-lactose monohydrate form (Desai and Nickerson 1964). A high degree of destabilization has been reported when 1/3 of lactose is in the α-form (Muir 1984). As lactose crystals are removed from solution, the freezing point increases, causing more nucleation and growth of the ice crystals and proteins destabilization by salting-out (Morr 1975).

Membrane filtration processes such as ultrafiltration (UF), reverse osmosis (RO) and nanofiltration can be used to remove ionic form of calcium, monovalent salts, lactose and/or water, respectively (Haenlein and Wendorff 2006; Mucchetti et al. 2000). The application of UF with concentration factors ranging between 1–3 times show an improvement of milk stability over storage time, that was found to be at least 3 times longer (Koschak et al. 1981). However, higher degrees of UF (4–5 times) that were able to remove high degree of soluble salts, in particular calcium (>20%), and lactose (>70%), were not able to prolong shelf life, resulting even in its reduction, as the equilibrium of colloidal to soluble calcium phosphate is affected (Lonergan, Fennema, and Amundson 1981; Koschak et al. 1981). Also RO demonstrated to be a good concentration technique that can be applied before milk's freezing; in this case, after 6 months frozen storage there were no differences in lipolysis or fat oxidation, nor physical stability (Voutsinas et al. 1996b). It has also been suggested that dialysis processes or ion depletion, may lead to an improvement of freezing stability (Lonergan, Fennema, and Amundson 1982).

Any ingredient that is able to decrease the rate of lactose crystallization would improve stability in frozen milk. For example, other added soluble carbohydrates (sucrose, glycerol, etc.) may act as cryo-protectants, delaying lactose crystallization, by increasing the viscosity of unfrozen solution (Desai, Nickerson, and Jennings 1961; Minson, Fennema, and Amundson 1981). Lactose hydrolysis has also been suggested to improve stability, as the monosaccharides glucose and galactose are characterized by a high solubility and they have no tendency to crystallize; a linear decrease of lactose

concentration showed to improve shelf life exponentially (Tumerman, Fram, and Cornely 1954). However, care needs to be taken for non-enzymatic oxidative Maillard reactions (Jansson et al. 2014).

Conditions that may involve supercooling, improve nucleation and minimize crystal growth, or that can produce a vitreous matrix with low molecular mobility, will be beneficial. Rapid freezing and cold storage at temperatures lower than −20 °C, can slow lactose crystallization; instead, storage temperature ranging between −2 °C and −12 °C cause detrimental effects on cow milk properties such as viscosity and solubility index (Koschak et al. 1981). These temperatures conditions increase molecular mobility of the unfrozen phase result in a lower stability of the milk system during frozen storage.

Butter freezing

Butter is a water-in-oil emulsion composed of more than 80% milk fat, and a water phase, that is dispersed into the fat matrix in the form of small spherical or oval droplets (Nahid et al. 2008). Butter is commonly frozen and stored in bulk before further processing. In this case, freezing can be applied to control the product peaks in terms of volumes that generally occur during winter, and to respond adequately to consumers' and market demand. The freezing process of butter can be advantageous to its quality. Despite butter can have a relatively long refrigerated shelf life (6–12 months), frozen storage has demonstrated to preserve better its aroma characteristics and the occurrence of off-flavors (Lozano et al. 2007).

As the major component is milk fat, butter quality after frozen storage is related to lipolysis and oxidation; these reactions result in modification of color, structure and sensory characteristics (rancidity and other off-flavors) (Krause et al. 2008). Moreover, textural and rheological changes during freezing and frozen storage can also be observed, due to a modification of the fat crystal structure into a more rigid one (De Man and Wood 1958); in fact, the rate of cooling and temperature fluctuations are crucial in defining the presence and relative abundance of sub-α, α, β', β crystal forms of milk fat (Lopez et al. 2002; Ronholt et al. 2012) and the microstructure of the crystalline network. Higher cooling rates promote a denser crystalline network composed by smaller crystals and the formation of metastable polymorphic forms such as sub-α and α form (Wiking et al. 2009).

Main factors affect the quality of butter during freezing, namely, the packaging or wrapping material, which controls oxygen and light contact with the product surface, thus regulating the rate of oxidation (Emmons et al. 1986; Lozano et al. 2007); the presence of metals in the contact materials that induce oxidation (Krause et al. 2008); water droplets distribution and size, promoting destabilization and microorganism growth after freezing/thawing; the freezing rate, affected by the size of the product (Krause et al. 2008). Storage in 25 kg blocks seem to be improving the stability of butter (Krause et al. 2008); packaging may also affect quality, with frozen butter packaged into aluminum foils showing a reduced oxidation and lipolysis caused by the light, compared to parchment paper (Lozano et al. 2007).

