Invited review: Heat stability of milk and concentrated milk: Past, present, and future research objectives

Joseph Dumpler,^{1*} Thom Huppertz,^{2,3} and Ulrich Kulozik⁴

¹Department of Food Science, Cornell University, Ithaca, NY 14853

²FrieslandCampina, Stationsplein 4, 3818 LE Amersfoort, The Netherlands

³Food Quality and Design, Wageningen University and Research, Bornse Weiland 9, 6708 WG Wageningen, The Netherlands

⁴Chair of Food and Bioprocess Engineering, Technical University of Munich, 85354 Freising-Weihenstephan, Germany

ABSTRACT

The ability of milk and concentrated milk to withstand a defined heat treatment without noticeable changes such as flocculation of protein is commonly denoted as heat stability. A heat treatment that exceeds the heat stability limit of milk or concentrated milk, which has a much lower heat stability, may result in undesired changes, such as separation of milk fat, grittiness, sediment formation, and phase separation. Most laboratory-scale batch heating methods were developed in the early 20th century to simulate commercial sterilization, and these methods have since been standardized. Heat stability studies have been motivated by different objectives during that time, addressing different processing issues and targets in the framework of available technology, legislation, and consumer demand. Although milk hygiene has improved during the last couple of decades, rendering milk less sensitive to coagulation, different standard methods suffered from poor comparability of results, even when comparing results for the same milk sample, indicating that unknown procedural steps affect heat stability. The prediction of heat stability of concentrated milk from the heat stability results of the corresponding unconcentrated milk for rapid quality testing purposes has been difficult, mainly due to different experimental conditions. The objective of this study is to review literature on heat stability, starting from studies in the early 20th century, to summarize the vast number of studies on compositional aspects of milk affecting heat stability, and to lead the way to the most recent work related to compositional changes in concentrates produced by membrane concentration and fractionation, respectively. Particular attention is paid to early and most recent developments and findings, such as the application of kinetic models to predict and limit protein

aggregation to assess and describe heat stability as a temperature-time-total milk solids continuum.

Key words: heat stability, concentrated milk, milk protein, casein micelle

INTRODUCTION

Heat stability of milk was historically defined as the time required to visually coagulate a sample at constant heating temperature in an oil bath for laboratory-scale assessment. This time was recorded and denoted as heat coagulation time as a means to describe the heat stability of milk and (reconstituted) milk concentrates. The term heat stability in the context of thermal processing for preservation refers to the ability of milk and concentrated milk to withstand a heat treatment, defined by heating temperature and duration, without coagulation. Coagulation is characterized by the formation of large proteinaceous flocs or dense particles that give rise to visible particles, a gritty mouthfeel, separation into a serum phase, separation of milk fat, and protein sediment during storage. In simple terms, we can categorize heat-induced coagulation as an undesired physical destabilization reaction in conflict with preservation of concentrated milk by heat to inactivate microorganisms, bacterial spores, and enzymes to increase the shelf life. Concentrated milk is preserved by either in-container autoclave sterilization (115–120°C for 5–15 min), or more recently, by continuous UHT (135–150°C for 1–10 s) sterilization. For evaporated milk manufacture, continuous heating conditions in the range of 125 to 135°C for 1 to 6 min can be applied to achieve similar product characteristics compared with in-container sterilization (Smith and Malmgren, 1999). The process for condensed milk sterilized in tin cans was patented by Gail Borden in the United States and England in the mid-19th century (Singh, 2004). Since then, heat and storage stability of these products have been an issue, especially in UHT-processed concentrated milk products (Smith and Malmgren, 1999; Dumpler and Kulozik, 2016; Chen et al., 2019).

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*Corresponding author: jd2228@cornell.edu

The various aspects and objectives of the heat stability of cow milk, other milks, and concentrated milk have been reviewed by Muir (1985) and Singh (2004) or summarized in book chapters by Singh (1995), O'Connell and Fox (2003), and Huppertz (2016). Recently, studies on milk-derived concentrates processed by membrane filtration have led to an increased interest in heat stability testing. Variations in composition of the concentrates, depending on the method of concentration or rather fractionation, can tremendously alter the heat and storage stability of the resulting concentrates. This might affect the possible shelf life due to a conflict of protein coagulation with the kinetics of microbial inactivation (Crowley et al., 2014, 2015a; Lin et al., 2016; Corredig et al., 2019; Singh et al., 2019). The kinetics and kinetic parameters of inactivation of microorganisms and bacterial spores in milk and other foods have been properly characterized (van Asselt et al., 2008). The kinetics of heat-induced chemical degradation reactions and denaturation of whey protein in milk have also been the subject of numerous studies (Kessler and Horak, 1981; Fink and Kessler, 1986; Dannenberg and Kessler, 1988; Morales et al., 1995; Saint Denis et al., 2001; Claeys et al., 2003). In spite of numerous studies on heat stability, the kinetics of heat-induced coagulation of milk proteins, useful for calculations to optimize process conditions, remained unknown. Therefore, the UHT heat treatment conditions of concentrated milk have been based on empiricism (Muir, 1984; Smith and Malmgren, 1999) until recently (Dumpler and Kulozik, 2015, 2016; Dumpler et al., 2018). A transfer of these bench-scale, batch heating results to state-of-the-art continuous-flow heating systems with notably shorter heating times and higher temperatures, however, remained challenging. Early studies of Holm et al. (1923), Webb et al. (1943), Davies and White (1966a), White and Davies (1966), and White and Sweetsur (1977) directed toward kinetic dependencies, which are especially useful for the design of HTST processing, fell behind studies investigating compositional aspects, sometimes in combination with preprocessing of milk. Recent studies suggested that a strikingly similar approach taken for UHT milk process optimization (i.e., minimizing chemical effects while maximizing microbial inactivation) enables resolution of the conflicting reactions of heat-induced coagulation and microbial inactivation to optimize concentrate quality and shelf life (Dumpler and Kulozik, 2015, 2016; Dumpler et al., 2018).

The objective of this work is to review the vast amount of literature on heat stability from studies in the early 20th century to the most recent work, including concentrates derived by membrane filtration and fractionation. This review intends to provide an

overview, point out the specific relevance of some of the most recent findings, and pave the way for a better understanding of the heat-induced coagulation process and its implications for shelf stable and functional dairy products and ingredients. It also intends to open the way for novel technologies to preserve milk concentrates by heat, using recently available and emerging thermal dairy processing equipment.

ASSESSMENT OF THE HEAT STABILITY OF CONCENTRATED MILK

The origin of scientific literature on the determination of the heat stability of milk and concentrated milk dates back to the early 20th century. Sommer and Hart (1919) investigated the relationship between titrable acidity, pH, natural variation in milk solids, and the heat stability of milk. This was done with the focus on the quality of raw milk for condenseries (i.e., manufacturers of evaporated milk). Sommer and Hart (1919) determined the heat stability by heating raw milk in small sealed glass tubes in a xylene vapor bath for rapid heat transfer at 136°C. The glass tubes were placed in a rack and tilted from time to time until visually detectable coagulation occurred. The entire timespan was then recorded as the coagulation time. Samples were heated in the xylene vapor whereby an equilibration time between the sample and the heating medium of ~1 min was achievable. The aim of Sommer and Hart (1919) was to predict the heat stability of the concentrate from the composition of unconcentrated milk. This experimental setup paved the way for later studies on heat stability of milk and concentrated milk. Studies on heat stability can be divided into 5 different categories. Remarkably, most of the work on heat stability focused on compositional effect on the heat stability of milk and concentrated milk (categories 3 and 4).

- 1) Improvement and comparison of experimental procedures on heat stability (Whitney et al., 1952; White and Davies, 1966; White and Sweetsur, 1977; Kneifel et al., 1987; Kelly and Roefs, 1990; Lehmann and Buckin, 2005).
- 2) Effects of heating temperature, total solids concentration, age of the milk, and composition of the concentrate on heat stability (Holm et al., 1923; Miller and Sommer, 1940; Davies and White, 1966b; Muir and Sweetsur, 1978, 1984; Dumpler and Kulozik, 2015, 2016; Dumpler, 2018; Dumpler et al., 2018).
- 3) Differences in composition of milk, resulting either from the stage of lactation, the genetics of an individual cow, or seasonal variation due to

herd synchronization (Holm et al., 1932; White and Davies, 1958; Rose, 1961a,b; Davies and White, 1966b; Holt et al., 1978b; Sweetsur and Muir, 1982; Singh and Tokley, 1990; Pouliot and Boulet, 1991; Chen et al., 2015).

- 4) The improvement or modification of the heat stability of milk and concentrated milk by pre- and postprocessing, processing sequence, additives, or milk fortification (Webb and Holm, 1932; Bell and Webb, 1943; Webb and Bell, 1943; Webb et al., 1943; Rose, 1962; Morrissey and O'Mahony, 1976; Muir and Sweetsur, 1976; Fox and Hearn, 1978a,b; Newstead et al., 1979; Pearce, 1979; Fox, 1981; Hardy et al., 1985; Dalgleish et al., 1987a,b; Augustin and Clarke, 1990; Singh and Tokley, 1990; Pouliot and Boulet, 1991; McCrae et al., 1994; Metwalli et al., 1996; O'Connell and Fox, 1999a; Tan-Kintia and Fox, 1999; Huppertz et al., 2004; Vyas and Tong, 2004; Singh et al., 2007; Sievanen et al., 2008; Omoarukhe et al., 2010; Lewis et al., 2011; de Kort et al., 2012; On-Nom et al., 2012; Chen et al., 2015).
- 5) Mechanistic insights into the factors affecting heat-induced coagulation (Rose and Tessier, 1959; Tessier and Rose, 1964; Sweetsur and White, 1975; Parker et al., 1979; Singh and Fox, 1985a,b, 1986, 1987a,b,c; van Boekel et al., 1989a,b,c; Nieuwenhuijse et al., 1992; O'Connell and Fox, 1999b, 2000; Dumpler et al., 2016).

In this review, we will further divide these overview categories into subjective and objective determination of the heat stability, individual constituents in milk, and processing of milk and their effect on the heat stability of milk and concentrated milks, mostly evaporated milk with legally defined composition. We will not discriminate unconcentrated from concentrated milk in general as the continuous removal of water from milk must lead to a steady change in milk properties in relation to heat coagulation. However, we will point out if unconcentrated or concentrated milk was used in referenced heat stability studies.

Subjective Determination of Heat Stability

The test method of Sommer and Hart (1919) was the archetype of all so-called subjective heat stability tests that were developed later on. The subjectivity of the test method refers to the discrimination between the uncoagulated and the coagulated state of a heated milk or concentrated milk sample by visual observation. Details of the procedure have been varied to result in a better detectability of the coagulation point to increase the precision of the test. In principle, a small

amount of sample is filled into a borosilicate glass tube, stoppered or screw capped, and immersed in a heat transfer medium. This tube is then tilted, rocked, or shaken to avoid burning of the milk onto the glass and to properly mix and evenly distribute the heat. The time between the immersion of the sample into the oil bath and the onset of the formation of visible protein particles (flocs) is called heat coagulation time. The length of the heat coagulation time is then a (relative) measure for heat stability. The basic method developed by Sommer and Hart (1919) was later modified, refined, and standardized by Davies and White (1966a) to become a standard method for heat stability testing. The experimental setup developed by Davies and White (1966a) is shown in Figure 1. Detailed descriptions of the individual methods can be found in the studies of Holm et al. (1932), Cole and Tarassuk (1946), Pyne and McHenry (1955), Rose and Tessier (1959), Belec and Jenness (1962), Davies and White (1966a), Farrell and Allgood (1970), and Kneifel et al. (1987). The test method developed by Davies and White (1966a) became the An Board Bainne method, which is the national standard method for heat stability testing suggested by the Irish Dairy Board. The Australian standard is based on the method of White and Davies (1966) and was developed to standardize the heat stability testing of reconstituted concentrated milk at 20% nonfat total solids. Variations in the observed heat coagulation time between these methods can be attributed to differences in the test setup and procedure. The different methods were also tested for comparability. The coagulation times of different samples each tested with 2 heat stability test methods gave a positive correlation, although it was found that the heat coagulation time of the same milk sample varied widely (Kneifel et al., 1987). Differences between individual test methods refer to the

- 1) medium for heat transfer (vegetable, mineral, or silicone oil, a metal block, vapor),
- 2) amount of sample and the size of the tube,
- 3) mode of movement of the tubes (tilting, rocking, shaking, or none),
- 4) procedure (a single tube or multiple tubes immersed at different times),
- 5) visual detection of coagulation at the glass surface or after removal from the glass in a Petri dish, and
- 6) measurement temperature (110–140°C).

