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Lilia M. Ahrné:

GELCOOK – Afklaring af mekanismen bag dannelse af kogestabile mælkegeler

GELCOOK – Elucidating the mechanism of formation of cookable milk gels

Mejeribrugets
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Final report

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1. Title of the project

Danish: Afklaring af mekanismen bag dannelse af kogestabile mælkegeler (GELCOOK)

English: Elucidating the mechanism of formation of cookable milk gels (GELCOOK)

2. Project manager

Professor Lilia M Ahrné, Copenhagen University, Department of Food Science, Ingredient and Dairy Technology, Rolighedsvej 26, Frederiksberg C, +45 93 56 55 07, Lilia@food.ku.dk

3. Other project staff

PhD student Anne Karin Laursen, Copenhagen University, Department of Food Science, Ingredient and Dairy Technology, Rolighedsvej 26, Frederiksberg C, +45 23 74 68 63,
Research Assistant Olivia Mærsk, Copenhagen University, Department of Food Science, Ingredient and Dairy Technology, Rolighedsvej 26, Frederiksberg C
Professor Rickard Ipsen, Department of Food Science, Ingredient and Dairy Technology, Rolighedsvej 26, Frederiksberg C, +45 23 74 68 63, jjkk@food.ku.dk (first year of the project)

4. Sources of funding

Milk Levy Fund
Arla Food AMBA (Dr Tijs Albert Maria Rover)

5. Project period

Project period with DDRF funding: 01-01-2020 - 31-12-2022

6. Project summary

Danish: Varme- og syre-inducerede mælkegeler smelter ikke ved tilberedning eller stegning og kan derfor betragtes som en ikke-smeltende ost, hvilket gør dem interessante som et mejeribaseret kødalternativ. På grund af manglende viden om hvorfor mælkesammensætning og syrningstemperatur påvirker egenskaberne af den dannede gel, har nærværende projekt til formål at belyse dannelsen af direkte syrnede mælkegeler ved temperaturer ≥60 °C. Projektets resultater er opsummeret i fire videnskabelige publikationer. Forsuringstemperaturen viste sig i høj grad at påvirke hastigheden af koagulum dannelse og kemiske interaktioner inden for proteinnetværket. Koagulatet var hovedsageligt styret af calciumbindinger (30-63 %), som steg 71-187 % afhængigt af pH med temperaturstigning fra 60 til 90 °C. Ændring af mælkesammensætningen, enten

ved at ændre mælkekilderne, dvs. komælk (KM) eller bøffelmælk (BM), eller ved tilsætning af valleprotein- eller kasein miceller, viste, at både koncentration og proteintype påvirkede den dannede gel. BM har et højere kaseinindhold (3,4-3,8 %) sammenlignet med KM (2,8-3,0 %), hvilket resulterer i en mindre fugtig, tættere og hårdere gel. Korrelerer godt med den tætte og hårde gel dannet, når kaseinindholdet i KM blev øget til 4,8 %. Forøgelse af valleprotein-indholdet fra kun 0,7 til 0,9 % resulterede i en væsentlig fugtigere og blødere gel. Ved kogning bliver gelen betydeligt blødere. Men først når kogevandet blev saltet, steg gelerne, der var forsuret ved ≥ 75 °C, i masse og var således i stand til at optage vand. Ved at bruge Low-Field nuklear magnetisk resonans (LF-NMR) blev der fundet en bedre forståelse af påvirkningen af gelernes struktur, som effekt af behandling eller ændret mælkesammensætning. Med stigende forsuringstemperatur medførte et generelt fald i proton afslapningstider hvilket indikerede en proteinstruktur, der voksede tættere. Resultaterne udgør et væsentligt fremskridt til viden om forholdet mellem sammensætning, proteininteraktioner og proteinforhold i varme- og syre-inducerede mælkegeler. Indsigterne fra projektet kan udnyttes og bruges af mejeriindustrien til at modulere varmesyre-inducerede gelers funktionalitet.

English: Heat and acid-induced milk gels do not melt or flow upon cooking or frying and can therefore be considered a non-melting cheese, making them interesting as a dairy-based meat alternative. Due to a lack of knowledge of why milk composition and acidification temperature affect the properties of the gel formed, the present project aims to elucidate the formation of directly acidified milk gels at temperatures ≥60 °C. The results of the project are summarized in four scientific publications. Acidification temperature was found to greatly impact the speed of coagulum formation and chemical interactions within the protein network. The coagulum was mainly governed by calcium bonds (30-63 %), which increased by 71-187 %, depending on pH with the increase of temperature from 60 to 90 °C. Altering the milk composition, either by changing the milk sources, i.e. cow milk (CM) or buffalo milk (BM), or by the addition of whey protein or casein micelles, showed that both concentration and protein type influenced the gels. BM has a higher casein content (3.4-3.8 % w/w) compared to CM (2.8-3.0 % w/w), resulting in a less moist, denser, and harder gel. Correlating well with the dense and hard gel formed when the casein content of CM increased to 4.8 % w/w. Increasing whey protein content from just 0.7 to 0.9 % w/w resulted in a significantly moister and softer gel. Upon cooking the gel becomes significantly softer. However, only when the cooking water was salted did the gels acidified at ≥ 75 °C increase in mass and thus were able to take up water. Using Low-Field nuclear magnetic resonance (LF-NMR) a better understanding of the structure of the gel as affected by processing or milk composition was found. With increasing acidification temperature, a general decrease in relaxation times indicated a protein structure growing denser. The findings of this project constitute a significant advancement to the body of knowledge on the relationship between composition, protein interactions, and protein ratio of heat and acid-induced milk gels. These insights can be exploited and used by the dairy industry for modulating acid milk gel functionality.