Freezing of mozzarella and pizza cheese

Mozzarella is a pasta filata type cheese; during its production the curd undergoes a stretching process which gives the cheese unique texture and melting properties. During this process, the curd is continuously kneaded and stretched at high temperature and the result is the formation of a smooth, fibrous and anisotropic matrix (Ribero, Rubiolo, and Zorrilla 2007). Mozzarella cheese from bovine milk is a commodity, produced, transformed and consumed worldwide. High value products are also manufactured using both water buffalo or cow milk, and in some of these cases, the production is controlled by Protected Designation of Origin (PDO) regulations in EU (Mozzarella di Bufala Campana; Mozzarella di Gioia del Colle) (European Commission 2020). Mozzarella cheese is obtained both by means of starter acidification or direct acidification with organic acids (Mucchetti, Pugliese, and Paciulli 2017). Bovine Mozzarella cheese is produced and categorized in two different groups (United States Food and Drug Administration 2019): High Moisture (HM) Mozzarella cheese (moisture content higher than 52% w/w but lower than 60% w/w) and Low Moisture (LM) Mozzarella cheese (moisture content between 45% and 52% w/w). The latter shows longer shelf life, and in various forms it is largely used as an ingredient of pizza and other preparations because of its unique functional properties such as meltability and stretchability (Bertola et al. 1996a). This product may also be modified in composition, and in this case, be called pizza cheese or cheese analog (Codex Alimentarius Commission 1978). US HM Mozzarella cheese has a moisture content lower than Italian Mozzarella cheese, usually ranging from 58 to 65%, with the latter that is stored and commercialized into a covering liquid to prevent moisture losses from the product (Mucchetti, Pugliese, and Paciulli 2017).

LM Mozzarella cheese is usually produced into blocks, ripened for a limited period (e.g. 2 weeks), and often further processed, after cutting it into slices, cubes or shredding it. LM Mozzarella cheese can be directly obtained by fluid milk renneting and/or by semi-finished curds that are stored and transported to the dairy to be stretched (Dalla Riva et al. 2017). On the contrary, HM Mozzarella cheese is a soft cheese, which is consumed fresh, and it can be produced in different shapes and sizes, sold in a covering liquid generally made of water, NaCl and organic acids (Mucchetti, Pugliese, and Paciulli 2017). The covering liquid has the function to avoid moisture losses and the formation of the rind in the outer part of the cheese. Because of the high moisture content and its texture, HM Mozzarella cheese is not suitable to be industrially shredded to be used in industrial applications (e.g. pizza or baking preparations), although a patent to perform this process when the cheese is in the frozen state has been reported (Coker et al. 2017).

Freezing of Mozzarella and pasta filata cheeses manufactured for pizza and baked foods production may be

beneficial to extend shelf life (Pilcher and Kindstedt 1990). Immediately after stretching, salting and cooling, the cheese structure requires some aging period (a few weeks) before developing the desired functional properties, such as meltability, stretchability, melting color, fat release. However, after reaching the desired characteristics, Mozzarella cheese then rapidly loses these properties during additional storage time (Kindstedt and Fox 1993). Several literature studies evaluated the possibility to apply the freezing process to LM Mozzarella cheese by assessing the change in the rheological and melting properties of the cheese, as these are key quality attributes for the product. It has been reported that textural properties do not change significantly after short-term freezing (Cervantes, Lund, and Olson 1983); however, the product shows higher values of hardness and adhesiveness after 3 months of frozen storage at −20 °C (Bertola et al. 1996b).

LM Mozzarella, refrigerated and frozen (blast frozen at −30 °C for 4.5 h, stored at −20° up to 90 days) dynamic rheological properties showed that while LM Mozzarella stored under refrigerated conditions showed a softening of its structure because of the activity of endogenous proteases on α_{s1} and β-caseins, frozen and thawed cheeses had an increase in elastic modulus (Diefes, Rizvi, and Bartsch 1993). This was also reported by others (Bertola et al. 1996b; Reid and Yan 2004) also for other cheeses (Alvarenga, Canada, and Sousa 2011; Alberini, Miccolo, and Rubiolo 2015). The increase in elasticity and hardness compared to fresh control cheese has been attributed to dehydration of the protein network. After thawing, the dense protein network is no longer able to re-adsorb bulk water, diminishing its lubricant effect and thus producing a harder cheese structure (Bertola et al. 1996b). A different study (Chaves and Grosso 1999) showed no effect of freezing after 15-days frozen storage and 29-days tempering time after thawing, for cheese with low moisture and a high salt content (44.3% and 1.45%, respectively). The composition of this cheese may have limited the residual enzymes' activity. Other authors also attributed to the increase in elasticity the increase in stretchability and decrease in meltability measured in frozen Mozzarella cheese (Oberg et al. 1992). Deep freezing of Mozzarella in brine solutions has also been tested (Ribero, Rubiolo, and Zorrilla 2007), and dehydration of the protein matrix seems to be reduced, with better preservation of the rheological properties. Microstructure analysis revealed large voids between protein fibers in frozen Mozzarella due to protein dehydration and ice crystals formation and growth (Graiver, Zaritzky, and Califano 2006; Kuo and Gunasekaran 2009), and after defrosting the structure appears more porous due to the presence of large serum pockets. These modifications may be responsible for the reduction of firmness and cohesiveness observed during long frozen storage times (up to 1 year) in LM part-skim Mozzarella (To et al. 2020).