The most important factor for the standardization of the heat stability assessment was the choice of the temperature of the heat transfer medium (i.e., the maximum sample temperature based on the expected heat stability). It was fixed to 140°C for unconcentrated

milk and 120°C for concentrated milk containing 18 to 21% nonfat total solids, as its expected heat stability is much lower. This standardization of temperature provides an advantage for comparability and simplicity of results of different milk samples but constitutes a major disadvantage when it comes to the kinetics of the coagulation process (i.e., the temperature, serum composition, and concentration dependency of the reaction). However, information on the temperature dependency of a physical or chemical reaction is of utmost importance for the prediction of the heat stability of various concentrates under modern continuous HTST heating conditions. The flexible and more rapid temperature adjustment in continuous heaters from pasteurization, high-heat treatment, and short holding times cannot be simulated or predicted by extrapolation from lower temperatures without knowing the kinetics (i.e., concentration, temperature, and time dependencies), due to the significantly different heating profile. The effects of temperature and total solids on the coagulation process had been investigated in early studies (Webb and Bell, 1943; Davies and White, 1966a) and recently by Dumpler and Kulozik (2015, 2016). In early studies, a first objective was to optimize measurement parameters of the subjective heat stability test and to investigate the kinetics of the onset of coagulation of the milk of individual cows. Figure 2 shows that the coagulation time decreases with increasing oil bath temperature. Davies and White (1966a) found that this dependency can be linearized in the range investigated when time is plotted on a logarithmic scale. The coagulum at the onset of coagulation, however, became less and less visible because the size of the clots decreased with decreasing temperature. This shows that the rate constant of heat-induced coagulation is considerably accelerated by an increase in temperature. The z -value (i.e., the increase in temperature required to reduce the coagulation time to 10% of the original time compared with the reference temperature) can be directly derived from the slope of the graphs shown here. The approximate z -values for the coagulation time varied between 15 and 27 K for milk of individual cows (Bright maid, milk 1) if linearized and commercial bulk milk, respectively. Figure 2 shows that pronounced compositional variations will not only affect the coagulation time at the measurement temperature chosen but also the temperature sensitivity of the reaction (i.e., the slope of the regression line). Using bulk skim milk and combining the observations of Davies and White (1966a) and Webb and Bell (1943) using a laboratory-scale heat stability test, both an increase in heating temperature and total solids content of skim milk concentrate resulted in a linear decrease in heat coagulation time in a wide range of temperatures between 85 and 142°C and of

total nonfat solids between unconcentrated milk to 35% total solids concentrate (Dumpler and Kulozik, 2015). Despite the limitations of the subjective test, it became widely used, as it is inexpensive, simple, and able to deliver valuable information about the heat stability and a relative comparison of milk samples. Temperature, composition, concentration effects, and the effect of various pretreatments on the onset of coagulation under in-container sterilization (i.e., low-temperature, long-time conditions) can be determined. Quantitative information about the amount of coagulated protein, the temperature and concentration-dependent course of coagulation, and its kinetics are not accessible, as the subjective heat stability test has a lengthy come-up time and is not an isothermal heat treatment, especially for short coagulation times. This led to the development of objective test methods to enable the prediction of HTST heating conditions above 100°C.

Objective Determination and Aggregation Kinetics

Objective methods to determine the heat stability can be divided into 2 categories. We can define the first category as automated methods that detect the onset of coagulation instead of visual observation by the naked eye. These methods were developed to replace

Figure 1. Copper-plated thermostatic paraffin bath used for the subjective heat-stability test of Davies and White (1966a). The sample tube attached to the carriage and the eccentric rocking the tube, a microscope (magnification 8.4), the immersion heaters, the stirrer, the thermometer indicating the paraffin bath temperature (left), and the thermometer for regulating the temperature (rear right-hand corner) are shown. The lamp illuminating the sample tube is not shown. Reproduced with permission from Cambridge University Press Ltd., UK, 1966 (<https://www.cambridge.org/core/journals/journal-of-dairy-research>).

Figure 2. The relation between the logarithm of coagulation time and temperature of 4 different types of milk (Davies and White, 1966a). Data were extracted using the digitizer of OriginPro 2017G (OriginLab Corp., Northampton, MA).

the subjective decision making on the onset of coagulation by physical parameters that are automatically measurable. The onset of coagulation of a milk sample was determined by changes in physical properties of the concentrate upon coagulation (e.g., by changes in viscosity; de Wit et al., 1986; Kelly and Roefs, 1990). Changes in viscosity due to gel or particle formation at the onset of coagulation were monitored by a heated falling-ball viscometer, the so-called Klaro-graph developed by de Wit et al. (1986), viscosity measurements of the concentrate after sterilization Kieseker (1988), or by an iron piston that is continuously moved by a ring magnet through the heated milk (Foissy and Kneifel, 1984; Kneifel et al., 1987). All the automated methods produced comparable results to the subjective method. Despite these advances, the subjective heat stability testing procedure remained the standard, possibly due to the simplicity and low cost of the method. Recently, an alternative approach for the objective determination of heat-induced changes in UHT milk during heat treatment leading to sediment formation during storage has been developed by Grewal et al. (2017). These authors used Fourier transform infrared (**FTIR**) spectroscopy in combination with principal component analysis. They found a strong correlation between the forma-

tion of sedimentable material ($2,760 \times g$ for 15 min) during storage and certain markers in the FTIR spectrum. Markoska et al. (2019) used a similar approach to quantify heat-induced changes in concentrated skim milk (9, 17, or 25% total solids) heated at 75 to 121°C and found that heat treatment of concentrated skim milk at 25% total solids affected the secondary structure of proteins more extensively compared with other samples and leads to more random protein structures. The FTIR spectroscopy might therefore be a useful tool to elucidate the extent of changes in the secondary structure of milk proteins during thermal processing and correlate them to the onset of coagulation and the quantity of aggregated protein.

The second category comprises methods that follow the process of heat-induced coagulation over time by determination of the remaining colloiddally soluble protein (i.e., casein micelles and whey proteins in milk that are suspended in solution due to Brownian motion and will not sediment unless aggregated to larger particles induced by heat). The separation of the aggregates is performed by filtration or centrifugation. The residual protein in the filtrate or supernatant was then determined and taken as a measure for the extent of heat-induced coagulation (Whitney et al., 1952; White and

Davies, 1966; White and Sweetsur, 1977; Nieuwenhuijse et al., 1991; O'Connell and Fox, 2000). These methods were not used widely, largely because numerous experiments per milk sample must be conducted separately for each heating time. The additional insights from a practical and scientific perspective gained by this method might not have justified the additional effort. The choice of the subjective test method for heat stability testing becomes evident in Figure 3, as the time without coagulation is followed by a sharp increase in coagulated protein that results in rapid detection of the coagulation point (dashed line). The graphs of coagulated protein can be divided into 3 phases: (1) the induction period or colloidal stability where no coagulation is detectable, (2) aggregation of proteins where the coagulum is formed, and (3) the stationary phase, where no further coagulation is detectable. The relative amount of coagulable protein over time indicates that it is the casein that coagulates. Using the additional information about the course of coagulation, White and Sweetsur (1977) modeled the kinetics of the coagulation of unconcentrated milk using a formal first-order reaction kinetics approach. These authors suggested that a kinetic description of the coagulation process was possible when the induction period and the stationary phase are excluded from the calculations. White and Sweetsur (1977) could derive an activation energy (E_A), rate constant (k), and a temperature factor (Q_{10}) using this formal reaction kinetic approach. However, they found that the residuals (i.e., the difference between the experimental data and graphs predicted by the model) were not evenly distributed over heating time. Hence, the non-normal distribution of residuals indicates that another model might be necessary to describe the data. The dependency of the course of coagulation of concentrated milk depending on pH was investigated by Nieuwenhuijse et al. (1991). They found that about half of the protein in concentrated milk from bulk milk was coagulable independent of pH. They also found that the onset of coagulation determined by the objective test method could be readily detected by the subjective test method. However, information on kinetics of the course of coagulation is lost using the subjective test method. Therefore, Dumpler et al. (2018) followed the coagulation process in their study using a similar approach as White and Davies (1966) using concentrated skim milk to vary total solids of the concentrate and heating temperature. Using laboratory-scale experiments, kinetic parameters of the coagulation reaction could be derived using an empirical Weibullian model to predict the heat coagulation process of concentrated skim milk on industrial scale continuous heat treatment. Figure 4 shows the lines of equal effect for con-

centrated skim milk calculated by means of the kinetic parameters. The graphs indicate 3 important aspects for the optimization of the heat treatment and shelf life of concentrated milk. First, the blue lines in Figure 5 separate the stability region from temperature-time combinations that will lead to considerable protein aggregation as sediment formation increases sharply when critical T-t-conditions are exceeded (Figure 3). If a certain level of microbial inactivation, such as 6 log reduction of *Bacillus cereus*, needs to be obtained at a given temperature and holding time while limiting aggregation of proteins to a minimum, the total solids content of the concentrate cannot exceed a certain value. For example, concentrates up to 30% total solids can be heat treated in the extended shelf life processing window. Concentrates up to 18% nonfat total solids, such as evaporated milk, can be sterilized. Concentrates in the range of concentration used for powder production can be high-heat treated (100–120°C for 1–15 s) to destroy vegetative microorganisms. Second, the z -value used to calculate the slope of the lines was 18.8 K ($Q_{10} = 3.4$), which corresponds to an activation energy of 161.4 kJ mol⁻¹. This Q_{10} value is in the range of values (3–4) stated in the literature (Davies and White, 1966a; Kessler, 1975; Walstra and Jenness, 1984; van Boekel et al., 1989a). Microbial inactivation at a given total solids content can be maximized by a reduction of the holding time to a minimum, pointing in directions of ultrashort pasteurization and ultrashort sterilization (USS) in the millisecond domain at high temperatures (Mathys, 2018). This indicates that microbial inactivation shows a stronger temperature dependency than the kinetics of micelle aggregation. Chemical effects will also be minimal as their temperature dependency is even lower, which leads to minimal chemical changes when micellar aggregation is limited by ultrashort pasteurization or USS heat treatment. A heat treatment in the USS processing range would result in long shelf life high-quality concentrates. A limitation of the diagram is that the thermal resistance of microorganisms might change as a result of water removal, and therefore, slightly higher temperature-time combinations might be necessary to achieve a desired level of inactivation. Attempts to increase the heat stability of the skim milk concentrate will lead to an increase in possible total solids content or heating temperature and holding time or both (Dumpler and Kulozik, 2016). Most interestingly, by comparing the heat coagulation T-t data framework determined for skim milk concentrates using a subjective heat stability test by Dumpler and Kulozik (2015) and the lines of equal effect for constant total solids in Figure 4, a quantitative estimation of the maximum T-t-combinations for the heat treatment of any milk

Figure 3. Aggregated protein in percent (●) and nonprotein nitrogen plus proteose peptone nitrogen (○) produced in normal separated bulk milk and milk from an individual cow after heating at 135°C for various periods. The vertical broken line indicates the visible onset of coagulation (White and Davies, 1966). Data were extracted using the digitizer of OriginPro 2017G (OriginLab Corp., Northampton, MA).

concentrate is possible. This especially applies when concentrates were obtained or modified in composition by membrane filtration, stabilizer addition, preheat treatment or any combination thereof. This transfer and prediction of applicable industrial continuous heating conditions by laboratory-scale experiments should be confirmed and addressed by future studies.

EFFECTS OF COMPOSITION ON HEAT STABILITY OF CONCENTRATED MILK

A century ago, Sommer and Hart (1919) found that titrable acidity and the pH of raw milk varied so widely that heat stability could not be related to the sensitivity of milk toward heat-induced coagulation. Variabilities in raw milk quality were found responsible for severe coagulation or gel formation during sterilization of the concentrated milk. They suggested that (1) casein required an optimum amount of calcium for maximum heat stability which is in equilibrium with magnesium, citrates, and phosphates present, (2) acid fermentation in poor quality raw milk reduced the heat stability of the concentrate due to a decrease in pH, and (3) natural variations in the amount of single milk constituents were partly responsible for variations in heat stability. In other words, water, calcium, and magnesium as divalent cations, the anions citrate and phosphate, and pH (i.e., the main constituents of the mineral equilibrium in milk) were considered as the major factors affecting the casein micelles' colloidal stability on heat treatment

of concentrated milk. We should keep these basic statements of Sommer and Hart (1919, 1922) in mind as the basic hypothesis when comparing later findings on the heat stability of milk and concentrated milk.

Lactose

Lactose interacts directly and indirectly with the milk proteins during heat treatment. It comprises about half of the skim milk solids. Lactose is a reducing disaccharide containing galactose and glucose linked by a β -(1, 4)-glycoside linkage. Prolonged heat treatment of milk at high temperature ($>100^{\circ}\text{C}$ for several minutes) results in the formation of early Maillard products. Lactulosyllysine is formed when the aldehyde of lactose reacts with lysine residues within the protein structure. Lactose and available lysine form a Schiff's base that is then further converted to the Amadori product of protein bound lactulosyllysine (van Boekel, 1998). As kinetic studies showed that the chemical reactions in liquid foods can be described by pseudo-first-order kinetics (i.e., no dependency of the velocity constants on the concentration of reactants), rate constants will not increase by removal of water from skim milk (Fink and Kessler, 1986; Morales et al., 1995; Claeys et al., 2003). However, the amount present in the concentrate by volume will increase. The glycation of caseins might influence the conformation and the colloidal stability of milk proteins (Tan-Kintia and Fox, 1999). It was assumed that a reaction that parallels the Maillard reaction during heat treatment of milk was the most

Figure 4. Lines of equal effect for concentrated skim milk (CSM) for a critical level of 2% of sedimentable protein of total protein depending on heating temperature, heating time, and total solids content of bulk CSM. The time-temperature range covered by experiments (dotted area) and extrapolated total solids contents (dotted lines) are shown. The red lines indicate 15 log *Escherichia coli* (Plu95%) and 6 log *Bacillus cereus* (mean). The UHT region (gray area) and certain microbial inactivation levels are indicated (Dumpler et al., 2018).

relevant in relation to heat stability of milk. The isomerization and subsequent thermal degradation of lactose also leads to a decrease in pH in milk and concentrated milk due to the formation of formic and acetic acid from lactose. The breakdown of lactose leads to the formation of advanced Maillard products and formic acid (Fox et al., 2015). The subsequent decrease in pH was assumed to negatively affect heat stability due to the reduction of the net negative charge of casein micelles in milk after prolonged heating (Anema and Klostermeyer, 1997). However, a clear correlation between the decrease in pH during heat treatment and the onset and propagation of coagulation could not be established, possibly due to many other heat-induced overlapping dissociation and aggregation reactions fol-

lowing different kinetics (Sweetsur and White, 1975; Horne and Muir, 1990). Considering the aggregation of casein micelles in concentrated skim milk heated by direct steam injection or continuous short time heating (Miller and Sommer, 1940; Dumpler and Kulozik, 2016) where chemical reactions and heat-induced acidification from lactose degradation are minimal, heat-induced acidity might adversely affect casein micelles only during heat stability testing or batch-sterilization processes. As its effect is then linked to the overall heat stability of the sample, heat-induced acidification can be seen as a net negative contributor but not as the reason for heat-induced coagulation, which must be the heat treatment and the induced structural changes on casein micelles themselves.

