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Lilia M. Ahrné:

PROCALCIUM – Calciums re-fordeling i ostemælk under forarbejdning

PROCALCIUM – Calcium dynamics during manufacturing of cheese

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Final report

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1. Title of the project

Danish: Calciums re-fordeling i ostemælk under forarbejdning (PROCALCIUM)

English: Calcium dynamics during manufacturing of cheese (PROCALCIUM)

2. Project manager

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4. Sources of funding

Milk Levy Fund
Arla Food AMBA

5. Project period

Project period with DDRF funding:	[Start 01/06/2020, End, 31/12/2022]
Revised, if necessary:	[Start 01/10/2020, End, 31/12/2024]

6. Project summary

Danish:
Calcium er et essentielt næringsstof, men bidrager også til de fleste mejeriprodukters tekstur, smag og funktionalitet. Dette projekt fokuserer på at skabe nye indblik i calciums fordeling mellem kolloid- og serumfaserne under mælkens forarbejdning til højværdi mælkeprodukter som for eksempel ost. Processeringstrin som filtrering af mælk, opkoncentration, pH-ændringer under syrning og salttilsætning, afkøling og opvarmning ændrer alle på fordelingen af calcium mellem serum- og kolloidalfasen, men en kinetisk beskrivelse af calciumfordelingen mangler. Projektets hypotese er, at en bedre forståelse af calciumfordelingens dynamik og kvantificering af kinetikken vil understøtte fremstilling af højværdiprodukter med forbedret funktionalitet samt kunne føre til optimering af mejeriprocesser gennem reduktion af produkttab. Projektets resultater vil blive demonstreret med henblik på at forbedre osteudbytte og skræddersy ostefunktionaliteter som strækningsog smelteevne. Der vil blive benyttet advancerede kemiske og in-situ struktur karakteriseringsmetoder i projektet.

English:
Calcium is an important nutrient but also contributes to the texture, taste and functionality of most dairy products. This project focuses on creating new insights regarding the content and status of calcium in colloidal and serum phases during the manufacturing of added-value dairy products, such as cheese. Process steps like milk preconditioning, up concentration, acidification, salting, cooling and heating, all change the distribution of calcium between the serum and colloidal phase, but a kinetic description of calcium distribution is basically lacking. The hypothesis of this project is that understanding of the dynamics of calcium distribution and quantification of the kinetics will support the manufacturing of added value products with improved functionality and contribute to optimization of the dairy processes by reducing product losses. The results of the project are now complete and fulfilled with a total of 4 publications in international journals and 1 still in preparation. The insights spanned from monitoring of calcium and minerals equilibria in milk (both serum and colloidal) over storage time, to monitoring such equilibria during membrane filtration, salting and acidification using common acidulant used by dairy industry.

7. Project aim

English:

The overall objective of this project is to understand how calcium dynamics can be controlled during processing as a way to increase yield and modulate the texture and functionality of added value dairy products, such as premium cheeses. This will be achieved by:

1. Set up state of the art techniques to understand the dynamics of calcium distribution as a function of temperature, milk upconcentration, pH and ionic strength (**WP1**).

2. A kinetic description of the dynamics of calcium distribution during processing (heating/cooling, UF filtration, and renneting/acidification) and storage (**WP2, WP3, WP4**).

3. Extracting knowledge about the relationships between calcium content and binding status, and curd yield, texture and functional properties of cheese (**WP4, WP5**).

Danish:

Projektets overordnede mål er at opnå en grundlæggende forståelse af, hvordan calciumdynamikken kan styres under mejeriteknologiske processer med henblik på at øge udbyttet samt målrettet påvirke tekstur og funktionalitet i mejeriprodukter med høj værditilvækst, herunder premiumoste. Dette opnås gennem:

1. Etablering af avancerede analytiske metoder til karakterisering af calciumfordelingens dynamik som funktion af temperatur, mælkekoncentrering, pH og ionstyrke (WP1).
2. Udvikling af kinetiske modeller, der beskriver calciumfordelingens dynamik under forarbejdning (opvarmning/køling, ultrafiltrering samt løbekoagulering/syrning) og efterfølgende lagring (WP2, WP3, WP4).
3. Generering af ny viden om sammenhængene mellem calciumindhold og calciumbindingsstatus samt ostekoagelets udbytte, teksturelle egenskaber og funktionalitet (WP4, WP5).