The effect of freezing in Mozzarella also depends on residual enzymatic activity. During freezing, damage can be caused to the casein network and to the LAB starter cells by the ice crystals; residual proteolytic enzymes will lead to a higher degree of proteolysis promoting a lowering of textural and rheological properties (Graiver, Zaritzky, and Califano 2006). Moreover, freezing damage to the matrix can make it more susceptible to subsequent proteolysis after thawing; non-protein nitrogen and soluble nitrogen at pH 4.6 are higher for frozen-thawed samples already at the initial time of ripening (Graiver, Zaritzky, and Califano 2006). It has been reported that the non-protein nitrogen content of frozen stored LM Mozzarella cheese ripened for 6 days before freezing is higher than the refrigerated control (Bertola et al. 1996b), highlighting a significant interaction between freezing, storage time and proteolysis (Alinovi et al. 2020c). Temperature abuses and freeze–thaw cycles will further affect proteolysis. The organic acid content, important in defining some of the flavor characteristics of the product, is similar in frozen LM Mozzarella cheese (at −20 °C and stored for 6 days) if compared with the refrigerated control during a subsequently overall ripening period of 41 days (Califano and Bevilacqua 1999).

Functional properties are a direct consequence of the rheological characteristics of the product. Conflicting results have been published, possibly because of differences in composition, processing history, and freezing process. Kuo and Gunasekaran (2003) stated that modifications in cheese structure during freezing and frozen storage increased the meltability and decreased the stretchability of LM Mozzarella cheese, as a consequence of protein damage. Bertola et al. (1996b) studied the effect of freezing conditions, frozen storage, ripening and aging time on functional properties of LM Mozzarella cheese such as free oil, meltability and apparent viscosity and they found out that, under their particular conditions (-20 °C in slow or fast conditions, using a cold chamber or ethylene glycol, respectively), they did not observe significant quality losses. Bunker (2016) did not observe modifications of these parameters as a function of the different freezing process tested (still-air freezing, air-blast freezing, immersion freezing at −30 °C and immersion freezing with ethanol at −70 °C). Reid and Yan (2004) observed that mozzarella frozen and stored at −28 °C for 3 months, an increase in stretchability and decrease in meltability, also confirming results reported by Oberg et al. (1992). Apostolopoulos and Marshall (1991) showed that the freezing of LM Mozzarella cheese did not have a significant effect on meltability but on the amount of free oil that was significantly higher. On the contrary, others (Diefes, Rizvi, and Bartsch 1993) reported a decrease of 14.7% of free oil for frozen stored LM Mozzarella cheese. Surface browning of frozen Mozzarella cheese was reported to be the equal (Oberg et al. 1992) or higher (Bunker 2016) than the control treatment.

The lack of homogeneity in results reported by these studies concerning LM Mozzarella cheese properties can be due to the different freezing processes (e.g. freezing temperatures; cheese size) and equipment used but also to the different cheese manufacture processes, composition and ingredient history (Alvarenga et al. 2013); in particular, Graiver, Zaritzky, and Califano (2006) applied a higher freezing temperature than Diefes, Rizvi, and Bartsch (1993) (-20 °C vs. −30 °C). This could promote the formation of bigger ice crystals causing more extensive damage to the

cheese structure. Most studies lacked an in-depth proteomic characterization, and it may be hypothesized that partial hydrolysis of the proteins may affect the structure of the protein network and functionalities such as stretching, melting and elasticity, that are critical when the cheese is used as food ingredient (e.g. pizza or baking applications).

It has been suggested that a period of tempering before or after freezing, may be necessary to soften the structure and recover optimal functional properties; while frozen storage leads to a more dehydrated protein matrix, with bigger serum channels and cracks as a consequence of ice crystallization, refrigerated storage acts in the opposite way, leading to a denser matrix because of water absorption and protein swelling (Laurienzo et al. 2008; Ribero, Rubiolo, and Zorrilla 2009).

Freezing of LM Mozzarella is carried out on whole blocks of cheese, or in portions of difference shape and sizes. In many food applications, Mozzarella is used in a shredded form and therefore many dairies shred the product prior to distribution to improve the ease of use. The higher freezing rate due to the small dimensions of the shredded cheese has an effect on the characteristics of the product: meltability and stretchability of the cheese will differ depending if the product was shredded or not, probably because of the higher freezing rate and the consequently lower damage to the cheese protein network (Oberg et al. 1992). Shredded cheese should be processed by applying a fluidized bed IQF method to prevent particles agglomeration; the frozen product can be used for pizza baking in frozen state or after thawing (Kielsmeier, Barz, and Allen 1990). A low air temperature (-35/-45 °C) causes a fast freezing and a high, fast temperature decrease in the outer part of the cheese piece, promoting the formation of a crust, icy layer that prevents agglomeration. Anticaking agents such as celluloses, starch or milk protein powders are added, to prevent agglomeration during freezing, storage, and prevent caking during defrosting (Mccadam 1965; Coker et al. 2017).

Low resolution Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI) were applied to investigate water distribution and molecular mobility in frozen Mozzarella (Kuo, Anderson, and Gunasekaran 2003). The water diffusion coefficient (D) increases with freezing at −21.5 °C, in accordance with findings on cream cheese formulations (Alinovi et al. 2020b), and after frozen storage for 1 or 4 weeks, and this change is attributed to the already described modifications of the protein matrix. The water is then less entrapped in the matrix. T_2 relaxation times were shifted to lower values and the population distribution was narrower, pointing to the exchange of water molecules between phases with different mobilities; protein damage caused by freezing and frozen storage could increase the number and accessibility of protein sites available for chemical exchange with water, lowering relaxation times and narrowing their distribution. If the microstructural damages caused by freezing are not severe, during tempering after thawing in LM Mozzarella, a decrease in D values and a reduction of more mobile serum fraction can be expected suggesting that the cheeses may be able to partly regain their initial state (Kuo, Anderson, and Gunasekaran 2003; To et al. 2020).