Caseins and Whey Proteins and Casein-Whey Protein Interactions

The caseins are the main milk protein fraction, comprising approximately 80% of all milk proteins. The caseins are classified as phosphoproteins, due to their ester phosphate groups within the amino acid sequence. In milk, 95% of this protein fraction is organized in micellar form as so-called casein micelles. About 94% of the casein micelle dry matter is formed by the 4 main caseins, which are denoted as κ -casein, α_{S2} -casein, α_{S1} -casein, and β -casein. The different genetic variants of the caseins differ in the number of ester phosphate groups and some amino acids within their primary sequence (Huppertz, 2013). Compared with the calcium-sensitive α_S - and β -caseins, κ -casein is insensitive to the relatively high level of soluble calcium within the serum phase of milk. κ -Casein is assumed to be mainly attached to the outer surface of the casein micelles as disulphide-linked oligomers (patches; Rasmussen et al., 1992). As a so-called hairy layer, these κ -casein patches form a negatively charged electrostatic and steric barrier toward heat-induced aggregation (Holt and Horne, 1996). κ -Casein is relatively loosely bound to the micelle surface, as it readily dissociates from casein micelles upon heating and an increase in pH (Anema and Klostermeyer, 1997; Anema, 1998). β -Casein is also assumed to be more loosely attached to the casein micelles, as it dissociates from casein micelles to a greater extent than α_S -caseins upon prolonged heating

(Anema, 1998; Anema and Li, 2000) and UHT conditions (Dumpler et al., 2016). The remaining 6% of the micelle dry matter is composed of inorganic calcium phosphate, calcium citrate, and complexed calcium ions, which are considered major building blocks of the stability of the casein micellar structure (Holt et al., 2013; Lenton et al., 2015). The high-heat stability at the natural pH of milk (about pH 6.6–6.8) and the high pH sensitivity of casein micelles are often attributed to the linkage between phosphoserine-containing caseins formed by calcium bridges and linkages by colloidal calcium phosphate within the casein micelles. The remaining internal stability of the casein complex is given by hydrogen bonds, electrostatic interactions, and hydrophobic bonds. Caseins were assumed not to show pronounced secondary and tertiary structural elements as α -helices or β -sheet structures and therefore, were denoted as rheomorphic proteins, making them very flexible in terms of conformation. They are assumed not to denature upon extensive heating (Holt and Sawyer, 1993). Heat treatment of casein micelles in concentrated milk resulted in an increase in the size of individual casein micelles and aggregation of casein micelles during UHT heat treatment. Based on the electron micrographs, Hostettler et al. (1968) stated that the increase in size of casein micelles during heat treatment was proportional to the concentration factor of the concentrated milk. The increase in size was also observed by Carroll et al. (1971). The contributing factors for the increase in (apparent) micelle size have been discussed as heating temperature and heating time, leading to whey protein and calcium phosphate deposition on the micelle surface (Carroll et al., 1971). These changes in turn reduce the amount of ionic calcium and pH, which are essential for the conformation of the caseins and the colloidal stability of the micelle. In addition, Dumpler et al. (2016) considered this increase in micelle size as an indicator for the loss of the native internal structure of the micelles and increase in the exposed surface of calcium-sensitive caseins. The coinciding reduction of the electrostatic and steric repulsion will make casein micelles more susceptible to low pH, high ionic strength, and high-calcium environments such as concentrated milk. Markoska et al. (2019) found the casein micelles to undergo dissociation that induced redistribution of structural elements upon heating unconcentrated milk at 121°C using FTIR, which confirms destabilizing structural changes within the casein micelle.

Whey proteins are globular proteins that are far more heat sensitive, compared with casein micelles. Complexes of denatured whey proteins and κ -casein on the surface of casein micelles can form a steric barrier on the surface of the casein micelles against aggregation at

Figure 5. Heat coagulation time (HCT) versus pH profile for type A milk, type B milk, concentrated milk at 20% nonfat total solids, and serum protein-free casein micelle dispersions heated at 140°C (Singh and Creamer, 1992). Data were extracted using the digitizer of OriginPro 2017G (OriginLab Corp., Northampton, MA).

pH <6.7. The natural pH range of unconcentrated milk is 6.6 to 6.8. In addition, the interaction of κ -casein and whey proteins changes physical characteristics, such as viscosity, color, and turbidity, as the size of casein micelles and the surface roughness increase due to adsorbed complexes (Anema and Li, 2003). At high pH (pH >6.7), these complexes will readily dissociate from the micelle surface when milk or concentrated milk is heated, resulting in κ -casein-depleted micelles that are susceptible to aggregation, depending on the serum conditions. At low pH (pH <6.7), denatured whey proteins remain attached to the casein micelle and aggregate together with casein micelles upon heat-induced aggregation (O'Connell and Fox, 2003). With decreasing pH, whey proteins were found to increasingly form heat-induced complexes with κ -casein, mainly at the surface of casein micelles in unconcentrated milk. An increasing amount of adsorbed whey proteins increases the heat stability of unconcentrated milk compared with whey protein-free milk or concentrated milk at pH <6.7. Due to these interactions, whey proteins were shown to influence the pH sensitivity of heat-induced coagulation of unconcentrated milk, possibly due to the formation of a steric barrier of denatured whey proteins toward the aggregation of casein micelles, especially at low pH (Rose, 1961a; Tessier and Rose, 1964; Fox and Hoynes, 1975). In contrast, the heat stability of concentrated milk was positively affected when the concentration of whey proteins was reduced and negatively affected by naturally occurring higher concentrations or the addition of whey proteins, especially β -lactoglobulin (Muir and Sweetsur, 1978). This was attributed to the increased extent of κ -casein dissociation upon addition of whey proteins or a crosslinking of casein micelles by reactive whey proteins that cannot be distinguished visually from heat-induced aggregation over the pH range of 6.5 to 7.3 (Newstead et al., 1977; Pearce, 1979; Singh and Creamer, 1992).

Acidity and pH

Rose (1961a, c) first described the marked effect of initial milk pH on heat stability of milk in the range of 6.2 to 7.4 after adjustment by acid or based addition. He defined 2 types of milk that differed in their heat coagulation time (**HCT**) pH profile as shown in Figure 5. Type A milk shows a maximum in HCT usually at the natural pH of milk at pH 6.6 to 6.8 and a local minimum at pH 6.9 to 7.0. Type B milk shows an increase in HCT with increasing pH. Most of the milk from individual cows and pooled bulk milk show the behavior of type A milk. Removal of serum proteins from type A milk results in milk that shows a pH dependency similar to type B milk. Partial removal of salts from

milk by dialysis against deionized water also leads to a conversion of a type A to a type B profile. These observations point out the implication of milk salts and whey proteins in the coagulum formation at pH >6.7 for type A milk, especially at the local minimum at around pH 6.9. A low heat stability of both milks at pH <6.9 was attributed to salt-induced coagulation. This means that the colloidal stability of casein micelles was assumed to be lowered by a decrease in surface charge of casein micelles, a reduced electrostatic repulsion, and a collapse of the hairy layer due to charge neutralization. This results in an increased tendency toward aggregation (van Boekel et al., 1989a). The dissolution of colloidal calcium phosphate (**CCP**) accompanied by a small increase in ionic strength, but most importantly an increase in calcium activity, could also contribute to an increase in attractive forces between casein micelles (Nieuwenhuijse et al., 1991; Dumpler et al., 2017).

The heat stability of concentrated milk at 20% non-fat total solids is low compared with unconcentrated milk. At pH >6.7, the extent of the dissociation of heat-induced whey protein κ -casein complexes is increased. This was correlated with a destabilization of casein micelles for both concentrated and unconcentrated milk (Singh, 2004). When the same measurement temperature is used, the HCT of concentrated milk at 20% total solids is approximately 25% of that of unconcentrated milk at the pH maximum as shown in Figure 5, indicating a relationship between the total solids content of the concentrate and the HCT. These observations point out that heat-induced coagulation is a complex, at least 2-stage process of destabilization and aggregation that is affected by multiple serum conditions. Thereby, the serum conditions such as pH, the calcium equilibrium, and other constituents influence the destabilization of the micelle by weakening the internal structure under elevated temperature, influence casein-whey protein interaction and dissociation, and affect heat-induced aggregation to form a coagulum. However, it is still unclear which reaction is rate determining. At different pH and solids concentration, different reactions such as chemical (e.g., cleavage of covalent bonds or crosslinking) or physical (e.g., dissociation of κ -casein, dissociation of the internal structure, or the aggregation of the micelles) might be rate determining. It remains uncertain at what level of structural change casein micelles tend to aggregate. In addition, aggregation might continue after cooling due to the damage affection micelle stability and might be linked to age-gelation of concentrated milk forming a gel network instead of particles. The characterization of the overall and remaining stability of casein micelles has been attempted by measuring the zeta-potential, but no clear relationship between surface charge and

casein micelle aggregation has been determined, in part due to the complexity of milk. Another important aspect is that the physicochemical properties and most serum changes (pH, calcium, charge of micelles, dissociation of caseins) cannot be determined at the level of the heating temperature. Some attempts have been made to determine ionic calcium and pH and high temperature. Innovative approaches to determine milk properties at high temperature will be important for novel insights into mechanisms of coagulation as the factors affecting heat stability (e.g., pH, ionic calcium, protein dissociation) change reversibly during heating.

Minerals and Mineral Balance

Milk salts make up to 7 to 8 g L⁻¹ in skim milk, whereby single constituents can vary considerably, especially in milk from mastitic or late-lactation cows compared with early-lactation cows. Milk salts are found in milk either soluble in the milk serum or as colloidal complexes associated with caseins within the casein micelle. About 65 to 70% of total calcium and 50% of total inorganic phosphate are present as CCP in milk due to the low solubility of calcium phosphate at the natural pH of milk. The need to form casein micelles in milk puts constraints on pH and ionic calcium because the self-assembly of caseins to casein micelles will be strongly dependent on pH and ionic calcium (Holt et al., 2013). Most of the phosphate is present as HPO₄²⁻ and H₂PO₄⁻ (in a ratio of 60:40). Most of the citrate is present as citrate³⁻ at the natural pH of milk (Fox et al., 2015). The affinity of the phosphate ion to soluble calcium decreases with increasing protonation by a decrease in pH. A decrease in pH increases the amount of calcium and phosphate present in the milk serum, as CCP is dissolved when HPO₄²⁻ is protonated to H₂PO₄⁻ (Frèche and Heughebaert, 1989). Heat treatment of milk results in a reduction of the serum calcium and phosphate level and an increase in CCP, due to the inverse solubility of calcium phosphate in milk with increasing temperature (Rose and Tessier, 1959; Anema, 2009; On-Nom et al., 2010). The calcium and phosphate levels in the serum phase are readily restored upon cooling, except for heat treatments at >95°C for several minutes (van Dijk and Hersevoort, 1992). Severe heat treatments such as in-container sterilization (120°C for 20 min) were reported to lead to an increase in CCP and a structural transformation of colloidal calcium phosphate into another calcium phosphate phase (i.e., another stoichiometric ratio of Ca/P; Holt, 1995). The precipitation of calcium phosphate during heat treatment of milk, and especially concentrated milk, leads to a decrease in pH, as protons are liberated from hydrogen and dihydrogen phosphate to form different

forms of crystalline calcium phosphate, which might negatively affect the colloidal stability of the casein micelles in milk (Nieuwenhuijse et al., 1988; Gaucheron, 2005). In some studies, the precipitation of calcium phosphate onto casein micelles during heat treatment was assumed to be one of the factors that cause heat-induced aggregation of casein micelles. It was assumed that precipitated positively charged calcium phosphate on the micelle surface reduces the net negative charge of casein micelles and therefore, promotes heat-induced coagulation (Singh, 1994; O'Connell and Fox, 2001). However, the precise location or the distribution of the newly formed calcium phosphate in milk upon heating and the effect of its phase transition to the insoluble crystalline or amorphous state remains uncertain. Possible precipitation onto the micelle surface, formation of nano-scale precipitate or crystalline calcium phosphate in the milk serum, or contribution to the growth or de novo formation of calcium phosphate nanocluster needs to be addressed. Future research work will be necessary, due to the reversibility of the process upon cooling. The presence of calcium in milk in at least 4 different states (CCP, Ca²⁺ complexed by proteins, calcium citrate and phosphate complexes, ionic calcium) in equilibrium makes it difficult to assess the proportion of a single form of calcium. Changes in pH, temperature, and overall composition, as will be discussed in more detail in the next section, will affect multiple forms of calcium at the same time and shift the salt balance to a new equilibrium. Changes in the mineral equilibrium of milk and concentrated milk in terms of ionic strength, soluble calcium, and pH will be especially relevant in the section on concentration of milk by membrane technology.