8. Background for the project

A variety of textures are created by transforming milk into cheese or other dairy products, using traditional and industrial techniques that are evolving to meet changing consumer demands. The new consumer trends and new export markets create a need for knowledge to tailor the functionality of dairy products. In several studies, calcium has been recognized to play an important role in structure formation of dairy products (Law & Leaver 1998, Metzger et al. 2000, Guinee et al. 2002, Joshi et al. 2003, Seth & Bajwa 2015).

Bovine milk contains 1.0 – 1.2 g calcium/kg milk distributed in a dynamic equilibrium between casein micelles and the serum phase (Lui et al. 2013). At neutral pH, approximately 30% of the calcium in the milk is present as supersaturated calcium phosphate in the serum phase, whereas the rest is associated with the casein micelles. Caseins have high binding capacity to calcium and prevent uncontrolled precipitation and growth of calcium phosphate by forming colloidal calcium phosphate (CCP) which are amorphous calcium phosphate nanocomplexes (Broyard and Gaucheron 2015). The calcium-binding capacity of casein is due to the phosphoseryl residues (Gaucheron 2005), and several studies have reported relevant equilibrium data about the calcium binding capacities of caseins, whey proteins and the influence of temperature, pH and ionic strength (e.g. Pappas and Rothwell, 1991, Swaisgood, 1993 Alvarez et al. 2007; Gaucheron et al. 1997; Parker & Dalgleish, 1981; Fuchs & Gessner, 2001). A more recent study by Mekmene and Gaucheron (2011) provided relevant insights into the calcium-binding capacities of phosphoserine, caseinophosphopeptides, beta-casein, caseinate, citrate and pyrophosphate but only at 22 °C, pH 8.10 and ionic strength 95 mM.

Thus, in milk, calcium is present in the serum and colloidal phase, both as free (ionic) and bound calcium to milk components. There is limited knowledge on how to control the dynamic calcium equilibrium between the different phases and speciation for the benefit of improved dairy product properties. Most of the present studies regarding calcium in milk focus on the total amount of calcium in the serum phase or calcium associated to the caseins and not on the dynamics of transfer between free ionic and bounded calcium (soluble/insoluble) in the two phases (serum and colloidal). Furthermore, a kinetic description of the dynamics of calcium distribution in response to heat treatment, concentration, changes in pH and ionic strength during processing and storage that open for a target process design is lacking. We propose in this project to tackle this knowledge gap, using advanced methodologies for fast kinetics (stopped flow spectroscopy) and in-situ structure characterization methods, such as scattering techniques and calcium imaging.

Previous studies at the University of Copenhagen and by a few other groups support the plausibility of our approach. The texture and heat stability of calcium enriched products was improved by reducing the concentration of ionic calcium by addition of citrates, phosphates and caseins (Mizuno and Lucey 2007, Ozcan et al. 2007, 2008), or changing pH and temperature (Karlsson et al. 2007, Koutina et al. 2014, 2015a, 2015b). Recently, Thybo (2019) (MSc thesis at UCPH) showed that during direct acidification of milk with lactic and citric acid most of the calcium is dissociated within 10 minutes and curd yield was highly influenced by the pH, as solubilization of colloidal calcium phosphate causes loss of minerals into the serum phase, decreasing both calcium and phosphates in the curd. Citrates shifts the binding of calcium away from the proteins affecting the temperature dependence of calcium binding (Rodrigo et al.2019). Hence, there is a need for systematic studies with improved methodologies to quantify and understand the dynamics of free and bound calcium in the serum and colloidal phases of milk and during cheese manufacturing steps like thermal treatment, upconcentration by membrane filtration, renneting, acidification and salt addition.