Recently, the effect of frozen storage up to 4 months on HM Mozzarella cheese water mobility was studied using low resolution NMR relaxometry (Alinovi et al. 2020a). Freezing caused dehydration of the caseins, and modified the relaxation times of the water molecules; in particular, the fastest T_2 relaxation component (∼1–2 ms) that represents the water protons population strongly bonded to the protein network (Ferrão et al. 2018) was affected. Along with the modification of the protein network, also changes in the structure and molecular mobility of the lipid fraction is observed with NMR (Sperry et al. 2018; Balthazar et al. 2017). In cheese characterized by a relatively high percentage of fat, such as Mozzarella, freezing can promote a destabilization and/or rupture of fat globules, causing a change in T_1 and T_2 NMR curves (Alinovi et al. 2020a). These modifications were confirmed by microstructural observations that showed the formation of large serum channels in samples subjected to freezing, compared with the fresh counterpart (Alinovi et al. 2020a).

These studies indicated that NMR may be a powerful technique to determine changes and quality of Mozzarella cheese after freezing, as the NMR relaxation in heterogeneous systems, such as cheese, is affected by the network structure, and each proton population is influenced by its surroundings (Gianferri et al. 2007; Silva et al. 2018). Alongside with NMR, also differential scanning calorimetry may prove to be an important technique to study water-related modifications of cheese products during freezing (Le Dean et al. 2001) by describing the water status from a thermodynamic perspective. Thus, more work should be carried out to fully interpret these dynamics within the complexity of the processing, storage history and anisotropy of the structures.

The effect of freezing on Italian HM Mozzarella cheese has also been studied, but not as extensively as for LM Mozzarella. It has recently been reported that different air blast freezing and thawing conditions (from −25 to −40 °C with different air velocities) do not affect HM Mozzarella cheese physico-chemical characteristics (Alinovi and Mucchetti 2020b). Conversely, there was a significant effect of the presence or absence of covering liquid during freezing. Cheeses frozen with covering liquid had a lower water holding capacity, showed softer texture and lower stiffness than for the same cheese frozen without the covering liquid. The latter displayed characteristics similar to those of the fresh controls. Authors concluded that freezing and thawing without the covering liquid was a preferable processing method to maintain HM Mozzarella quality, followed by an overnight equilibration period with a new covering liquid (Alinovi and Mucchetti 2020b).

There is a difference in quality after freezing of HM Mozzarella, for both conventional and blast freezing (Conte et al. 2017), as shown by a study for 2 months of storage, using microtomography and sensory analysis. In both cases, there is a gradual decrease in overall sensory quality, quickly reaching a critical value of acceptability (59–60 days for 30 g

size, 25–27 days for 250 g size). Microtomography observations indicated that frozen storage decreases the pores dimensions. During thawing or subsequent refrigerated storage, there will be a weight gain due to water absorption (Laurienzo et al. 2008; Ribero, Rubiolo, and Zorrilla 2009; Alinovi and Mucchetti 2020b). On the contrary, Alinovi et al. observed an increase of pore size volume during frozen storage of HM Mozzarella, that was related the change of water repartition between the serum and the protein network (Alinovi et al. 2020a), as also previously discussed for LM Mozzarella cheese.

Water mobility and temperature may not completely limit the residual activity of enzymes during frozen storage. In HM Mozzarella, proteolytic and oxidative reactions still occur, and after 4-months of storage changes in textural characteristics and visual appearance can be detected (Alinovi et al. 2020b; Alinovi et al. 2020a). Moreover, also color characteristics, that are important parameters determining visual acceptance of the product, showed changes during frozen storage. The decrease in lightness is caused by structural reorganization of the matrix during frozen storage and/or by differences in the amount and distribution of free water on the surface (Alinovi et al. 2020c).

No information is available on the effects of long frozen storage periods on the characteristics of Mozzarella cheese (e.g. more than 6 months), nor on the effect of long storage times on the physical and chemical properties after thawing. It may be possible to hypothesize that the shelf life can be lowered by the modifications caused by freezing and prolonged storage.

Freezing of non-bovine cheeses: caprine and ovine milk cheeses

Freezing has been used to resolve seasonality for high value cheeses produced from small ruminants' milk (Alvarenga et al. 2013). The product is frozen immediately after production (Casla et al. 1995; Fontecha et al. 1994) or after being fully ripened (Tejada et al. 2000; Tejada et al. 2002), depending on the type of cheese.