Additives and Milk Fortification

In industrial practice, the main target was to improve the heat stability of milk concentrated by reverse osmosis (RO) and evaporation using permitted additives. The destabilizing effect of increasing concentrations of serum calcium with increasing concentration of milk solids on the heat stability of milk was to some extent reduced by the addition of citrates or phosphates to milk before heat treatment, due to the complexation of the soluble calcium (Singh et al., 2007; Boumpa et al., 2008; de Kort et al., 2012). Therefore, citrates, phosphates, and polyphosphates are frequently used as stabilizers in the manufacture of evaporated milk to enable sterilization without sediment formation during storage. However, the amount and type of stabilizer used, together with the adjustment of pH by addition of bicarbonates to reduce ionic calcium in concentrated milk and to compensate for seasonal variations in milk

composition, is mostly of empirical nature until today (Pouliot and Boulet, 1991). The subjective heat stability test is mostly used to test and optimize the heat stability before commercial production. In research, other chemicals were also used to investigate and compare quantitative effects of these additives, as well as for targeted investigations on the mechanisms of heat-induced coagulation. Additives to enhance heat stability or to investigate the effect of the modification on proteins or the serum composition of concentrated milk are summarized in Table 1. Studies on targeted modification of the serum composition (dialysis, serum exchange), protein modification (crosslinking, derivatization of amino acid side chains), removal of whey proteins or addition of κ -casein, addition of urea and many others resulting in a shift in the HCT-pH profile or a change from a type A to a type B milk and vice versa are numerous (Tessier and Rose, 1964; Muir and Sweetser, 1976; Muir et al., 1979; Singh and Fox, 1985a,b, 1986; Dalglish et al., 1987b; Singh and Fox, 1987a,b,c; Metwalli and van Boekel, 1996; O'Connell and Fox, 2000). Apart from research on the general effect of calcium on the heat stability of milk and concentrated milk, calcium fortification of UHT milks, a more recent trend, has increased the number of studies on the effect of calcium addition to unconcentrated milk. The destabilizing effect of soluble calcium on the colloidal stability of casein micelles, especially when heated, has long been known. Addition of calcium to milk usually lowers the pH, increases the amount of ionic calcium and CCP, reduces the amount of soluble phosphate in the milk serum, and thereby reduces the heat stability. The way excess soluble calcium destabilizes casein micelles is not fully understood, partly due to the interaction of different forms of calcium in milk that aggravates research on cause and effect relationships. There have been numerous studies on the effect of calcium from various sources on the heat stability of milk and concentrated milk (i.e., calcium addition as soluble calcium chloride, calcium lactate, and calcium gluconate or as insoluble calcium carbonate, calcium sulfate, and calcium phosphate; Rose, 1961c; Tessier and Rose, 1961; Vyas and Tong, 2004; Singh et al., 2007; Sievanen et al., 2008; Omoarukhe et al., 2010; On-Nom et al., 2012). There is consensus about the destabilizing effect of calcium from soluble salts on the colloidal stability of milk. However, the use of soluble forms of calcium avoids the problem of sedimentation of calcium salt crystals during storage. The addition of soluble salts had a minor effect on heat and storage stability of milk, but insoluble calcium salts had no measurable effect. Heat treatment of calcium-fortified milk using soluble calcium salts generally led to gel formation or the formation of protein particles

that were sedimentable by centrifugation or sedimented during storage of milk. It is evident that calcium fortification of milk with either soluble or insoluble calcium remains challenging. Even natural variations of soluble calcium of milk from individual cows showed a distinct correlation with the heat stability of milk (Pyne and McHenry, 1955). By contrast, a reduction in soluble calcium in reconstituted concentrated milk before heat treatment led to an increase in heat stability (Faka et al., 2009). Serum calcium in concentrated milk was associated with salt-induced coagulation of casein micelles in concentrated milk and was considered as one of the most important factors in reducing the heat stability of concentrated milk compared with unconcentrated milk (Nieuwenhuijse et al., 1991). The destabilizing effect of calcium addition can be fully compensated by addition of phosphates or citrates, whereby a certain ratio results in optimal heat stability (Sommer and Hart, 1922; Miller and Sommer, 1940; Horne and Muir, 1990).

EFFECTS OF PROCESSING ON HEAT STABILITY OF CONCENTRATED MILK

Processing of concentrated milk to the final shelf stable evaporated milk products includes multiple processing steps that can be arranged in different order and will thereby affect the stability of the concentrated milk toward the final heat treatment, mostly an in-container or more recently a UHT sterilization process. The heat stability of concentrated milks is low, and seasonal variations in milk composition result in considerable differences in the heat stability. This seasonal variation is especially pronounced in milk production regions with pasture-based milk production (Holt et al., 1978b). Low heat stability means that milk concentrated to 18 to 21% nonfat total solids was found to coagulate during sterilization even after preheating of the milk depending on season (Singh and Tokley, 1990; Pouliot and Boulet, 1991). This indicates that a nonfat total solids content of 18 to 21% of evaporated milk at the fixed heating intensity for sterilization represents a borderline case in terms of the conflict between microbial inactivation and the stability of the concentrate toward heat. At higher total solids content, all concentrates will be unstable toward sterilization even after preheating of milk and stabilizer addition (Webb et al., 1943; Walstra and Jenness, 1984). Therefore, technological means including processing steps, their order, and the use of additives were often directed toward a control or an improvement of the heat stability of concentrated milk to achieve sterilization of evaporated milk without coagulation of caseins and sediment formation. The sequence of processing does have an effect on the appear-

Table 1. Summary of the purpose and outcomes of the supplementation of milk or concentrated milk with various chemicals in relation to heat stability testing¹

Additive	Milk type	Purpose	Outcomes	References
NaOH, HCl	Skim, whole milk, and concentrate	Investigation of the effect of pH on the heat stability of milk and concentrated milk (HCT-pH profiles)	Changes of HCT by adjustment of pH due to protein charge and changes in the mineral equilibrium	Rose (1961a) Sweetsur and White (1975) Singh and Fox (1986) Tan-Kintia and Fox (1999)
Phosphates, citrates, EDTA	Concentrated whole milk (31% total solids) Concentrated micellar casein solutions	Compensate for seasonal variation and increase heat stability by complexing agents	Complexation of soluble calcium that increase HCT depending on pH and total solids	Pouliot and Boulet (1991) de Kort et al. (2012)
Phospholipids from natural sources (buttermilk)	Recombined evaporated milk and reconstituted skim milk	Investigation of the effect of buttermilk in combination with preheat treatment of milk on seasonal variation	Moderate increase in HCT in combination with preheat treatment of milk	Singh and Tokley (1990)
Oxidizing agents, 5 mM KBrO ₄ , KIO ₃ , ioadosobezoate	Milk, serum protein-free casein micelles	Modification/oxidation of the charge of amino acid residues	Changes in the HCT-pH profile, overall destabilizing effect	Singh and Fox (1987b)
N-ethyl maleimide, β-mercaptoethanol, dithiothreitol, cysteine		Blockage of thiol-disulphide exchange reaction during heating, reduction/cleavage of inter- and intramolecular disulphide bridges	Enhanced dissociation of κ-casein and reduction of the heat stability of milk	Singh and Fox (1987b)
Transglutaminase	Unconcentrated and concentrated skim milk	Investigation of the HCT-pH profile depending on protein crosslinking in relation to κ-casein dissociation	Reduction of the dissociation of caseins from casein micelles, mainly κ-casein by enzymatic crosslinking; improvement of HCT-pH dependent	Huppertz (2014) O'Sullivan et al. (2002) O'Sullivan et al. (2001)
Formaldehyde and dialdehydes	Skim milk and concentrated skim milk	Effects of chemical crosslinking on the shape of the HCT-pH profile	Increase heat stability, especially at pH >6.7 due to reduced dissociation of κ-casein; minimum in the HCT-pH-profile diminishes	Holt et al. (1978a) Singh and Fox (1985b) Muir et al. (1979)
κ-Casein addition and hydrolysis of κ-casein by chymosin	Whole milk	Investigation of the effect of κ-casein hydrolysis by chymosin on heat coagulation time and the HCT-pH profile	Stabilization of milk by κ-casein and destabilization of casein micelles by renneting toward heating	Fox and Hearn (1978c) Tessier and Rose (1964)
β-Lactoglobulin (addition and removal)	Wole milk, serum protein-free casein micelles, concentrated milk	Investigation of the effect of κ-casein and β-lactoglobulin on heat coagulation time and the HCT-pH profile	Serum protein removal and κ-casein addition results in a conversion of a type A into a type B HCT-pH profile	Tessier and Rose (1964) Singh and Fox (1987c) Pearce (1979)
Ethanol	Not specified	Demonstration of the similarity of the sigmoidal curves obtained for ethanol stability with heat coagulation temperatures (at constant holding time; Miller and Sommer, 1940)	Reduction of the heat stability with increasing ethanol concentration	Horne and Muir (1990) O'Connell et al. (2001)
Removal and addition of urea (5–10 mM)	Skim milk Concentrated skim milk	Demonstrate the effect of urea on the HCT-pH profile and find mechanistic explanations	Increase in the dissociation of protein from the micelles upon heating and increase in the heat stability of milk (buffering effect of NH ₄ ⁺ formed upon urea decomposition); no effect on concentrated milk	Dalgleish et al. (1987b) Metwalli et al. (1996) Metwalli and van Boekel (1996) Muir and Sweetsur (1976) Muir et al. (1979)
Polyphenols from coffee, tea, oak bark	Skim milk Concentrated milk	Assess the heat stability of milk as these constituents might enhance the nutritional value and shelf life of milk	Improvement of the heat stability by slight reduction in calcium activity and derivatization of κ-casein to inhibit its dissociation	O'Connell and Fox (1999a) O'Connell and Fox (1999b)

¹HCT = heat coagulation time.

ance of the final products, as each processing step (i.e., preheat treatment, stabilizer addition, homogenization, and sterilization) affects the results of the following step(s) and the stability of the concentrated milk.

Preheat Treatment of Milk

Forewarming or preheating of unconcentrated milk was found to increase the heat stability of concentrated milk prepared therefrom and is an integral part of concentrated milk processing before sterilization. The increase in heat stability by preheating can be determined by the subjective heat stability test. As shown in Figure 6, heat stability was found to be sufficient for in-container sterilization (usually 115–120°C for 12–20 min) at the optimum (native) pH after preheat treatment of unconcentrated milk. Preheating conditions of milk vary widely and were often directed toward a complete denaturation of whey proteins to avoid pseudo-coagulation by crosslinking of casein micelles by denatured whey proteins. The reduction of the amount of reactive whey proteins that could participate in the coagulation of casein micelles in concentrated milk and the coverage of casein micelles with denatured whey proteins were assumed to reduce the rate of heat-induced aggregation during sterilization at pH <6.7 (Tessier and Rose, 1964). Preheating of milk also results in a shift of the maximum of the HCT-pH profile to the acidic side (pH <6.6; Rose, 1962). Due to the seasonal (i.e., compositional) variation in the HCT-pH profile, the effectiveness of the preheat treatment was found to vary (Singh and Tokley, 1990). Usual preheating conditions for milk used vary widely and are more of an empirical nature than directed due to a full understanding of the underlying mechanisms. Heating conditions resulting in a notable increase in heat stability are 90°C for 10 min, 110 to 120°C for 1 to 5 min, 140°C for 5 s, or 130 to 150°C for 1 to 5 min (Webb et al., 1943; Newstead et al., 1979; Singh and Tokley, 1990; Fox et al., 2015). Concentration of milk by evaporation at 55 to 85°C led to a shift in the heat stability maximum to a more acidic pH, due to possible heat-induced changes in milk depending on residence time and temperatures of the milk in the evaporator compared with concentration of milk by RO (Dumpler and Kulozik, 2015). All these preheating conditions were tested in the context of the subjective heat stability test and in-container sterilization. Very little is known about the effectiveness of preheating in terms of an increase in the heat stability of heated concentrated milk toward continuous UHT heat treatment. Quantitative data for the increased temperature-time combinations possible without coagulation of concentrated

milk depending on total solids content were based on analysis of the viscosity, particle size, or the amount of sediment in the container of the concentrates after heat treatment (Kieseker, 1988; Smith and Malmgren, 1999). Dumpler and Kulozik (2016) used heat treatment of concentrated skim milk by direct steam injection to investigate the quantitative effect of preheating the milk. The amount of sedimentable protein depending on temperature-time combinations with and without preheat treatment of the milk was determined. UHT preheat treatment of skim milk before concentration by RO increased the heat stability of 26% total solids concentrate by 8°C at a constant holding time and a maximum of 2% sedimentable protein of total protein. The corresponding 3-fold to 5-fold increase in the possible holding time at the same temperature is in the range of what has been found for in-container sterilization at the pH of maximum stability (Figure 6). The additional microbial inactivation that can be achieved by increasing the heat stability to that extent by UHT preheating is considerable and might enable the complete inactivation of all pathogens in milk concentrates which are relevant for subsequently spray dried milk powders. The combination of UHT preheating (i.e., the sterilization of the milk) with a high-heat treatment in between evaporator effects might be suitable to achieve very high-quality milk concentrates for liquid concentrates or powders.

Figure 6. Examples for HCT versus pH profiles for concentrated milk at 18 to 20% nonfat total solids heated at 120°C prepared from either preheated (e.g., 110°C–120°C for 120–180 s) or nonpreheated milk (Singh and Tokley, 1990). Data were extracted from Singh and Creamer (1992) using the digitizer of OriginPro 2017G (OriginLab Corp., Northampton, MA).