9. Sub-activities in the entire project period

The project was carried out as a postdoc project. Assistant professor Giovanni Barone carried out the initial and final part of the project (from 01/10/2020 to 01/12/2021), and postdoc Yuan Jiang carried out the remaining time (from 01/01/2022 to 01/10/23) and the last period Giovanni Barone.

To reach the objectives settled, the project was divided into four workpackages (WP):

WP1. Methods to quantify and understand the dynamics of calcium in milk (Months: 1-12).
D1.1. Review paper published about calcium-binding and methodologies to understand and quantify calcium dynamics during processing

WP2. Calcium dynamics and kinetics of pasteurized milk during storage (Months: 6-12).
D2.1 Scientific paper published on dynamics and kinetics of calcium in milk pasteurized during storage

WP3. Calcium dynamics in UF milk concentrates during filtration and salting (Months: 10-24).
D3.1 Scientific paper published about calcium distribution after ultrafiltration
D3.2. Scientific paper published about calcium distribution under dead end and cross flow filtration
D3.3 Scientific paper published about effect of salting

WP4. **Calcium dynamics during renneting and direct acidification (Months: 16-36).**
D4.1. Scientific paper in preparation to be submitted for publication regarding the dynamics of calcium during acidification of milk and the consequences for gelation

WP5: **Extracting knowledge about the relationships between calcium content and binding status, and curd yield, texture and functional properties of cheese.**

10. Deviations

In general, the project was carried out as planned, although some changes in the project plan were implemented based on research questions that were raised from the results obtained. The changes implemented were agreed on in the project group, as follows:

WP1 was performed as planned

WP2. Focused only on pasteurized milk and changes in heating and cooling temperature were not considered relevant.

WP3. This work package was extended, resulting in two extra deliverables. So, mineral profiles during cross flow filtration at different temperatures were performed as planned, although the effect of storage temperature was not prioritized. In addition, a comparison was made between ultrafiltration by dead end and cross flow that resulted in very interesting results. Another study was done to assess the potential of controlling the ionic strength by salt addition. More extensive work was done to understand the role of beta casein.

WP4. Was perform in large extended as planned, with focus given to effects of milk storage on rennet gelation.

WP5. Was carried out alongside the project. Furthermore, due to the interest a new project was proposed MinCurd that is now coordinated by Assistant Professor Giovanni Barone.

The project has delivered both an extensive toolbox and new insight with industrial relevance regarding mineral partition between colloidal and serum phase have been relevant for Arla developing new cheese making process and improving ongoing.

11. Project results

The project results will be presented based on the work packages previously outlined.

***WP1:* Methods to quantify and understand the dynamics of calcium in milk**

In this workpackage state-of-the-art techniques were set up to understand the dynamics of calcium distribution as a function of temperature, milk concentration, pH and ionic strength.

The initial stage consisted of the identification of suitable and specific methods to be used in the project. The methods were selected based on physical chemistry and structural for understanding calcium partitioning and speciation. The information was gathered and formulated in the form of a review paper and published as:

> Barone, G., Yazdi, S. R., Lillevang, S. K. & Ahrne, L., (2021). Calcium: A comprehensive review on quantification, interaction with milk proteins and implications for processing of dairy products, Comprehensive Reviews in Food Science and Food Safety. 20, 6, p. 5616-5640.

An overview of total and ionic calcium content in different dairy products and possible methods for determining calcium partitioning in milk (serum to colloidal and vice versa) were provided (table 1). Finally, a comprehensive schematic of how common dairy processing can affect calcium partitioning and speciation was proposed (Fig 1). The publication of the needed information has been useful in establishing the experimental plans of the next work packages.