The effect of frozen storage (2 days, 3, 6 months at −20 °C) of soft cheeses from ewe's milk before ripening period (28 days) has been reported (Van Hekken, Tunick, and Park 2005). Proteolysis slightly varied with control cheese showing higher concentration of peptides in the molecular weight range between (22.5-15 kDa), mostly assigned to β-casein hydrolysis; only the short-term frozen storage slightly modified the structure of the cheese, by reducing the elastic behavior of the body. It is important to note that the final pH values of the cheeses were in the range between 4.05 and 4.14, and such low pH value causes a significant reduction in neutral enzymes activity (e.g. plasmin). Martín-Hernández et al. (1990) evaluated the effect of freezing (-40 °C using a plate freezer) and frozen storage (4 months) on four different caprine cheeses: a rennet-coagulated fresh cheese, a washed curd cheese made with a partial substitution of a part of the whey with 5% NaCl solution, a soft cheese made with surface flora (*Penicillium candidum* spores) and a semi-hard, Majorero-type cheese; all these cheeses were made using pasteurized milk with the addition of animal rennet and, with the exception of the rennet-coagulated fresh cheese, with addition of LAB cultures. In all the cases, with the exception of the rennet-coagulated fresh cheese, free nitrogen values were significantly higher in the frozen cheeses than their respectively controls, after the refrigerated ripening time. The higher proteolysis was related to the presence of enzymes from microbial lysis. A lower level of α_s-casein breakdown was measured in the frozen-stored cheeses and was attributed to the lower residual rennet (animal extract) activity, and to a decrease in protein accessibility due to the rearrangements in the network (Martín-Hernández et al. 1990). Lipolysis was not influenced by frozen storage in all cases, with the exception of the soft cheese with surface flora; in this case, frozen cheese showed a higher free fatty acids index. Sensory analysis demonstrated that the structure was acceptable in all the cheeses with the exception of frozen fresh cheese, due to a higher brittleness and graininess compared to control. The rennet-coagulated fresh cheese, with a moisture content of about 60% showed very little freezing stability.

Freezing of soft goat cheeses (-20 °C) and subsequent refrigerated storage (14 and 28 days) affect the organic acids composition as a result of different bacterial fermentation during ripening (Park and Drake 2005; Park and Lee 2006). Despite the change in organic acids, sensory perception of goat cheese has not been shown to be different after the frozen storage period in these high moisture and mild cheeses. These results were confirmed by a following work that showed that freezing (-20 °C for 24 h) followed by ripening for 30 days did not impact over the majority of flavor attributes with the exception of waxy/goaty and brothy flavors that decreased and increased, respectively following 3–6 months of frozen storage (Park, Gerard, and Drake 2006); overall freshness was largely unaffected. Longer storage, up to 5 years, caused significant reduction of freshness perception of caprine cheese (Park 2013).

Storage studies on semi hard ewe's milk cheeses have also been reported (Fontecha, Bellanato, and Juarez 1993; Fontecha et al. 1994; Fontecha et al. 1996). The effect of freezing (-35 °C and −80 °C using a plate freezer and liquid nitrogen, respectively) and frozen storage (-20 °C for 4 months) of young semi hard ewe's cheese promoted an increase in proteolysis during the following ripening (up to 90 days at 11 °C), as already evidenced in the case of caprine cheeses (Martín-Hernández et al. 1990); water holding capacity and a_w values in frozen/thawed cheeses were respectively higher and lower because of proteolysis, which caused the release of low molecular weight peptides. This observation is related to the presence of residual viable microbial population after storage. The caseinolytic and aminopeptidase activity of *L. lactis subsp, lactis* and *L. lactis subsp, cremoris* is affected by frozen storage but not by the freezing process itself (Casla et al. 1995). Changes in proteolysis were not influenced by the different freezing methods. However, slower freezing rates combined with prolonged frozen storage (4 months) were responsible for the presence of cracks and voids in the frozen cheeses as also reported in the case

of Mozzarella cheese (Graiver, Zaritzky, and Califano 2006; Kuo and Gunasekaran 2009), that caused also a softening of cheese body already after only 2 days of ripening (Fontecha et al. 1996; Fontecha et al. 1994).

Near Infrared and Raman spectra of frozen, non-ripened cheese samples highlight an increase of protein unordered structure following freezing, while control cheeses show a higher proportion of β-sheet and α-helix structures (Fontecha, Bellanato, and Juarez 1993); frozen storage does not seem to cause additional changes. The same work also demonstrated that two different freezing methods cause differences in the spectra of cheeses during ripening, with the young cheese frozen at −35 °C showing a larger development of unordered structure than the young cheese frozen at −80 °C using liquid nitrogen. This difference could be due to the larger ice crystals formation and the slower freezing rate as well as an increased proteolysis (Fontecha, Bellanato, and Juarez 1993).

Tejada et al. (2000, 2002) evaluated the effect of freezing (-20 °C and −82 °C) and prolonged frozen storage (up to 9 months) on the characteristics of a ewe's milk cheese manufactured using a vegetable coagulant; differently than the previously mentioned studies, the freezing process was applied on the cheeses ready for consumption after a 90-days ripening period. Chemical analyses after thawing did not show any significant difference, also in agreement with Prados et al. (2006) that studied the effect of freezing and 9-months frozen storage on characteristics of fully ripened Manchego-type cheese. The number, size and distribution of the eyes were significantly reduced after 90 days of storage in freezing conditions, probably due to the disruption of the structure, similarly to what reported by Conte et al. (2017).

Alvarenga, Canada, and Sousa (2011) evaluated the effect of freezing (-20 °C and −30 °C) after 28, 35 and 42 days of ripening and frozen storage (12 months at −10 and −20 °C) on Serpa cheese. While the primary proteolysis was inhibited by frozen storage, the secondary proteolysis still proceeded, as frozen cheeses had higher values of non-protein nitrogen. Physical analyses of the cheese showed an increase in hardness after frozen storage, as previously reported by Diefes, Rizvi, and Bartsch (1993).