Concentration by Evaporation

After preheating, milk is usually concentrated by evaporation or RO. Little quantitative data exist on the decrease in HCT with increasing heating temperature and total solids content for concentrates from preheated and nonpreheated milk. However, it was stated by Walstra and Jenness (1984) that milk concentrated to >40% total solids coagulates at temperatures of <100°C, which results in gel formation rather than formation of distinct particles. The prediction of the heat stability of a concentrated milk samples after determination of the heat stability of the unconcentrated milk was stated to be insufficient for predictions (Singh, 2004), although early studies indicated a linear relationship between the total solids content of the concentrate and the HCT (Webb and Bell, 1943; Webb et al., 1943). This might be partly due to standardization of the heat stability tests for unconcentrated (140°C) and concentrated milk (120°C) and the total solids standards for evaporated milk. In addition, the evaporation process at elevated temperature affects the pH sensitivity of concentrated milk compared with unconcentrated milk, which might result in the denaturation of whey proteins and changes in the milk salt equilibrium during evaporation. Concentration of milk by RO might be a technique to avoid these changes. When we argue that the only difference in composition of milk and concentrated milk is the water content, there should be a detectable correlation and a continuum, both, for the decrease in heat stability with increasing total solids content in terms of possible heating times and temperature as shown by (Dumpler and Kulozik, 2015, 2016; Dumpler et al., 2018). Factors contributing to heat-induced coagulation, such as increased soluble serum calcium, whey protein, and a reduction in pH are continuously changing and the collision of casein micelles by temperature-dependent Brownian motion becomes more likely as the average distance between micelles is reduced. With increasing concentration of milk solids, both structural changes affecting the colloidal stability of the micelles (loosening of the micelle structure and dissociation) and the aggregation of the micelles due to unfavorable serum conditions occur at an increased rate.

Concentration by Membrane Technology

Concentration of milk by porous membranes such as ultra- or microfiltration results in higher heat stability of the milk protein concentrate when the same factor of concentration is compared with milk concentrated by RO or evaporation. This is due to several reasons: (1) a reduced concentration of soluble salts, especially

soluble calcium, as the serum composition of milk concentrated by porous membranes is similar to unconcentrated milk; (2) a pH that is closer to the pH of the maximum stability of milk; and (3) a reduced amount of whey proteins when microfiltration is applied, which increases the heat stability at pH >6.7 and results in a HCT-pH profile of type B milk. Ultrafiltration of milk in diafiltration mode using deionized water further increased the HCT of the final concentrate possibly due to an increase in pH and reduced ionic strength (Sweetsur and Muir, 1980; Muir and Sweetsur, 1984). However, later studies found that demineralization of milk by excessive diafiltration can lead to changes in casein micelle structure depending on the diafiltration medium (Alexander et al., 2011; Reitmaier et al., 2020), moderate to very poor heat stability of milk protein concentrates (Crowley et al., 2014, 2015a), and changes in the functional properties of the reconstituted powders (Crowley et al., 2015b, 2018). The need for a heat treatment to obtain high-quality milk protein powders or after reconstitution for high milk protein beverages has led to several studies using the subjective heat stability test for the characterization of milk protein concentrates. Diafiltration media that maintain the serum composition and pH of milk, such as simulated milk ultrafiltrate without lactose, could lead to optimal heat stability of the concentrate and further insights into heat-induced changes in whey protein-free milk systems with modified serum composition (Dumpler et al., 2017).

Milk Fat and Homogenization

Milk fat or lipid droplets in the unhomogenized state have little effect on the heat stability of milk, as indicated by the similar coagulation times of unconcentrated skim milk and whole milk. Addition of unhomogenized milk fat globules to concentrated milk only reduces the volume available for casein micelles and milk salts at the same total nonfat milk solids level. This is why it's important to compare heat stability results based on the true nonfat milk solids in the aqueous phase (Dumpler, 2018). Homogenization of skim milk in the usual range for milk (≤ 35 MPa) had little effect on the heat stability of concentrated skim milk (Sweetsur and Muir, 1983). Much higher pressures as used for ultra-high-pressure homogenization (>180 MPa) are required to affect the heat stability of skim milk (Sandra and Dalgleish, 2005). Homogenization of milk entails the disruption of fat globules from around 2 to 10 μm to a size less than 2 μm and the subsequent adsorption of casein micelles and denatured whey proteins on the newly formed oil-water interface. This secondary layer

inhibits the aggregation, coalescence, and creaming of fat globules during storage. From these observations, we can conclude that the interaction of casein micelles with fat globules at the oil-water interface results in structural rearrangements that render the casein micelles more susceptible for heat-induced coagulation. Homogenization after the final heat treatment of concentrated milk containing milk fat, is usually preferred for evaporated milk manufacture by the UHT process. This is because homogenization of milk before concentrating or homogenization of the concentrate results in a significant decrease in heat stability of the final concentrates. It was suggested that the protein layer of caseins and whey proteins on the surface of the newly formed fat droplets were responsible for a reduction in heat stability. The destabilizing effect of homogenization of milk or the concentrate increases with increasing homogenization pressure and temperature (i.e., with increasing fat globule surface area at the natural pH of milk; Sweetsur and Muir, 1983; Muir, 1984; McCrae et al., 1994; Hinrichs et al., 1998). Using UHT heat treatment in combination with aseptic homogenization, the conflict between the creaming of the concentrate during storage and the heat stability of concentrate can be avoided as homogenization is performed after UHT sterilization. When in-container sterilization of the concentrated milk is used for preservation, homogenization needs to be performed before sterilization, at best before preheat treatment and concentration (Whiteley and Muir, 1996). Optimal homogenization conditions should not only prevent creaming during storage but should also keep the concentrate sufficiently heat stable for sterilization. The opposing effects of homogenization in relation to heat stability and creaming make the optimization of homogenization conditions challenging, especially considering additional effects such as seasonal variations and stabilizer addition. If too low homogenization pressures are used to keep the concentrate sufficiently heat stable, creaming and cream plug formation will occur within the containers. If homogenization conditions destabilize the concentrate, immediate gel or sediment formation during storage might be observed.

Protein Hydrolysis and Crosslinking of Proteins

The role of κ -casein for the stabilization of casein micelles against spontaneous or heat-induced aggregation was demonstrated by studies investigating the effect of enzymatic hydrolysis of κ -casein by renneting of milk or the addition of κ -casein to milk on the heat stability at different pH. However, it was observed that renneting for 20 min at 30°C was necessary to significantly reduce

the heat stability of milk (Fox and Hearn, 1978c). Tessier and Rose (1964) found that the addition of κ -casein resulted in an increase in heat stability over the entire pH range investigated. No correlation has been found between the degree of glycosylation of κ -casein and heat stability (Robitaille and Ayers, 1995).

Nonenzymatic covalent crosslinking of proteins results in lysinoalanine (from serine and lysine), lanthionine, histidinoalanine, and other unidentified reaction products. The presence of lactose increases the rate of nonenzymatic crosslinking due to Maillard reactions (Zin El-Din and Aoki, 1993; Henle et al., 1996; van Boekel, 1998). The kinetics of covalent crosslinking of milk proteins are known for whey protein-free micellar casein and the implication of crosslinking on heat stability was discussed (Bulca et al., 2016). A relationship between lactose-induced crosslinking of caseins and heat-induced aggregation has been suggested to contribute to heat-induced coagulation upon prolonged heating (van Boekel et al., 1989a) but might be less relevant for HTST processing (Dumpler and Kulozik, 2016). Because very close and prolonged contact between micelles is necessary for covalent crosslinking, it is likely to be a secondary effect but not a cause for heat-induced coagulation of concentrated and unconcentrated milk. Treatment of milk with transglutaminase (TGase) resulted in the conversion of a type A heat stability profile into a type B milk profile and progressively increases the heat stability of reconstituted skim milk and skim milk concentrate (22.5% total solids) on the alkaline side of the HCT-pH profile (pH > 6.6) (O'Sullivan et al., 2001). A preheat treatment of the milk before incubation with TGase to denature whey proteins further enhanced the heat stability of milk (O'Sullivan et al., 2002). Huppertz (2014) found similar trends and could show that the heat stability increases with increasing degree of crosslinking, i.e., increasing incubation time at pH 6.9. However, a crosslinking degree above 80% was necessary to increase the heat stability of skim milk at its natural pH as it was reduced by TGase treatment at lower degrees of crosslinking (Huppertz, 2014). This was attributed to a higher susceptibility of the micelles to heat-induced acidification. Both studies found the heat stability of concentrated milk (18/22.5% total solids) at or below its natural pH could not be improved by TGase treatment, but greatly improved by adjustment to pH >6.8.

High Hydrostatic Pressure

The high hydrostatic pressure processing (HPP) induced dissociation of the casein micelle structure has been studied to some extent. Reversible and irrevers-

ible structural changes have been characterized. It was found that pressures >280 MPa lead to an irreversible dissociation of the casein micelles, leading to a reduced light scattering of the milk due to a reduction in particle size whereby changes occur gradually with increasing pressure. Pressures in the range of 100 to 250 MPa lead to a reversible dissociation of the casein micelles in milk. Pressures below 50 MPa led to no noticeable changes in skim milk (Gebhardt et al., 2006, 2011). However, little is known about the effects of high hydrostatic pressure-induced changes on the heat stability of milk and concentrated milk. Huppertz et al. (2004) found that HPP at 250 MPa and 600 MPa for 30 min reduced the heat stability of milk and concentrated milk at pH <6.9, which indicates that reassembled micelles are less stable than native micelles. Heat stability was increased at higher pH both for unconcentrated and concentrated milk by HPP. A preheat treatment of skim milk before HPP reduced its effectiveness to increase the heat stability at pH >6.9. Serum protein-free milk showed no measurable changes in heat stability upon HPP treatment. The results indicate that the rapid heat stability testing can be a valuable tool to investigate HPP-induced changes in milk, as the effects of various constituents of milk on the HCT-pH profile are well understood.

CONCLUSIONS AND FUTURE PERSPECTIVES

Numerous studies have investigated the effects of composition and pretreatment of milk and concentrated milk and its effect on the HCT-pH profile. The motivation for these studies originated from the fact that poor quality raw milk showed a low pH (pH <6.6), preheat treatment shifts the optimal pH to a more acidic pH, and the addition of citrates and phosphates as stabilizers changes the pH. Studies from the 1960s to the 1980s aimed at attributing the shape of the HCT-pH profile to individual milk constituents and the factors affecting the shape of the profile are largely understood. Heat stability testing measures a certain level of coagulation, neglecting the course of the entire coagulation process. To classify the subjective heat stability test, we need to perform a short experiment of thought. If we would transfer this approach to microbial inactivation, we would use a fixed temperature and investigate the pH and other serum composition at which a 5-log reduction is or is not obtained depending on serum composition. We would need to precisely define these conditions and allow for no other than the ones specified, especially the required heating time. The poor predictive power of this case-by-case approach for the transfer to other equipment than a batch system becomes evident, partly

explaining the vast amount of literature on HCT-pH profiles. It appears obvious that the investigation of the kinetics of microbial inactivation, the changes in kinetics depending on environmental conditions and food material, and the prediction of inactivation effects based on modeling have been more successful and have become the standard to ensure food safety while maximizing product quality. A miniaturized approach of Miller and Sommer (1940) and Dumpler and Kulozik (2016) using continuous heating, possibly a micro process engineering approach, might be a valuable tool to achieve further insights into the kinetics of heat-induced structural changes in milk. Ultra-short-time pasteurization and sterilization (150–180°C for 1–0.01 s) might be capable to reduce heat-induced changes on casein micelles and subsequent aggregation, especially under unfavorable serum conditions as found in milk concentrates to extend their shelf life. Future directions also point at the use of advanced heat stability testing to predict the behavior of concentrated milk obtained by membrane filtration with different protein profiles and serum composition under continuous heating conditions. These concentrates gain increasing attention from industry and research as a liquid concentrate but are mainly spray dried to powder for export markets. Considering the increasing importance of these concentrates and the decline of evaporated milk market share, a differentiation between the heat-induced coagulation of unconcentrated and concentrated (evaporated milk) milk, associated mechanisms, and effects appears no longer advisable, especially due to the difficulties to assign concentrates obtained by membrane filtration to these categories. A kinetic heating temperature-time-composition continuum might be an appropriate approach to determine critical processing limits for any kind of concentrate.