Table 1. Total and ionic calcium values for a range of dairy products and the most commonly used analytical techniques for their determination

	Product	Technique	Calcium content	Reference
			(mg/L)	
Total calcium	Liquid milk	Titration	1010–1360	Cardwell et al. (1990); Dolores Alvarez Jiménez et al. (1988)
		ICP-OES	1078–1922	Murcia et al. (1999); Zwierzchowski and Ametaj (2019)
		AAS	1118-1461	Cerbulis and Farrell (1976); ; Sowmya et al. (2015)
	Milk powder ingredients		(mg/100 g)	
		ICP-MS	1272–1350	Chen and Jiang (2002); Herwig et al. (2011); Barone et al. (2020a)
		ICP-OES	882–1506	McKinstry et al. (1999); Sikand et al. (2011)
		AAS	960–1300	Noël et al. (2008);
		XFR	1070–1330	McCarthy et al. (2020)
	Cheeses		(mg/100 g)	
		Titration	670–842	Kindstedt and Kosikowski (1985)
		ICP-OES	570–1700	Bilandžić et al. (2015); Manuelian et al. (2017)
		AAS	608–1107	Pollman (1991); Hassan et al. (2004)
Ionic calcium	Liquid milk		Molarity (mM)	
		Titration	2.5–3.4	Rose and Tessier (1959)
		Selective electrode	1.07–2.60	Augustin and Clarke (1990); M. J. Lin et al. (2006)
		Ion-exchange chromatography	2.02–2.32	Christianson et al. (1954); Cataldi et al. (2003)
	Cheeses			
		Titration	0.85–1.52	Hassan et al. (2004); Wolfschoon-Pombo and Andlinger (2013)

Abbreviations: AAS, atomic absorption spectroscopy; ICP-OES, inductively coupled plasma-optical emission spectrometry; XFR, X-ray fluorescence; ICP-MS, inductively coupled plasma mass spectrometry.

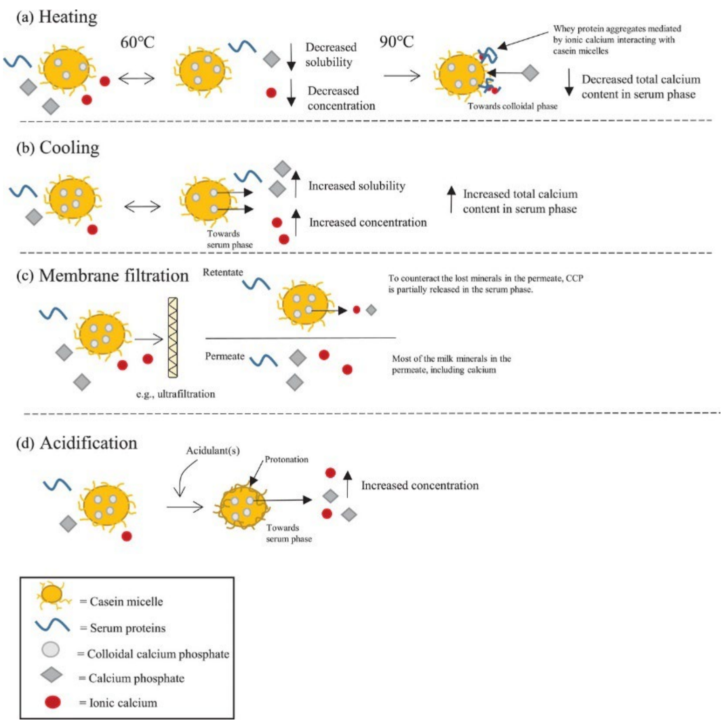

Figure 1. Schematic representation of calcium partitioning during common processing practises of bovine milk such as (a) heating, (b) cooling, (c) membrane filtration, and (d) acidification

***WP2*: Calcium dynamics and kinetics during heating and storage of milk**

In milk, calcium exists associated with caseins (called colloidal calcium) or in the serum phase (ionic calcium or solubilized by chemical bounding to salts). The calcium equilibrium between these two forms affects how milk behaves during storage and processing. Furthermore, β-casein is also known to change between the colloidal and serum phase due to temperature changes. Thus, the objective of this work was to provide a better understanding of Ca and β-casein partitioning between the serum and the colloidal phase during storage of pasteurized milk from 4°C to 35°. Such partition is strongly associated with Ca and binding of Ca to proteins and salt anions during cheese manufacturing and thus affect the yield, textures and functionalities of various cheese such as mozzarella, yellow cheeses and mold cheeses.