7. Project aim

Danish:
Projektets formål er at:

1. Opnå en grundlæggende forståelse af, hvordan inducerede variationer i forarbejdningen (varmebehandling, pH, syrningshastighed, presning, shear-behandling mv.) påvirker sammensætning, mikrostruktur, reologi og tekstur i syre- og varmegelerede kødalternativer baseret på komælk.
2. Klarlægge de faktorer, der påvirker tilberedningsegenskaberne af syre- og varmeinducerede mælkegeler, samt forstå, hvordan den sensoriske kvalitet – særligt teksturen – kan maksimeres.

3. Belyse forskellene i mikrostruktur, tekstur og sensorisk kvalitet mellem syre- og varmeinducerede mælkegeler fremstillet af mælk fra forskellige arter (bøffel og ko).

English:
The objective of this project is:

1. Achieve a fundamental understanding of how induced variations in processing (heat, pH, acidification rate, pressing, shearing etc.) affect composition, microstructure, rheology and texture of acid and heat gelled meat alternatives from cow milk.
2. Clarifying the factors influencing cookability of acid and heat induced milk gels and understanding how to maximize the sensory quality especially in terms of texture.
3. To elucidate the differences in microstructure, texture and sensory quality of acid and heat induced milk gels prepared using milk from different species (buffalo and cow).

The project was planned as a PhD project.

8. Background for the project

Numerous novel protein sources have been and are investigated to produce sustainable food products to accommodate the growing world population and the general increase in consumer wealth. However, as consumers, we are habitual beings, and studies show that product familiarity is needed for the consumer to make sustainable choices. In this regard, familiar flavors and textures could come from cheese and cheese-like products.

Examples of already existing dairy products with meat substituting qualities are the Mexican queso blanco which is enjoyed both fried and fresh, the same accounts for the Indian paneer which is widely used as a meat alternative in curries. In Italy, ricotta cheese is also produced through a similar process, but from the whey side-stream of mozzarella production. These cheeses are non-melting, as they do not flow or melt upon heating, making them suitable as a total or partial meat substitute.

The Indian cheese-type paneer is a fresh cheese with a short shelf-life. It is produced from heat treated (≥85 °C ~ 5-15 min) milk by direct acidification at temperatures ≥60 °C and is therefore considered heat and acid-induced milk gel. It is milky white, has a firm texture, and mild flavor of cooked milk. Traditionally paneer is produced from buffalo milk (BM) or a mix of cow milk (CM) and BM. Literature on paneer is most often concerned with the optimization of the manufacturing process to reach high textural and sensorial quality paneer, from the milk of buffalo, cow, or other ruminants (Arvind et al., 2019; Desai et al., 1991; S. Singh & Kanawjia, 1988; Tellabati et al., 2022; Vishweshwaraiah & Anantakrishnan, 1985). The intrinsic differences in composition and compositional properties of CM and BM, result in very different paneer characteristics in terms of texture and flavor. To accommodate a growing demand for paneer, BM production did not suffice, why many studies have been conducted to optimize the production of paneer from CM to achieve similar properties to BM paneer (Arvind et al., 2019; Desai et al., 1991; Masud et al., 1992; Mistry et al., 1992; S. Singh & Kanawjia, 1988; Vishweshwaraiah & Anantakrishnan, 1985).

However, there are only limited literature describing the influence of processing on the microstructure, chemical and functional properties, and cookability of CM paneer (Desai et al., 1991; Kaláb et al., 1988; Kaushik et al., 2004; Mistry et al., 1992; H. K. Sharma et al., 1998).

Microstructurally heat and acid-induced milk gels can be described as emulsion-filled gels. The protein-based gel is filled with serum, present continuously in the gel, and milk fat droplets, embedded in the continuous gel structure (Dickinson, 2012). The protein gel is formed through the coagulation of aggregated whey protein (WP) and WP and casein (CN) aggregates, resembling the formation of yogurt. However, heat and acid-induced milk gels differ in microstructure and texture from yogurt, being a dense and solid-like gel. In texture and solid content, heat and acid-induced gels resemble soft yellow cheese. However, heat and acid-induced milk gels have a much more inhomogeneous and open pore-filled structure filled with serum or fat, compared to yellow cheese. The reason for these differences arises from the structure formation process of acidification at temperatures ≥60 °C, which is largely not understood.