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REFERENCES

- Alexander, M., M.-P. Nieh, M. A. Ferrer, and M. Corredig. 2011. Changes in the calcium cluster distribution of ultrafiltered and diafiltered fresh skim milk as observed by Small Angle Neutron Scattering. *J. Dairy Res.* 78:349–356. <https://doi.org/10.1017/S0022029911000409>.
- Anema, S. G. 1998. Effect of milk concentration on heat-induced, pH-dependent dissociation of casein from micelles in reconstituted skim milk at temperatures between 20 and 120°C. *J. Agric. Food Chem.* 46:2299–2305. <https://doi.org/10.1021/jf970909+>.
- Anema, S. G. 2009. Effect of milk solids concentration on the pH, soluble calcium and soluble phosphate levels of milk during heating. *Dairy Sci. Technol.* 89:501–510. <https://doi.org/10.1051/dst/2009026>.
- Anema, S. G., and H. Klostermeyer. 1997. Heat-induced, pH-dependent dissociation of casein micelles on heating reconstituted skim milk at temperatures below 100 °C. *J. Agric. Food Chem.* 45:1108–1115. <https://doi.org/10.1021/jf960507m>.
- Anema, S. G., and Y. Li. 2000. Further studies on the heat-induced, pH-dependent dissociation of casein from the micelles in reconstituted skim milk. *Lebensm. Wiss. Technol.* 33:335–343. <https://doi.org/10.1006/fstl.2000.0665>.
- Anema, S. G., and Y. Li. 2003. Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *J. Dairy Res.* 70:73–83.
- Augustin, M. A., and P. T. Clarke. 1990. Effects of added salts on the heat stability of recombined concentrated milk. *J. Dairy Res.* 57:213–226. <https://doi.org/10.1017/S0022029900026820>.
- Belec, J., and R. Jenness. 1962. Dephosphorization of casein by heat treatment. II. In skimmilks. *J. Dairy Sci.* 45:20–26.
- Bell, R. W., and B. H. Webb. 1943. Relationships between high temperature forewarming and the color and heat stability of evaporated milks of different solids content. *J. Dairy Sci.* 26:579–585. [https://doi.org/10.3168/jds.S0022-0302\(43\)92757-2](https://doi.org/10.3168/jds.S0022-0302(43)92757-2).
- Boumpa, T., A. Tsioulpas, A. S. Grandison, and M. J. Lewis. 2008. Effects of phosphates and citrates on sediment formation in UHT goats' milk. *J. Dairy Res.* 75:160–166. <https://doi.org/10.1017/S0022029908003166>.
- Bulca, S., J. Dumpler, and U. Kulozik. 2016. Kinetic description of heat-induced cross-linking reactions of whey protein-free casein solutions. *Int. J. Dairy Technol.* 69:489–496.
- Carroll, R. J., M. P. Thompson, and P. Melnychyn. 1971. Gelation of concentrated skimmilk: Electron microscopic study. *J. Dairy Sci.* 54:1245–1252. [https://doi.org/10.3168/jds.S0022-0302\(71\)86015-0](https://doi.org/10.3168/jds.S0022-0302(71)86015-0).
- Chen, B., A. S. Grandison, and M. J. Lewis. 2015. Effect of seasonal variation on some physical properties and heat stability of milk subjected to ultra-high temperature and in-container sterilisation. *Food Chem.* 181:227–234. <https://doi.org/10.1016/j.foodchem.2015.02.072>.
- Chen, B. Y., F. Y. Ren, A. S. Grandison, and M. J. Lewis. 2019. Comparison of heat stability of cow's milk subjected to ultra-high temperature and in-container sterilisation. *Int. J. Dairy Technol.* 72:364–372. <https://doi.org/10.1111/1471-0307.12609>.
- Claeys, W. L., A. M. Van Loey, and M. E. Hendrickx. 2003. Kinetics of hydroxymethylfurfural, lactulose and furosine formation in milk with different fat content. *J. Dairy Res.* 70:85–90. <https://doi.org/10.1017/S0022029902005897>.
- Cole, W. C., and N. P. Tarassuk. 1946. Heat coagulation of milk. *J. Dairy Sci.* 29:421–429.
- Corredig, M., P. K. Nair, Y. Li, H. Eshpari, and Z. Zhao. 2019. Invited review: Understanding the behavior of caseins in milk concentrates. *J. Dairy Sci.* 102:4772–4782. <https://doi.org/10.3168/jds.2018-15943>.
- Crowley, S. V., M. Boudin, B. Chen, I. Gazi, T. Huppertz, A. L. Kelly, and J. A. O'Mahony. 2015a. Stability of milk protein concentrate suspensions to in-container sterilisation heating conditions. *Int. Dairy J.* 50:45–49. <https://doi.org/10.1016/j.idairyj.2015.05.009>.
- Crowley, S. V., E. Burlot, J. V. C. Silva, N. A. McCarthy, H. B. Wijayanti, M. A. Fenelon, A. L. Kelly, and J. A. O'Mahony. 2018. Rehydration behaviour of spray-dried micellar casein concentrates produced using microfiltration of skim milk at cold or warm temperatures. *Int. Dairy J.* 81:72–79. <https://doi.org/10.1016/j.idairyj.2018.01.005>.
- Crowley, S. V., B. Desautel, I. Gazi, A. L. Kelly, T. Huppertz, and J. A. O'Mahony. 2015b. Rehydration characteristics of milk protein concentrate powders. *J. Food Eng.* 149:105–113. <https://doi.org/10.1016/j.jfoodeng.2014.09.033>.
- Crowley, S. V., M. Megemont, I. Gazi, A. L. Kelly, T. Huppertz, and J. A. O'Mahony. 2014. Heat stability of reconstituted milk protein concentrate powders. *Int. Dairy J.* 37:104–110. <https://doi.org/10.1016/j.idairyj.2014.03.005>.
- Dalgleish, D. G., Y. Pouliot, and P. Paquin. 1987a. Studies on the heat stability of milk: I. Behaviour of divalent cations and phosphate in milks heated in a stainless steel system. *J. Dairy Res.* 54:29–37. <https://doi.org/10.1017/S0022029900025176>.
- Dalgleish, D. G., Y. Pouliot, and P. Paquin. 1987b. Studies on the heat stability of milk: II. Association and dissociation of particles and the effect of added urea. *J. Dairy Res.* 54:39–49. <https://doi.org/10.1017/S0022029900025188>.
- Dannenbergh, F., and H.-G. Kessler. 1988. Reaction kinetics of the denaturation of whey proteins in milk. *J. Food Sci.* 53:258–263. <https://doi.org/10.1111/j.1365-2621.1988.tb10223.x>.
- Davies, D. T., and J. C. D. White. 1966a. The stability of milk protein to heat: I. Subjective measurement of heat stability of milk. *J. Dairy Res.* 33:67–81. <https://doi.org/10.1017/S0022029900011730>.
- Davies, D. T., and J. C. D. White. 1966b. The stability of milk protein to heat: II. Effect on heat stability of ageing milk at different temperatures. *J. Dairy Res.* 33:83–91. <https://doi.org/10.1017/S0022029900011742>.
- de Kort, E., M. Minor, T. Snoeren, T. van Hooijdonk, and E. van der Linden. 2012. Effect of calcium chelators on heat coagulation and heat-induced changes of concentrated micellar casein solutions: The role of calcium-ion activity and micellar integrity. *Int. Dairy J.* 26:112–119. <https://doi.org/10.1016/j.idairyj.2012.03.014>.
- de Wit, J. N., G. Klarenbeek, and C. de Graaf. 1986. The Klarograph: a new instrument for measuring the viscosity, density and heat stability of milk and milk concentrates at temperatures up to 140°C. *Voedingsmiddelentechnologie* 19:25–27.
- Dumpler, J. 2018. Heat Stability of Concentrated Milk Systems: Kinetics of the Dissociation and Aggregation in High Heated Concentrated Milk Systems. Springer Spektrum, Wiesbaden, Germany.
- Dumpler, J., I. Kieferle, H. Wohlschläger, and U. Kulozik. 2017. Milk ultrafiltrate analysis by ion chromatography and calcium activity for SMUF preparation for different scientific purposes and prediction of its supersaturation. *Int. Dairy J.* 68:60–69. <https://doi.org/10.1016/j.idairyj.2016.12.009>.
- Dumpler, J., and U. Kulozik. 2015. Heat stability of concentrated skim milk as a function of heating time and temperature on a laboratory scale – Improved methodology and kinetic relationship. *Int. Dairy J.* 49:111–117. <https://doi.org/10.1016/j.idairyj.2015.05.005>.
- Dumpler, J., and U. Kulozik. 2016. Heat-induced coagulation of concentrated skim milk heated by direct steam injection. *Int. Dairy J.* 59:62–71. <https://doi.org/10.1016/j.idairyj.2016.03.009>.
- Dumpler, J., F. Peraus, V. Depping, B. Stefánsdóttir, M. Grunow, and U. Kulozik. 2018. Modelling of heat stability and heat-induced aggregation of casein micelles in concentrated skim milk using a Weibullian model. *Int. J. Dairy Technol.* 71:601–612. <https://doi.org/10.1111/1471-0307.12501>.
- Dumpler, J., H. Wohlschläger, and U. Kulozik. 2017. Dissociation and coagulation of caseins and whey proteins in concentrated skim milk heated by direct steam injection. *Dairy Sci. Technol.* 96:807–826. <https://doi.org/10.1007/s13594-016-0304-3>.
- Faka, M., M. J. Lewis, A. S. Grandison, and H. Deeth. 2009. The effect of free Ca²⁺ on the heat stability and other characteristics of low-heat skim milk powder. *Int. Dairy J.* 19:386–392. <https://doi.org/10.1016/j.idairyj.2008.12.006>.

- Farrell, G. C., and R. G. Allgood. 1970. Notes on the method for determining the heat stability of skim milk powder. *Aust. J. Dairy Technol.* 25:121–122.
- Fink, R., and H.-G. Kessler. 1986. HMF values in heat treated and stored milk. *Milchwissenschaft* 41:638–641.
- Foissy, H., and W. Kneifel. 1984. An automatic method for measuring the heat coagulation time of milk powder solutions. *J. Dairy Res.* 51:325–329. <https://doi.org/10.1017/S0022029900023591>.
- Fox, P. F. 1981. Heat-induced changes in milk preceding coagulation. *J. Dairy Sci.* 64:2127–2137. [https://doi.org/10.3168/jds.S0022-0302\(81\)82819-6](https://doi.org/10.3168/jds.S0022-0302(81)82819-6).
- Fox, P. F., and C. M. Hearn. 1978a. Heat stability of milk: Influence of denaturable proteins and detergents on pH sensitivity. *J. Dairy Res.* 45:159–172. <https://doi.org/10.1017/S0022029900016332>.
- Fox, P. F., and C. M. Hearn. 1978b. Heat stability of milk: Influence of dilution and dialysis against water. *J. Dairy Res.* 45:149–157. <https://doi.org/10.1017/S0022029900016320>.
- Fox, P. F., and C. M. Hearn. 1978c. Heat stability of milk: Influence of κ -casein hydrolysis. *J. Dairy Res.* 45:173–181. <https://doi.org/10.1017/S0022029900016344>.
- Fox, P. F., and M. C. T. Hoynes. 1975. Heat stability of milk: Influence of colloidal calcium phosphate and β -lactoglobulin. *J. Dairy Res.* 42:427–435. <https://doi.org/10.1017/S0022029900015466>.
- Fox, P. F., T. Uniacke-Lowe, P. L. H. McSweeney, and J. A. O'Mahony. 2015. Heat-induced changes in milk. Pages 345–375 in *Dairy Chemistry and Biochemistry*. Springer International Publishing, Cham, Switzerland.
- Frèche, M., and J. C. Heughebaert. 1989. Calcium phosphate precipitation in the 60–80°C range. *J. Cryst. Growth* 94:947–954. [https://doi.org/10.1016/0022-0248\(89\)90128-0](https://doi.org/10.1016/0022-0248(89)90128-0).
- Gaucheron, F. 2005. The minerals of milk. *Reprod. Nutr. Dev.* 45:473–483. <https://doi.org/10.1051/rnd:2005030>.
- Gebhardt, R., W. Doster, J. Friedrich, and U. Kulozik. 2006. Size distribution of pressure-decomposed casein micelles studied by dynamic light scattering and AFM. *Eur. Biophys. J.* 35:503–509. <https://doi.org/10.1007/s00249-006-0058-6>.
- Gebhardt, R., N. Takeda, U. Kulozik, and W. Doster. 2011. Structure and stabilizing interactions of casein micelles probed by high-pressure light scattering and FTIR. *J. Phys. Chem. B* 115:2349–2359. <https://doi.org/10.1021/jp107622d>.
- Grewal, M. K., J. Chandrapala, O. Donkor, V. Apostolopoulos, and T. Vasiljevic. 2017. Predicting sediment formation in ultra high temperature-treated whole and skim milk using attenuated total reflectance-Fourier transform infrared spectroscopy. *Int. Dairy J.* 74:39–48. <https://doi.org/10.1016/j.idairyj.2017.01.004>.
- Hardy, E. E., A. W. M. Sweetsur, I. G. West, and D. D. Muir. 1985. Heat stability of concentrated milk: Enhancement of initial heat stability by incorporation of food grade lecithin. *Int. J. Food Sci. Technol.* 20:97–105. <https://doi.org/10.1111/j.1365-2621.1985.tb01908.x>.
- Henle, T., U. P. Schwarzenbolz, and H. Klostermeyer. 1996. Irreversible crosslinking of casein during storage of UHT-treated skim milk. Pages 290–298 in *Heat Treatments & Alternative Methods*. International Dairy Federation, Brussels, Belgium.
- Hinrichs, J., C. Boheim, and H.-G. Kessler. 1998. UHT-Erhitzen von Nanofiltrations-Milchkonzentraten. *Deutsche Milchwirtschaft* 49:307–309.
- Holm, G. E., E. F. Deysher, and F. R. Evans. 1923. The relationships of concentration and time to the temperature of coagulation of evaporated skim and whole milk. *J. Dairy Sci.* 6:556–568. [https://doi.org/10.3168/jds.S0022-0302\(23\)94119-6](https://doi.org/10.3168/jds.S0022-0302(23)94119-6).
- Holm, G. E., B. H. Webb, and E. F. Deysher. 1932. The heat coagulation of milk: 1.—Variations in the compositions, heat stability, and other tests of milks from cows during the course of a lactation period. *J. Dairy Sci.* 15:331–343. [https://doi.org/10.3168/jds.S0022-0302\(32\)93421-3](https://doi.org/10.3168/jds.S0022-0302(32)93421-3).
- Holt, C. 1995. Effect of heating and cooling on the milk salts and their interaction with casein. Heat-Induced Changes in Milk – International Dairy Federation Special Issue 9501, 2nd ed. P. F. Fox, ed. International Dairy Federation, Brussels, Belgium.
- Holt, C., J. A. Carver, H. Ecroyd, and D. C. Thorn. 2013. Invited review: Caseins and the casein micelle: Their biological functions, structures, and behavior in foods. *J. Dairy Sci.* 96:6127–6146. <https://doi.org/10.3168/jds.2013-6831>.
- Holt, C., and D. S. Horne. 1996. The hairy casein micelle: Evolution of the concept and its implications for dairy technology. *Neth. Milk Dairy J.* 50:85–111.
- Holt, C., D. D. Muir, and A. W. M. Sweetsur. 1978a. The heat stability of milk and concentrated milk containing added aldehydes and sugars. *J. Dairy Res.* 45:47–52. <https://doi.org/10.1017/S0022029900016186>.
- Holt, C., D. D. Muir, and A. W. M. Sweetsur. 1978b. Seasonal changes in the heat stability of milk from creamery silos in south-west Scotland. *J. Dairy Res.* 45:183–190. <https://doi.org/10.1017/S0022029900016356>.
- Holt, C., and L. Sawyer. 1993. Caseins as rheomorphic proteins: Interpretation of primary and secondary structures of the α_{S1} -, β - and κ -caseins. *J. Chem. Soc. Faraday Trans.* 89:2683–2692. <https://doi.org/10.1039/FT9938902683>.
- Horne, D. S., and D. D. Muir. 1990. Alcohol and heat stability of milk protein. *J. Dairy Sci.* 73:3613–3626. [https://doi.org/10.3168/jds.S0022-0302\(90\)79064-9](https://doi.org/10.3168/jds.S0022-0302(90)79064-9).
- Hostettler, H., J. Stein, and K. Imhof. 1968. Ueber die Nachdickung und Gelierung UHT-sterilisierter ungezuckerter Milchkonzentrate bei der Lagerung. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene 59:60–77.
- Huppertz, T. 2013. Chemistry of the caseins. Pages 135–160 in *Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects*, 4th edition. P. L. H. McSweeney and P. F. Fox, ed. Springer US, Boston, MA.
- Huppertz, T. 2014. Heat stability of transglutaminase-treated milk. *Int. Dairy J.* 38:183–186. <https://doi.org/10.1016/j.idairyj.2013.10.014>.
- Huppertz, T. 2016. Heat stability of milk. Pages 179–196 in *Advanced Dairy Chemistry: Volume 1B: Proteins: Applied Aspects*. P. L. H. McSweeney and J. A. O'Mahony, ed. Springer New York, New York, NY.
- Huppertz, T., S. Grosman, P. F. Fox, and A. L. Kelly. 2004. Heat and ethanol stabilities of high-pressure-treated bovine milk. *Int. Dairy J.* 14:125–133. [https://doi.org/10.1016/S0958-6946\(03\)00170-5](https://doi.org/10.1016/S0958-6946(03)00170-5).
- Kelly, P. M., and S. P. F. M. Roefs. 1990. Comparison of a klarograph and oil-bath methods for the measurement of the heat stability of reconstituted milks. *Int. J. Food Sci. Technol.* 14:132.
- Kessler, H. G. 1975. Technologischer Stand der H-Milch-Herstellung. *Deutsche Molkerei-Zeitung* 50:1646–1650.
- Kessler, H.-G., and P. Horak. 1981. Objective evaluation of UHT-milk-heating by standardization of bacteriological and chemical effects. *Milchwissenschaft* 36:129–133.
- Kieseker, F. G., and B. Aitken. 1988. An objective method for determination of heat stability of milk powders. *Aust. J. Dairy Technol.* 43:26–31.
- Kneifel, W., H. Foissy, and H. Goller. 1987. Comparison of the An Bord Baine method, the Australian Standard method and an automatic procedure for determining the heat-coagulation time of 20% reconstituted milk. *Int. J. Dairy Technol.* 40:42–45. <https://doi.org/10.1111/j.1471-0307.1987.tb02834.x>.
- Lehmann, L., and V. Buckin. 2005. Determination of the heat stability profiles of concentrated milk and milk ingredients using high resolution ultrasonic spectroscopy. *J. Dairy Sci.* 88:3121–3129. [https://doi.org/10.3168/jds.S0022-0302\(05\)72994-5](https://doi.org/10.3168/jds.S0022-0302(05)72994-5).
- Lenton, S., T. Nylander, S. C. Teixeira, and C. Holt. 2015. A review of the biology of calcium phosphate sequestration with special reference to milk. *Dairy Sci. Technol.* 95:3–14. <https://doi.org/10.1007/s13594-014-0177-2>.
- Lewis, M. J., A. Grandison, M.-J. Lin, and A. Tsioulpas. 2011. Ionic calcium and pH as predictors of stability of milk to UHT processing. *Milchwissenschaft* 66:197–200.
- Lin, Y., A. L. Kelly, J. A. O'Mahony, and T. P. Guinee. 2016. Fortification of milk protein content with different dairy protein powders alters its compositional, rennet gelation, heat stability and ethanol