Furthermore, mathematical kinetic models were developed to describe calcium partition between the colloidal and serum phase, which provide a first approach to a more detailed modelling involving the different casein forms. The results obtained in this study provide fundamental and novel insights into the relationship between dairy calcium equilibria in the colloidal and serum phase of milk. Some examples of kinetics data are provided to described that at:

- Low temperatures (4°C–10°C), more calcium moved to the serum phase over time.
- At higher temperatures > 20°C, calcium remained in the casein micelles.
- Calcium dissociation from the micelles seems to be coupled with β-casein

The results of this study were published in:

> Jiang, Y., Barone, G., Rauh, V., Lillevang, S. K., Skibsted, L. H. & Ahrne, L., (2023). Temperature effects on calcium partition kinetics in pasteurised skim milk during storage, International Dairy Journal. 137, 9 p., 105518.

WP3: A kinetic description of the dynamics of calcium distribution during UF filtration and salting

Three studies were conducted in this work package. Two regarding ultrafiltration (A, B) and one regarding salting (C).

A) Effect of Ultrafiltration temperature

In this study, the influence of temperature (i.e., 10, 25 and 55 °C) on minerals contents and physicochemical properties of retentates and permeate produced during ultrafiltration (UF) of skim milk were investigated at different volumetric concentration ratios (VCR: 1.0, 1.5, 2.0, 2.5 and 3.0). Mineral content in UF retentates was significantly lower ($p < 0.05$) at 10 °C when compared to 55 °C at all the VCRs studied, with an average reduction of 17 %; while, in respective permeate, mineral content was higher at 55 °C. Ionic calcium concentration in the retentate increased along the VCR and decreased with increasing temperature (2.35-2.78 mM and 0.19-0.43 mM at 10 and 55 °C, respectively). UF of milk temperature at 10 °C resulted in a low permeate flux rate along the VCR, but with better flux recovery after cleaning when compared to 55 °C. The results obtained in this study provide new insights into the complex relationships between the milk temperature and milk mineral partitioning on UF performance and physiochemical properties of retentates and permeates, that is exploitable for the production of dairy retentates having tailored mineral content and functionality.

The study has been published as:

> Barone, G., Guadagnucci, S., Rauh, V., Lillevang, S. K., Caboni, M. F. & Ahrné, L., (2025). Minerals content and physicochemical properties of skim milk retentates and permeates during ultrafiltration at different temperatures, International Dairy Journal. 161, 9 p., 106138.

B) Effect of ultrafiltration set up

The filtration set up has a strong effect on the calcium content of retentates and permeates. In this study, the partitioning of minerals in ultrafiltration (UF) streams of skim milk using cross-flow (CF-UF) and dead-end (DE-UF) were investigated using 50 kDa membrane at different temperatures (5, 10, 25, 35, 55°C). CF-UF showed higher protein retention (6.02–10.9%) compared with DE-UF (3.44–4.70%), along with more retention of calcium, with values ranging from 29.8 to 41.5 and 57.8 to 112 mM for DE-UF and CF-UF, respectively. Ionic calcium in permeates varied less for CF-UF (2.48–1.18 mM) than for DE-UF (3.11–0.98 mM). The insights provided in this study can be exploited to tailor adjustments of minerals in milk streams using UF.

Further results can be found in the publication:

Jiang, Y., Rauh, V., Lillevang, S. K., Skibsted, L. H., Lipnizki, F., Barone, G. & Ahrné, L., (2024). The influence of different ultrafiltration set-ups on the mineral partitioning between skim milk streams, International Journal of Dairy Technology. 77, 3, p. 884 – 892.

C) Salting influence on Calcium partitioning

In this study the influence of increased ionic strength was investigated by addition of NaCl (80–580 mM) to pasteurised skim milk on minerals partitioning, casein micelle properties and β-casein partitioning as a function of temperatures (i.e., 4, 10, 25, and 55 °C). Increasing ionic strength influenced the partition of divalent minerals in serum phase, especially total and ionic calcium concentration in the serum phase, while it had no effect on monovalent mineral partitioning. Adding more salt increased the amount of calcium and phosphorus in the serum phase of the milk (table 2). pH significantly decreased with increasing of ionic strength due to changes in mineral equilibria, and increased protein content in the milk serum phase, especially at low temperatures. The results obtained in this study provide new insights into mineral and protein partitioning of milk as influenced by ionic strength relevant for control of mineral distribution and for the development of new dairy products.