Freezing of other cheeses

Moisture, fat and salt content play a critical role in defining the physical chemical and sensory characteristics of frozen Cheddar cheese (Kasprzak, Wendorff, and Chen 1994). Firmness decreased after freezing/thawing when the moisture content was over 35% as a consequence of ice crystals damage. This was also confirmed with textural and microstructural observations in another study (Reid and Yan 2004). Proteolytic activities continue during storage at −18 °C, and the rate of proteolysis further increases after thawing due to rearrangements in the protein network and release of enzymes from ruptured bacterial cells. Meltability and texture are not significantly affected after freezing (Reid and Yan 2004). It has been reported that cheeses with a moisture in fat-free cheese between 55–57%, corresponding to the firm semi-hard designation of Codex Alimentarius general cheese standard 283-1978 (Codex Alimentarius Commission 1978), are the most suitable to be frozen, as determined a higher degree of textural acceptability (Kasprzak, Wendorff, and Chen 1994).

Simov and Ivanov (2005)studied the effect of freezing (at −16 °C in still air freezing conditions) and frozen storage (-10 °C/-12 °C up to 12 months) of young, semi ripened and ripened Kashkaval cheeses. In this case also proteolysis of the cheese was significantly delayed but not arrested by frozen storage. The highest increase in non-casein and non-protein nitrogen was observed in young frozen cheeses, because of the higher pH value, which favors the activity of enzymes during the step of ripening after thawing in comparison with the control. A similar trend was also reported for a soft, semi cooked, short ripened cheese like Port Salut Argentino cheese (Verdini and Rubiolo 2002), that showed a higher ripening index and higher concentration of hydrophobic peptides also when immediately analyzed after thawing because high residual rennet activity and the presence of peptidases from damaged LAB cells (Verdini, Zorrilla, and Rubiolo 2005).

On the contrary freezing may be applied to stop mold induced proteolysis of ripened blue cheese, reducing the rate of aging during shelf life or to store young cheeses in order to mitigate the effects of seasonal variations in milk availability. Freezing of unripened Cabrales cheese (-40 °C using a plate freezer) delayed mold growth and the rate of primary and secondary proteolysis during the subsequent 8-months ripening of thawed cheeses as a consequence of freezing damages (Ramos et al. 1987).

Freezing may become a solution to improving shelf life of fresh, unripened cheese. However, high moisture content presents challenges as freezing may result in a detrimental effect of cheese body and texture, as observed for Italian HM Mozzarella cheese. This was shown for example, for freezing of Cottage cheese (Emmons, Beckett, and Tape 1968), which results in a mealy, fragile appearance after thawing, with extensive whey separation. Reformulation may be needed to improve the freezing stability of such products. Also, in the case of cream cheeses, freezing is an industrially applied method to increase shelf life and improve convenience.

To date, there is scarcity of fundamental studies on the effects of freezing and thawing on fresh cheeses characteristics, despite the widespread practice in the industry, to achieve longer shelf life of the cheeses. In cream cheese products, to preserve proteins functionality and structure, as well as cheese characteristics, and to limit denaturation, different cryoprotectants and stabilizers have been evaluated and used; also, antioxidants can be incorporated to minimize oxidative reactions against proteins and fat during storage. A reduction of freezing-induced modifications can be achieved by incorporation of sorbitol (typically 4% each), polyphosphate, glycoproteins, polysaccharides, polyols, sucrose and other sugars (Dey et al. 2019; Xiong 1997). The type and quantity of sugars and stabilizers can be critical in determining the freezing stability of cream cheese

formulations, in terms of microstructural changes and water status and mobility (Alinovi et al. 2020b).

Freezing of cheese curd

Curd is the intermediate product of rennet or acid coagulation milk, that is obtained after whey drainage. Curd is industrially produced as a semi-finished product and it is immediately packaged with the aim of regularly supplying cheesemaking sites lowering the costs and volumes that are involved in milk transportation (Barone et al. 2017a; Barone et al. 2017b; Barone et al. 2017c). Freezing of curd may be preferred to refrigerated storage of curd to extend the shelf life of this kind of semi-finished product (Pazzola et al. 2013). Moreover, curd's freezing can be an alternative to milk's freezing, as it reduces the warehousing needs and allows first step of manufacturing to occur closer to the milk production sites. Freezing of curds will therefore be an opportunity to delocalize cheesemaking in production areas closer to the end user, trading an intermediate product, and allowing to produce on demand, fresh product closer to the consumer, for example, in-store. Furthermore, cheese curd freezing may a be a tool for controlling milk's surplus by increasing storability.

Curd's freezing is applied to promote cheese productions that are seasonally limited in term of quantity (caprine, sheep, buffalo milk) (Lu and Miller 2019) or in the case of cow's milk, frozen curds are mainly used as an ingredient for producing pasta filata and/or processed cheeses.