- stability characteristics. *Int. Dairy J.* 61:220–227. <https://doi.org/10.1016/j.idairyj.2016.06.010>.
- Markoska, T., T. Huppertz, M. K. Grewal, and T. Vasiljevic. 2019. Structural changes of milk proteins during heating of concentrated skim milk determined using FTIR. *Int. Dairy J.* 89:21–30. <https://doi.org/10.1016/j.idairyj.2018.08.010>.
- Mathys, A. 2018. Perspective of micro process engineering for thermal food treatment. *Front. Nutr.* 5:24. <https://doi.org/10.3389/fnut.2018.00024>.
- McCrae, C. H., D. Hirst, A. J. R. Law, and D. D. Muir. 1994. Heat stability of homogenized milk: Role of interfacial protein. *J. Dairy Res.* 61:507–516. <https://doi.org/10.1017/S0022029900028430>.
- Metwalli, A. A. M., N. H. Metwalli, and M. A. J. S. van Boekel. 1996. Effect of urea on heat-induced changes in milk. *Neth. Milk Dairy J.* 50:427–457.
- Metwalli, A. A. M., and M. A. J. S. van Boekel. 1996. Effect of urea on heat coagulation of milk. *Neth. Milk Dairy J.* 50:335–340.
- Miller, P. G., and H. H. Sommer. 1940. The coagulation temperature of milk as affected by pH, salts, evaporation and previous heat treatment. *J. Dairy Sci.* 23:405–421. [https://doi.org/10.3168/jds.S0022-0302\(40\)95542-4](https://doi.org/10.3168/jds.S0022-0302(40)95542-4).
- Morales, F.-J., C. Romero, and S. Jiménez-Pérez. 1995. New methodologies for kinetic study of 5-(Hydroxymethyl)-furfural formation and reactive lysine blockage in heat-treated milk and model systems. *J. Food Prot.* 58:310–315. <https://doi.org/10.4315/0362-028X-58.3.310>.
- Morrissey, P. A., and F. O'Mahony. 1976. Heat stability of forewarmed milks: Influence of κ -casein, serum proteins and divalent cations. *J. Dairy Res.* 43:267–274. <https://doi.org/10.1017/S002202990001582X>.
- Muir, D. D. 1984. UHT-sterilized milk concentrate: A review of practical methods of production. *Int. J. Dairy Technol.* 37:135–141. <https://doi.org/10.1111/j.1471-0307.1984.tb00520.x>.
- Muir, D. D. 1985. Heat stability of milk and concentrated milk. *Int. J. Biochem.* 17:291–299. [https://doi.org/10.1016/0020-711X\(85\)90203-4](https://doi.org/10.1016/0020-711X(85)90203-4).
- Muir, D. D., and A. W. M. Sweetsur. 1976. The influence of naturally occurring levels of urea on the heat stability of bulk milk. *J. Dairy Res.* 43:495–499. <https://doi.org/10.1017/S0022029900016083>.
- Muir, D. D., and A. W. M. Sweetsur. 1978. The effect of concentration on heat stability of skim-milk. *J. Dairy Res.* 45:37–45. <https://doi.org/10.1017/S0022029900016174>.
- Muir, D. D., and A. W. M. Sweetsur. 1984. Optimization of the heat stability of protein-rich concentrates prepared by ultrafiltration of skim-milk. *Int. J. Food Sci. Technol.* 19:263–271. <https://doi.org/10.1111/j.1365-2621.1984.tb00349.x>.
- Muir, D. D., A. W. M. Sweetsur, and C. Holt. 1979. The synergic effect of urea and aldehydes on the heat stability of concentrated skim-milk. *J. Dairy Res.* 46:381–384. <https://doi.org/10.1017/S0022029900017350>.
- Newstead, D. F., E. F. Conaghan, and A. J. Baldwin. 1979. Studies on the induction of heat stability in evaporated milk by preheating: Effects of milk concentration, homogenization and whey proteins. *J. Dairy Res.* 46:387–391. <https://doi.org/10.1017/S0022029900017374>.
- Newstead, D. F., W. B. Sanderson, and E. F. Conaghan. 1977. Effects of whey protein concentration and heat treatment on the heat stability of concentrated and unconcentrated milk. *N. Z. J. Dairy Sci. Technol.* 12:29–36.
- Nieuwenhuijse, J. A., A. Sjollem, M. A. J. S. van Boekel, T. van Vliet, and P. Walstra. 1991. The heat stability of concentrated skim milk. *Neth. Milk Dairy J.* 45:193–224.
- Nieuwenhuijse, J. A., W. Timmermans, and P. Walstra. 1988. Calcium and phosphate partitions during the manufacture of sterilized concentrated milk and their relations to the heat stability. *Neth. Milk Dairy J.* 42:387–421.
- Nieuwenhuijse, J. A., T. van Vliet, and P. Walstra. 1992. Kinetic aspects of the heat induced coagulation of concentrated skim milk. *Neth. Milk Dairy J.* 46:45–68.
- O'Connell, J. E., and P. F. Fox. 1999a. Effect of extracts of oak (*Quercus petraea*) bark, oak leaves, aloe vera (*Curacao aloe*), coconut shell and wine on the colloidal stability of milk and concentrated milk. *Food Chem.* 66:93–96. [https://doi.org/10.1016/S0308-8146\(98\)00248-9](https://doi.org/10.1016/S0308-8146(98)00248-9).
- O'Connell, J. E., and P. F. Fox. 1999b. Proposed mechanism for the effect of polyphenols on the heat stability of milk. *Int. Dairy J.* 9:523–536. [https://doi.org/10.1016/S0958-6946\(99\)00124-7](https://doi.org/10.1016/S0958-6946(99)00124-7).
- O'Connell, J. E., and P. F. Fox. 2000. The two-stage coagulation of milk proteins in the minimum of the heat coagulation time-pH profile of milk: Effect of casein micelle size. *J. Dairy Sci.* 83:378–386. [https://doi.org/10.3168/jds.S0022-0302\(00\)74892-2](https://doi.org/10.3168/jds.S0022-0302(00)74892-2).
- O'Connell, J. E., and P. F. Fox. 2001. Effect of β -lactoglobulin and precipitation of calcium phosphate on the thermal coagulation of milk. *J. Dairy Res.* 68:81–94. <https://doi.org/10.1017/S0022029900004556>.
- O'Connell, J. E., and P. F. Fox. 2003. Heat-induced coagulation of milk. Pages 879–945 in *Advanced Dairy Chemistry: Volume 1: Proteins*, Parts A&B. P. F. Fox and P. L. H. McSweeney, ed. Springer US, Boston, MA.
- O'Connell, J. E., A. L. Kelly, P. F. Fox, and K. G. de Kruif. 2001. Mechanism for the ethanol-dependent heat-induced dissociation of casein micelles. *J. Agric. Food Chem.* 49:4424–4428. <https://doi.org/10.1021/jf001479h>.
- O'Sullivan, M. M., A. L. Kelly, and P. F. Fox. 2002. Effect of transglutaminase on the heat stability of milk: A possible mechanism. *J. Dairy Sci.* 85:1–7. [https://doi.org/10.3168/jds.S0022-0302\(02\)74045-9](https://doi.org/10.3168/jds.S0022-0302(02)74045-9).
- O'Sullivan, M. M., P. C. Lorenzen, J. E. O'Connell, A. L. Kelly, E. Schlimme, and P. F. Fox. 2001. Short communication: Influence of transglutaminase on the heat stability of milk. *J. Dairy Sci.* 84:1331–1334. [https://doi.org/10.3168/jds.S0022-0302\(01\)70162-2](https://doi.org/10.3168/jds.S0022-0302(01)70162-2).
- Omoarukhe, E. D., N. On-Nom, A. S. Grandison, and M. J. Lewis. 2010. Effects of different calcium salts on properties of milk related to heat stability. *Int. J. Dairy Technol.* 63:504–511. <https://doi.org/10.1111/j.1471-0307.2010.00613.x>.
- On-Nom, N., A. S. Grandison, and M. J. Lewis. 2010. Measurement of ionic calcium, pH, and soluble divalent cations in milk at high temperature. *J. Dairy Sci.* 93:515–523. <https://doi.org/10.3168/jds.2009-2634>.
- On-Nom, N., A. S. Grandison, and M. J. Lewis. 2012. Heat stability of milk supplemented with calcium chloride. *J. Dairy Sci.* 95:1623–1631. <https://doi.org/10.3168/jds.2011-4697>.
- Parker, T. G., D. S. Horne, and D. G. Dalgleish. 1979. Theory for the heat-induced coagulation of a type A milk. *J. Dairy Res.* 46:377–380. <https://doi.org/10.1017/S0022029900017349>.
- Pearce, R. J. 1979. Heat stability in concentrated and non-concentrated milks – the effect of urea and β -lactoglobulin levels and the influence of preheating. *J. Dairy Res.* 46:385–386. <https://doi.org/10.1017/S0022029900017362>.
- Pouliot, Y., and M. Boulet. 1991. Seasonal variations in the heat stability of concentrated milk: Effect of added phosphates and pH adjustment. *J. Dairy Sci.* 74:1157–1162. [https://doi.org/10.3168/jds.S0022-0302\(91\)78268-4](https://doi.org/10.3168/jds.S0022-0302(91)78268-4).
- Pyne, G. T., and K. A. McHenry. 1955. 572. The heat coagulation of milk. *J. Dairy Res.* 22:60–68. <https://doi.org/10.1017/S002202990000755X>.
- Rasmussen, L. K., P. Hojrup, and T. E. Petersen. 1992. The multimeric structure and disulfide-bonding pattern of bovine κ -casein. *Eur. J. Biochem.* 207:215–222. <https://doi.org/10.1111/j.1432-1033.1992.tb17040.x>.
- Reitmaier, M., B. Barbosa, S. Sigler, H.-J. Heidebrecht, and U. Kulozik. 2020. Impact of different aqueous phases on casein micelles: Kinetics of physicochemical changes under variation of water hardness and diafiltration conditions. *Int. Dairy J.* 109:104776. <https://doi.org/10.1016/j.idairyj.2020.104776>.
- Robitaille, G., and C. Ayers. 1995. Effects of κ -casein glycosylation on heat stability of milk. *Food Res. Int.* 28:17–21. [https://doi.org/10.1016/0963-9969\(95\)93326-P](https://doi.org/10.1016/0963-9969(95)93326-P).
- Rose, D. 1961a. Factors affecting the pH-sensitivity of the heat stability of milk from individual cows. *J. Dairy Sci.* 44:1405–1413. [https://doi.org/10.3168/jds.S0022-0302\(61\)89901-3](https://doi.org/10.3168/jds.S0022-0302(61)89901-3).