Table 2. Mineral concentration (mM) of serum phase from skim milk at different temperatures (4, 10, 25 and 55C) as a function of increasing ionic strength due to addition of NaCl.

Ion	T (°C)	Ionic strength (mM)					
		80	165	208	250	507	593
Calcium	4	19.2 ± 2.28^{a}	22.3 ± 3.04^{b}	23.2 ± 3.26^{c}	24.0 ± 1.51^{d}	24.8 ± 1.16^{d}	25.2 ± 1.54^{e}
	10	17.0 ± 2.93^{a}	18.7 ± 3.29^{b}	18.6 ± 3.01^{b}	19.0 ± 3.56^{c}	20.3 ± 4.34^{d}	21.4 ± 5.90^{e}
	25	12.1 ± 0.57^{a}	13.4 ± 4.89^{b}	14.5 ± 2.21^{c}	15.5 ± 4.96^{d}	18.0 ± 1.63^{e}	19.0 ± 1.01^{f}
	55	11.5 ± 1.61^{a}	12.2 ± 1.16^{b}	13.8 ± 1.93^{c}	14.3 ± 2.04^{d}	15.0 ± 1.62^{e}	16.9 ± 2.10^{f}
Phosphorus	4	25.3 ± 0.51^{a}	26.2 ± 0.64^{b}	27.7 ± 2.46^{c}	28.2 ± 2.60^{d}	30.5 ± 0.66^{e}	29.1 ± 1.22^{f}
	10	21.7 ± 1.70^{a}	23.7 ± 1.91^{b}	23.5 ± 1.51^{ab}	23.3 ± 3.01^{ab}	23.6 ± 2.85^{b}	26.1 ± 3.27^{c}
	25	16.3 ± 2.35^{a}	18.7 ± 2.72^{b}	19.5 ± 3.51^{c}	22.9 ± 1.46^{d}	24.0 ± 4.07^{e}	24.4 ± 1.92^{e}
	55	22.2 ± 1.25^{b}	21.3 ± 1.70^{a}	22.5 ± 2.33^{c}	21.2 ± 2.04^{a}	22.0 ± 0.43^{b}	23.2 ± 0.83^{d}
Magnesium	4	5.10 ± 1.01^{a}	5.47 ± 1.27^{b}	5.24 ± 1.21^{c}	5.15 ± 1.49^{d}	5.72 ± 1.29^{e}	5.36 ± 2.10^{f}
	10	4.27 ± 1.33^{b}	4.63 ± 1.36^{d}	4.54 ± 1.30^{c}	3.87 ± 1.49^{a}	4.55 ± 0.64^{c}	4.87 ± 1.75^{e}
	25	4.87 ± 0.21^{d}	3.92 ± 1.42^{b}	3.88 ± 2.24^{a}	4.17 ± 1.19^{c}	5.08 ± 0.27^{e}	5.10 ± 1.05^{e}
	55	4.88 ± 0.54^{a}	4.87 ± 1.16^{ab}	4.96 ± 1.42^{bc}	4.97 ± 1.13^{ab}	5.07 ± 0.66^{c}	5.32 ± 1.77^{d}
Potassium	4	44.3 ± 2.64^{cd}	44.7 ± 1.97ec	42.4 ± 3.44^{ab}	42.1 ± 1.00^{abc}	44.4 ± 1.48^{d}	41.2 ± 2.49^{a}
	10	41.8 ± 2.63^{ab}	43.2 ± 1.22^{cd}	42.7 ± 2.84^{ab}	42.8 ± 2.27^{bc}	41.3 ± 2.64^{a}	43.8 ± 1.27^{d}
	25	41.2 ± 2.92^{b}	43.5 ± 1.72^{c}	40.5 ± 1.57^{b}	43.7 ± 2.78^{c}	39.7 ± 1.63^{a}	39.9 ± 2.13^{a}
	55	43.0 ± 2.38^{ab}	41.6 ± 2.67^{a}	42.5 ± 3.87^{ab}	43.0 ± 4.36^{bc}	42.4 ± 3.42^{ab}	45.2 ± 3.29^{d}
Sodium	4	18.7 ± 2.37^{a}	105 ± 5.21^{b}	141 ± 9.09^{c}	182 ± 16.6^{d}	447 ± 10.3^{e}	491 ± 28.2^{f}
	10	16.2 ± 1.65^{a}	100 ± 2.71^{b}	138 ± 13.5^{c}	180 ± 17.6^{d}	402 ± 15.9^{e}	511 ± 43.6^{f}
	25	18.3 ± 2.06^{a}	98.6 ± 6.42^{b}	141 ± 10.9^{c}	155 ± 15.7^{d}	430 ± 18.2^{e}	493 ± 40.9^{f}
	55	19.0 ± 2.49^{a}	102 ± 8.04^{b}	143 ± 8.21^{c}	187 ± 14.3^{d}	438 ± 17.5^{e}	540 ± 46.1^{f}