An important parameter that has to be considered in relation with the cheese to be produced, is the pH of frozen cheese curds. The pH at the end of the coagulation process should be in the range between 6.3–6.6 if the type of cheese requires a rennet-type coagulation, while in the case of cheeses characterized by acid-type coagulation, the pH of the curd should reach the isoelectric point of caseins after lactic acidification processes (range 4.6–4.9) (Belitz, Grosch, and Schieberle 2009; Hori 1982). In industrial processes milk acidification made by LAB can be integrated or substituted by the addition of acidulants (e.g. lactic, citric acid, glucono delta lactone); this step can affect the amount of residual enzymes in the curd, and helps to control pH during thawing (Bansal, Fox, and Mcsweeney 2007). The type of acidification applied to modify the pH value of the frozen curd is of particular importance for products intended for pasta-filata cheese production, as it regulates the degree of demineralization of casein (calcium removal), that is one of the main factors determining stretching behavior and final textural properties of the cheese (Sheehan and Guinee 2004). Generally, curd's pH is kept slightly higher than the desired pH for the next technological operation (i.e. curd's stretching in the case of Mozzarella cheese) to avoid over-acidification (Vélez et al. 2015).

Also, draining may be an important step in the production of curds to be frozen, as it regulates the amount of water to be frozen and thus the time required for process, and the properties of the curd as an ingredient for further processing. Whey drainage can be obtained by the pressing or, in the case of fresh acid coagulated cheeses, traditionally by natural draining that can last up to 12–15 h until a dry matter content around 40% is reached (Portmann 1970) or actually by separation or membrane filtration (Mucchetti et al. 2000). pH and moisture content are critical parameters that have to accurately decided before performing freezing, as high values of these two parameters create favorable conditions for proteolytic or lipolytic activities (Alonso et al. 2011; Picon, Gaya, et al. 2010). The size of the curds may also be critical depending on the process.

The degree of integrity of casein is an important factor that has to be defined in view of the functional properties of the final cheese to be produced; highly hydrolyzed casein chains cause generally low water absorption, while more preserved and intact casein proteins maximize curd's attitude to be stretched and increase water absorption in the case of pasta filata type cheeses (Barbieri et al. 2014). Barone et al. (2017b) showed that deep-frozen cow's curds stored at −18 °C had a lower protein degradation even compared to refrigerated curds after a short shelf life period; this feature makes frozen curd a better ingredient than refrigerated curd for high moisture products. However, long frozen shelf life causes a softening of the casein structure, because of the residual activity of proteases and physical damage of ice crystals.

To minimize curd's softening during storage and improve storability, renneting agents characterized by a low nonspecific proteolytic activity, as camel chymosin (Alinovi, Cordioli, et al. 2018) and thermophilic starters with a low proteolytic activity should be preferred (Johnson 2014).

However, as well as in the case of Mozzarella cheese intended for pizza production, a tempering period may be required before further processing, if the body is too firm, to reach optimal functional properties and best final product quality. In fact, as in the case of cheeses during frozen storage, the modification of protein structure and the redistribution of the water molecules within the protein network can lead to a hard curd structure (Kljajevic et al. 2017).

Several research studies evaluated the applicability of frozen curds for the manufacture of different kind of cheeses.

Hori (1982) studied the feasibility of the freezing and thawing processes to store curds intended for cream cheese production; curds were treated by applying 5 different freezing (still air at −20 °C and −80 °C, CO_2 at −80 °C, N_2 vapors at −196 °C and ethanol immersion at −80 °C) and thawing processes (still air at 0 °C and 12 °C, water immersion at 7 °C, hot water immersion at 50 °C and 80 °C), corresponding to different freezing and thawing rates. Results showed an increase in textural hardness with decreasing freezing rate and with increasing thawing rate, respectively (Hori 1982). Textural results were in accordance with NMR T_2 relaxation time of bound water, that followed the same trend described for textural parameters, giving information about the extent of freezing and thawing injury, that were maximum at the lowest freezing and thawing rates, with a larger effect of thawing than freezing (Hori 1982).

While the increasing freezing damage is an expected result with the lower freezing rate, it is not always true with lower thawing rate. In fact, while slow thawing enhances the rate of physical and chemical modifications that occur in

the phase transition temperature range, it has been reported that slow thawing may have beneficial effects to the structure, for example, re-equilibration of minerals (in particular calcium and phosphorous) and the reabsorption of unbound water (Haard 1997). In fact, the amount of bound water decreased with the decreasing freezing rate and increased with the decreasing thawing rate.

A process for preparing Mozzarella cheese from single deep frozen curd by IQF technique without the need of any fresh curd has been patented (Zambrini and Bernardi 2017); the curd of appropriate size (longest side smaller than 10 cm, preferably 2 cm) can be individually frozen (IQF) and stored for 1 year or more. Curd portions can be thawed in line using water-steam used for steam stretching or with the aid of microwave or dielectric heating; the final cheese product can be obtained with only marginal mass losses and without the production of any by-product if the correct amount of added water is fully adsorbed by the curd during stretching (Zambrini and Bernardi 2017).

Frozen and thawed curds can also be used to produce fresh cheese to be further ripened (Portmann 1970). The presence and activity of bacteria and enzymes is of key importance to develop the optimal cheese texture and flavor during ripening. For example, Vélez et al. (2015) demonstrated, with a mini-curd model of young hard-cooked cheese (i.e. Reggianito Argentino) that no significant differences were found in pH, temperature during manufacture and composition between the cheese manufactured using frozen curd and the fresh control. As the curd for this kind of cheese is typically rennet-type, the procedure involved the standardization of pH at 6.40 by means of lactic acid and the addition of the commercial starter; the curd particles are stored frozen (-18 °C) before the acidification process is carried out later, after thawing, until a pH of 5.6 is reached.