- Rose, D. 1961b. Variations in the heat stability and composition of milk from individual cows during lactation. *J. Dairy Sci.* 44:430–441. [https://doi.org/10.3168/jds.S0022-0302\(61\)89760-9](https://doi.org/10.3168/jds.S0022-0302(61)89760-9).
- Rose, D. 1961c. β -Lactoglobulin and pH-sensitivity of the heat stability of evaporated milk. *J. Dairy Sci.* 44:1763. [https://doi.org/10.3168/jds.S0022-0302\(61\)89952-9](https://doi.org/10.3168/jds.S0022-0302(61)89952-9).
- Rose, D. 1962. Factors affecting the heat stability of milk. *J. Dairy Sci.* 45:1305–1311. [https://doi.org/10.3168/jds.S0022-0302\(62\)89617-9](https://doi.org/10.3168/jds.S0022-0302(62)89617-9).
- Rose, D., and H. Tessier. 1959. Composition of ultrafiltrates from milk heated at 80 to 230° F in relation to heat stability. *J. Dairy Sci.* 42:969–980. [https://doi.org/10.3168/jds.S0022-0302\(59\)90680-0](https://doi.org/10.3168/jds.S0022-0302(59)90680-0).
- Saint Denis, T., G. Humbert, and J.-L. Gaillard. 2001. Heat inactivation of native plasmin, plasminogen and plasminogen activators in bovine milk: A revisited study. *Lait* 81:715–729. <https://doi.org/10.1051/lait:2001159>.
- Sandra, S., and D. G. Dalgleish. 2005. Effects of ultra-high-pressure homogenization and heating on structural properties of casein micelles in reconstituted skim milk powder. *Int. Dairy J.* 15:1095–1104. <https://doi.org/10.1016/j.idairyj.2004.11.015>.
- Sievanen, K., T. Huppertz, A. L. Kelly, and P. F. Fox. 2008. Influence of added calcium chloride on the heat stability of unconcentrated and concentrated bovine milk. *Int. J. Dairy Technol.* 61:151–155. <https://doi.org/10.1111/j.1471-0307.2008.00391.x>.
- Singh, G., S. Arora, G. S. Sharma, J. S. Sindhu, V. K. Kansal, and R. B. Sangwan. 2007. Heat stability and calcium bioavailability of calcium-fortified milk. *LWT Food Sci Technol.* 40:625–631. <https://doi.org/10.1016/j.lwt.2006.03.009>.
- Singh, H. 1994. Cross-linking of milk proteins on heating concentrated milk at 120°C. *Int. Dairy J.* 4:477–489. [https://doi.org/10.1016/0958-6946\(94\)90020-5](https://doi.org/10.1016/0958-6946(94)90020-5).
- Singh, H. 1995. Heat stability of concentrated milk. Pages 256–278 in *Heat-Induced Changes in Milk*, 2nd ed. P. F. Fox, ed. International Dairy Federation, Brussels, Belgium.
- Singh, H. 2004. Heat stability of milk. *Int. J. Dairy Technol.* 57:111–119. <https://doi.org/10.1111/j.1471-0307.2004.00143.x>.
- Singh, H., and L. K. Creamer. 1992. Heat stability of milk. Pages 621–656 in *Advanced Dairy Chemistry: Volume 1 – Proteins*. P. F. Fox, ed. Elsevier Applied Science, London.
- Singh, H., and P. F. Fox. 1985a. Heat stability of milk: pH-dependent dissociation of micellar κ -casein on heating milk at ultra high temperatures. *J. Dairy Res.* 52:529–538. <https://doi.org/10.1017/S0022029900024481>.
- Singh, H., and P. F. Fox. 1985b. Heat stability of milk: The mechanism of stabilization by formaldehyde. *J. Dairy Res.* 52:65–76. <https://doi.org/10.1017/S0022029900023906>.
- Singh, H., and P. F. Fox. 1986. Heat stability of milk: Further studies on the pH-dependent dissociation of micellar κ -casein. *J. Dairy Res.* 53:237–248. <https://doi.org/10.1017/S0022029900024845>.
- Singh, H., and P. F. Fox. 1987a. Heat stability of milk: Influence of colloidal and soluble salts and protein modification on the pH-dependent dissociation of micellar κ -casein. *J. Dairy Res.* 54:523–534. <https://doi.org/10.1017/S0022029900025723>.
- Singh, H., and P. F. Fox. 1987b. Heat stability of milk: Influence of modifying sulphhydryl-disulphide interactions on the heat coagulation time–pH profile. *J. Dairy Res.* 54:347–359. <https://doi.org/10.1017/S0022029900025528>.
- Singh, H., and P. F. Fox. 1987c. Heat stability of milk: Role of β -lactoglobulin in the pH-dependent dissociation of micellar κ -casein. *J. Dairy Res.* 54:509–521. <https://doi.org/10.1017/S0022029900025711>.
- Singh, H., and R. P. Tokley. 1990. Effects of preheat treatments and buttermilk addition on the seasonal variations in the heat stability of recombined evaporated milk and reconstituted concentrated milk. *Aust. J. Dairy Technol.* 45:10–16.
- Singh, J., S. Prakash, B. Bhandari, and N. Bansal. 2019. Ultra high temperature (UHT) stability of casein-whey protein mixtures at high protein content: Heat induced protein interactions. *Food Res. Int.* 116:103–113. <https://doi.org/10.1016/j.foodres.2018.12.049>.
- Smith, J. F., and B. Malmgren. 1999. UHT RCM. UHT recombined concentrated milk. Pages 43–48 in *Proc. 3rd International Symposium on Recombined Milk & Milk Products: Special issue 9902*. International Dairy Federation, Brussels, Belgium.
- Sommer, H. H., and E. B. Hart. 1919. The heat coagulation of milk. *J. Biol. Chem.* 40:137–151.
- Sommer, H. H., and E. B. Hart. 1922. The heat coagulation of milk. *J. Dairy Sci.* 5:525–543. [https://doi.org/10.3168/jds.S0022-0302\(22\)94183-9](https://doi.org/10.3168/jds.S0022-0302(22)94183-9).
- Sweetsur, A. W. M., and D. D. Muir. 1980. Effect of concentration by ultrafiltration on the heat stability of skim-milk. *J. Dairy Res.* 47:327–335. <https://doi.org/10.1017/S002202990002121X>.
- Sweetsur, A. W. M., and D. D. Muir. 1982. Natural variation in heat stability of concentrated milk before and after homogenization. *Int. J. Dairy Technol.* 35:120–126. <https://doi.org/10.1111/j.1471-0307.1982.tb02204.x>.
- Sweetsur, A. W. M., and D. D. Muir. 1983. Effect of homogenization on the heat stability of milk. *J. Dairy Res.* 50:291–300. <https://doi.org/10.1017/S0022029900023128>.
- Sweetsur, A. W. M., and J. C. D. White. 1975. Studies on the heat stability of milk protein: III. Effect of heat-induced acidity in milk. *J. Dairy Res.* 42:73–88. <https://doi.org/10.1017/S0022029900015119>.
- Tan-Kintia, R. H., and P. F. Fox. 1999. Effect of various preheat treatments on the heat stability of unconcentrated milk. *Int. Dairy J.* 9:219–225. [https://doi.org/10.1016/S0958-6946\(99\)00064-3](https://doi.org/10.1016/S0958-6946(99)00064-3).
- Tessier, H., and D. Rose. 1961. Heat stability of casein in the presence of calcium and other salts. *J. Dairy Sci.* 44:1238–1246. [https://doi.org/10.3168/jds.S0022-0302\(61\)89874-3](https://doi.org/10.3168/jds.S0022-0302(61)89874-3).
- Tessier, H., and D. Rose. 1964. Influence of κ -casein and β -lactoglobulin on the heat stability of skimmilk. *J. Dairy Sci.* 47:1047–1051. [https://doi.org/10.3168/jds.S0022-0302\(64\)88843-3](https://doi.org/10.3168/jds.S0022-0302(64)88843-3).
- van Asselt, A. J., A. P. J. Sweere, H. S. Rollema, and P. de Jong. 2008. Extreme high-temperature treatment of milk with respect to plasmin inactivation. *Int. Dairy J.* 18:531–538. <https://doi.org/10.1016/j.idairyj.2007.11.019>.
- van Boekel, M. A. J. S. 1998. Effect of heating on Maillard reactions in milk. *Food Chem.* 62:403–414. [https://doi.org/10.1016/S0308-8146\(98\)00075-2](https://doi.org/10.1016/S0308-8146(98)00075-2).
- van Boekel, M. A. J. S., J. A. Nieuwenhuijse, and P. Walstra. 1989a. The heat coagulation of milk. 1. Mechanisms. *Neth. Milk Dairy J.* 43:97–127.
- van Boekel, M. A. J. S., J. A. Nieuwenhuijse, and P. Walstra. 1989b. The heat coagulation of milk. 2. Kinetic studies. *Neth. Milk Dairy J.* 43:129–146.
- van Boekel, M. A. J. S., J. A. Nieuwenhuijse, and P. Walstra. 1989c. The heat coagulation of milk. 3. Comparison of theory and experiment. *Neth. Milk Dairy J.* 43:147–162.
- van Dijk, H. J. M., and A. Hersevoort. 1992. The properties of casein micelles. 5. The determination of heat-induced calcium phosphate precipitations in milk. *Neth. Milk Dairy J.* 46:69–76.
- Vyas, H. K., and P. S. Tong. 2004. Impact of source and level of calcium fortification on the heat stability of reconstituted skim milk powder. *J. Dairy Sci.* 87:1177–1180. [https://doi.org/10.3168/jds.S0022-0302\(04\)73266-X](https://doi.org/10.3168/jds.S0022-0302(04)73266-X).
- Walstra, P., and R. Jenness. 1984. *Dairy Chemistry and Physics*. John Wiley & Sons, New York, NY.
- Webb, B. H., and R. W. Bell. 1943. The effect of high-temperature-short-time heating of concentrated milk upon its heat stability. *J. Dairy Sci.* 26:1071–1077. [https://doi.org/10.3168/jds.S0022-0302\(43\)92809-7](https://doi.org/10.3168/jds.S0022-0302(43)92809-7).
- Webb, B. H., R. W. Bell, E. F. Deysher, and G. E. Holm. 1943. The effect of various degrees of forewarming upon the heat stability of milks of different solids concentrations. *J. Dairy Sci.* 26:571–578. [https://doi.org/10.3168/jds.S0022-0302\(43\)92756-0](https://doi.org/10.3168/jds.S0022-0302(43)92756-0).
- Webb, B. H., and G. E. Holm. 1932. The heat coagulation of milk: II. The influence of various added salts upon the heat stabilities of milks of different concentrations. *J. Dairy Sci.* 15:345–366. [https://doi.org/10.3168/jds.S0022-0302\(32\)93423-7](https://doi.org/10.3168/jds.S0022-0302(32)93423-7).
- White, J. C. D., and D. T. Davies. 1958. The relation between the chemical composition of milk and the stability of the caseinate complex: IV. Coagulation by heat. *J. Dairy Res.* 25:281–296. <https://doi.org/10.1017/S0022029900009274>.

- White, J. C. D., and D. T. Davies. 1966. The stability of milk protein to heat: III. Objective measurement of heat stability of milk. *J. Dairy Res.* 33:93–102. <https://doi.org/10.1017/S0022029900011754>.
- White, J. C. D., and A. W. M. Sweetsur. 1977. Kinetics of the heat-induced aggregation of milk protein. *J. Dairy Res.* 44:237–247. <https://doi.org/10.1017/S0022029900020161>.
- Whiteley, A. J., and D. D. Muir. 1996. Heat stability of homogenized concentrated milk: 1. Comparison of Microfluidizer with a valve homogenizer. *Milchwissenschaft* 51:320–323.
- Whitney, R. M., K. Paulson, and G. K. Murthy. 1952. A test of the heat stability of milk proteins for use in research. *J. Dairy Sci.* 35:937–948. [https://doi.org/10.3168/jds.S0022-0302\(52\)93779-X](https://doi.org/10.3168/jds.S0022-0302(52)93779-X).
- Zin El-Din, M., and T. Aoki. 1993. Polymerization of casein on heating milk. *Int. Dairy J.* 3:581–588. [https://doi.org/10.1016/0958-6946\(93\)90101-5](https://doi.org/10.1016/0958-6946(93)90101-5).

ORCIDS

Joseph Dumpler <https://orcid.org/0000-0002-7877-2479>

Thom Huppertz <https://orcid.org/0000-0003-4142-9359>

Ulrich Kulozik <https://orcid.org/0000-0001-9598-9242>