[a] Values followed by different superscript letters in the same row are significantly different ($p < 0.05$).

The results of this work are published in:

Barone, G., Cirrincione, F., Jiang, Y., Rauh, V., Lillevang, S. K., Caboni, M. F., Skibsted, L. H. & Ahrné, L. (2023). Influence of ionic strength and temperature on mineral and protein partitioning of skim milk, International Dairy Journal. 147, 7 p., 105764.

WP4. Calcium dynamics of acidified milk during storage

One key process in dairy production is acidification, where acids lower milk's pH which influence proteins interactions and induce gelation. The objective of the study was to understand the calcium dynamics of pasteurized skim milk acidified to pH ~ 5.8 with citric acid and lactic acid during storage at 5 °C up to 24 h and consequences for renneting. The results showed significant differences between ionic calcium in milk acidified with lactic acid and citric acid during storage at 5 °C for 24 hours. Lactic acid (LA) resulted in high ionic calcium (Ca^{2+}) in serum phase of milk and promotes faster aggregation and larger structures, particularly evident after 24 hours. Citric acid (CA), due to its calcium-binding capacity, resulted in low ionic calcium level in serum phase and more bound fraction, leading to less aggregation or more compact structures after 24 hours compared to lactic acid as observed by Small Angle X-ray analysis (SAXS). This has consequences for the rennet-induced gelation of milk as shown in Fig 2. Storage of CA acidified milk delays extensively renneting time after storage which is not observed for LA.

This study provided better understanding of the differences between the two acids as well as new insights for selecting the right acid to achieve specific gel properties, improving consistency and functionality in dairy products.

The manuscript will be published with the title of: "Dynamic Structural and Physicochemical Changes of Partial Acidified Milk: The Role of Citric and Lactic Acid for Casein Micelle Structure and Gel Formation"