In the case of caprine curds, Sendra et al. (1999), did not observe significant changes in the main components of sheep's milk cheese made with frozen curd or in the lipolytic activity. Moreover, temperature variations associated to curd's frozen storage did not cause a significant change in composition, proteolysis, lipolysis or fat oxidation, and pH; only changes in microstructure were observed in frozen curds, but were partially overcame by the ripening process of the cheese (Sendra et al. 2002). On the contrary, other authors (Alichanidis et al. 1981) demonstrated poor quality for cheese (Teleme cheese) made by frozen curd (-40 °C per 12 h), stored at −20 °C up to 6 months, due to extensive proteolysis, accelerated ripening, a lower water holding and moisture content and a crumbly texture. A higher pH of the frozen-curd and a higher extent of proteolysis was the cause for the quality challenges of the cheese made with frozen curd compared to control.

Several works studied the effect of freezing (-52 °C) and frozen storage (-24 °C for 4 months) on cheeses made by mixtures of frozen and fresh curds; in these works, goat's curd freezing and frozen storage was combined with the application of high-pressure treatments (Alonso et al. 2013; Alonso et al. 2011; Campos et al. 2011; Picon et al. 2013), pasteurization (Alonso et al. 2013), different curd's scalding times (Picon, Alonso, et al. 2010) and different pressing times (Picon, Gaya, et al. 2010) to observe possible differences during a 60-days ripening period of Hispánico cheese.

No influence of freezing process in terms of LAB, gram negative, mesophilic, coliforms and staphylococci bacterial counts was observed (Alonso et al. 2011; Campos et al. 2011; Picon, Gaya, et al. 2010). However, differences were found in the *Lactobacillus* population and in particular of *Lactobacillus plantarum* during ripening time; because of its complex set of peptidases that contribute to the formation of volatile compounds and flavors, differences in terms of flavor and taste within the control and the frozen cheeses may be present. Accordingly, a higher degree of proteolysis, aminopeptidase and esterase activity in in the cheese made with frozen stored curd was present (Picon, Alonso, et al. 2010), that can be partially attributed to changes in the enzymatic activity of LAB subsequently frozen storage, membrane rupture and enzymes leakage. On the contrary, other studies did not highlight significant differences with the exception of free amino acids content, that was significantly higher in the cheese made with frozen stored curd (Picon, Gaya, et al. 2010). These modifications contributed to statistical differences of volatile and flavor compounds, but too low to cause apparent sensory changes (Picon, Gaya, et al. 2010; Picon, Alonso, et al. 2010; Alonso et al. 2011) with a few exceptions, that led to a stronger, but less balanced flavor intensity (Alonso et al. 2013).

As frozen stored curds for cheesemaking are used by cheese producers to reduce production costs and increase margins, their application can be perceived as a negative factor by consumers in the case of traditional, high quality products. In particular, freezing and frozen storage of curds is not allowed for some PDO cheeses that have to follow EU regulations. Authors studied the possibility to trace the use of frozen stored curds in cheesemaking by measuring the presence of proteolytic markers generated by the residual activity of renneting agents and plasmin (Di Luccia et al. 2009; Manzo et al. 2017). Research studies have suggested that the presence of the fragment f(24–199) of α_{s1}-casein can be used as a marker to reveal the use of frozen stored curd in HM Mozzarella and in PDO Buffalo Mozzarella cheese making (Faccia et al. 2014; Petrella et al. 2015); plasmin activity against β-casein leads to the formation of γ_1, γ_2, γ_3-caseins fragments (f(29–209), f(106–209), and f(108–209)) in the case of bovine milk, while an additional fragment f(69–209) can be released and detected in the case of buffalo milk. Fragment f(24–199) of α_{s1}-casein is the primary proteolytic product generated by the residual activity of renneting agents and in particular calf chymosin. As Mozzarella cheese curd temperature during stretching can inactivate most of chymosin present in the curd, a long term frozen storage of the frozen cheese curd before stretching can favor the formation of the fragment f(24–199) of α_{s1}-casein, hence, this peptide can be used as a molecular marker of the use of a frozen cheese curd (Faccia et al. 2014).

Conclusions and outlook

Freezing of milk and dairy products is an effective method to reduce milk's seasonal availability, and to extend shelf life

and market reach both for high value products or for market commodities, such as Mozzarella or cheese curds. Several factors need to be controlled to obtain products with desired characteristics and without textural and sensory defects. First and foremost, the physical properties of the product can be modified as a function of how freezing and frozen storage are managed, affecting the amount of frozen water, size of ice crystals, and water mobility. Dehydration, rearrangement and aggregation of caseins change the structure of the protein network and can have important detrimental effects on the textural, functional properties of the cheese or cheese curd (water holding capacity, color, stretchability, hardness etc.). To obtain high quality frozen products or fresh products from frozen ingredients, studies aimed to improve freezing technologies and management of the processes has to be carried out; a more in depth of the proteomics and water status of frozen dairy products is crucial for a better understanding of the mechanisms behind the changes in the structure properties.

In this view, the improvement of frozen dairy products quality characteristics should also be the result of an integrated process able to manage all the unit operations, that have to be optimized to prevent/reduce the microbial load, and the unwanted enzymatic activities, that can modify the sensorial properties and the physico-chemical stability of the products.

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