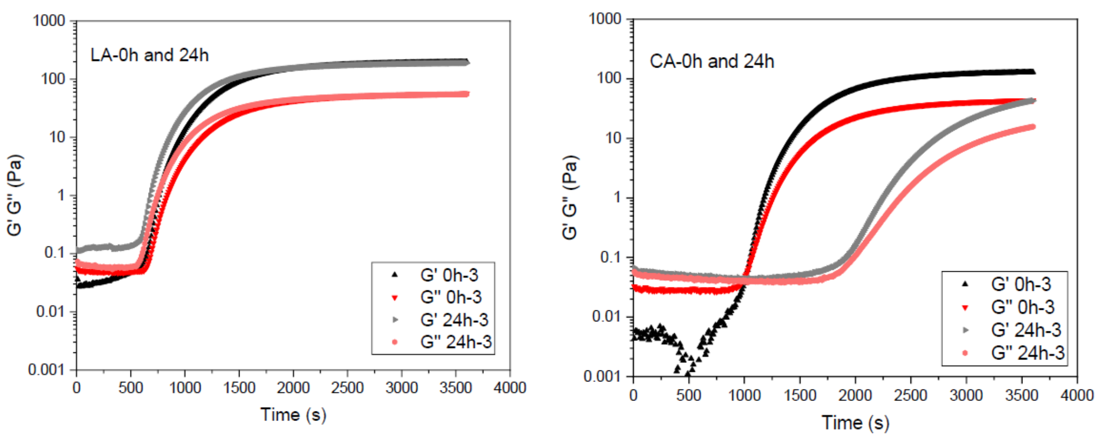

Figure 2. Rennet-induced gelation process of pasteurized acidified milk (left plot, with lactic acid) and (right plot with citric acid) not stored after acidification (0h) and stored for 24h monitored during a time sweep in a rheometer.

WP5: Extracting knowledge about the relationships between calcium content and binding status, and curd yield, texture and functional properties of cheese.

The results of the project have been implemented at Arla during the project and tested in Arla's pilot plant to optimize production and enhance cheese quality.

Optimizing Curd Yield and Texture: By adjusting calcium chloride levels and pH, we fine-tuned milk coagulation to improve curd formation and minimize whey protein loss. Textural modifications were achieved by altering calcium-protein interactions through controlled acidification and heat treatments, ensuring desired firmness and elasticity.

Enhancing Functional Properties: Calcium influences meltability and stretchability, essential for processed dairy applications. Renneting conditions and maturation protocols were optimized to enhance cheese performance and consistency.

12. The relevance of the results, including relevance for the dairy industry

Findings were translated into potential Arla's production processes, reducing variability and improving efficiency. These advancements support the development of cheese varieties with tailored texture and functional attributes, aligning with industry demands. Moreover, the knowledge generated by ProCalcium has provided fundamentals building of a new project called MinCurd also funded by the Milk Levy Fund and Arla Foods.

13. Communication and knowledge sharing about the project

Papers in international journals:

Barone, G., Cirrincione, F., Jiang, Y., Rauh, V., Lillevang, S. K., Caboni, M. F., Skibsted, L. H., & Ahrné, L. (2023). Influence of ionic strength and temperature on mineral and protein partitioning of skim milk. International Dairy Journal, 105764. https://doi.org/10.1016/j.idairyj.2023.105764.

Barone, G., Guadagnucci, S., Rauh, V., Lillevang, S. K., Caboni, M. F., & Ahrné, L. (2025). Minerals content and physicochemical properties of skim milk retentates and permeates during ultrafiltration at different temperatures. International Dairy Journal, 161, 106138.

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Easily read papers:

Calcium and Cheese, Calcium dynamics during manufacturing of cheese (2020), Mælkeritidende, October 2020. **https://mejeri.dk/media/15rkxeqy/lilia-ahrne_-procalcium.pdf**

Student theses:

Federica Cirrincione; Title: 'Influence of ionic strength and temperature on mineral and protein partitioning of skim milk'; Bologna University, Italy.

Sara Guadagnucci; Title: 'Minerals content and physicochemical properties of skim milk retentates and permeates during ultrafiltration at different temperatures'; Bologna University, Italy.

Oral presentations at scientific conferences, symposiums etc.:

1) *12th Nizo Conference, Innovation in Dairy Ingredients OCTOBER 2021.*
   Title of oral presentation: Calcium and b-casein partitioning in milk during processing: a kinetic study.
2) Nordic filtration Symposia, DECEMBER 2024.
   Title of oral presentation: The influence of different ultrafiltration set-ups on the mineral partitioning between skim milk stream.
3) *13th Nizo Conference, Innovation in Dairy Ingredients OCTOBER 2023.*
   Title of oral presentation: Minerals content and physicochemical properties of skim milk retentates and permeates during ultrafiltration at different temperatures.

14. Contribution to master and PhD education

In total, two master students were educated, with their final reports being essential for publication purposes.

15. New contacts/projects

The project has implemented a new toolbox at KU that has been used in a large number of ongoing projects and education. Furthermore, the project will continue in MinCurd.

16. List of references

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