9. Sub-activities in the entire project period

WP 1: Investigating fundamental aspects of combined heat and acidification of milk (Months: 1-12)

D1.1 Scientific paper on effect of heating and direct acidification on protein aggregation

WP 2: Understanding how processing affects microstructure in cookable milk gels (Months: 6-18)

D2.1 Scientific paper on microstructure of milk gels made using heating combined with direct acidification

WP3: Cookability and sensory properties of milk gels made by heating combined with direct acidification (Month: 24-36)

D3.1 Scientific paper on cookability of milk gels made using heating and direct acidification

D3.2 PhD thesis written and submitted

WP4: Upscaling of process for cookable milk gels (Months: 30-33)

D4.1 Pilot scale experiments performed using selected process parameters.

10. Deviations

The project did to a large extent run as planned in the initial expression of interest to DDRF. Although, due to covid and lack of commitment from India, and limited exchange of knowledge occurred with India. The activities in each work package were divided in a different way than the proposal to fulfill the expected final output of the project. The scale up of the project in WP4 was performed at Arla during the exchange of research environment of Anne Katrin Laursen.

11. Project results

WP 1: Investigating fundamental aspects of combined heat and acidification of milk

T2.1 Influence of milk source, fat content, and heat load on the texture, composition, and microstructure of heat and acid-induced gels from cow or buffalo milk

Objective: Investigation of the effect of milk composition by characterization of the compositional, textural, and microstructural differences in heat and acid-induced milk gels produced from cow milk (CM) and Buffalo milk (BM), with various fat content, under the same processing conditions

Main results: The compositional analysis showed that the moisture contents of the CM gels were higher than that of the BM gels, while fat and protein content were significantly higher for the BM gels, independent of processing conditions. Due to the naturally higher protein content of BM, the calcium content was, as expected, also the highest in these gels. The intensity of the heat treatment at 90°C up to 12 min affected mainly moisture and protein content. The texture analysis results (TPA) showed that initial fat content had the biggest influence on the cheese texture. As would be expected, increasing the initial fat resulted in a softer gel, because the milk was not homogenized, the protein and fat did not interact and the fat was mainly present in the gel as an inert filler, softening the gel.

In conclusion, this section shows that the differences in acidification speed and heat sensitivity between CM and BM are highly related to the difference in CN content and the derived mineral content, mainly calcium. Furthermore, we observed that fat played a minor role in the texture and composition of gels produced from milk heated for a short time (0 min) but highly affected the moisture content and texture at a long heat treatment time (12 min). This was attributed to the fat primarily acting as an inert filler, interrupting and therefore reducing the hardness of the gel structure. Thus, more fat would have a larger influence on the texture of the gel and deserve further studies.

The results of this work are published in:

Laursen, A. K., Rovers, T. A. M., Ipsen, R., & Ahrné, L. (2022). Texture and microstructure of heat and acid-induced gels from buffalo and cow milk: Effect of thermal treatment and fat content of milk. International Dairy Journal, 129, 105299. https://doi.org/10.1016/j.idairyj.2021.105299.

T 2.2 Effect of milk protein type and concentration on the texture, composition, and microstructure of heat and acid-induced milk gels

Objective: Investigation of the effect of milk composition by modifying casein to whey protein ratio, and concentration on textural and microstructural properties of heat and acid-induced milk gels.

Main results: Increasing whey protein isolate content (WPI) resulted in higher moisture content and decreased calcium content in the gels. The addition of MCI increased the content of calcium slightly, whereas WPI lowered (diluted) the mineral content. Fat content showed an inverse U-shape, with the highest fat content in the gel made with natural CN:WP, 4.55, and CN:WP of 5.25, while the lowest fat content was observed at CN:WP of 0.85. However, the addition of MCI resulted in a significantly higher fat content for the extreme addition of 2 % MCI (CN:WP 7.33).

The compositional results give indications that increasing WP content would result in a more pore-filled gel structure with high moisture and a dense and dry CN gel. CLSM micrographs presented in Figure 1 (ABC) clearly show that the addition of MCI increases gel density (Figure 1A), whereas increasing WPI content results in a more porous structure (Figure 1C). In Figure 1 (DEF) the visual gel structure without magnification is also presented. From these it is further obvious that increasing CN content by adding 2 % MCI, results in also a visually compact and dense structure, seemingly formed by smaller curds (Figure 1D). Whereas the addition of 2 % WPI gives a more coherent-looking structure with many small holes and a few larger holes (Figure 1F). The merging of the curds does however not seem to be affected by the curd size. The texture measurements showed increasing protein content, independent of protein type. Indicating that moisture content has little effect on the hardness of the gels, instead, the rigidity and type of protein bonds probably are the source of the effect.

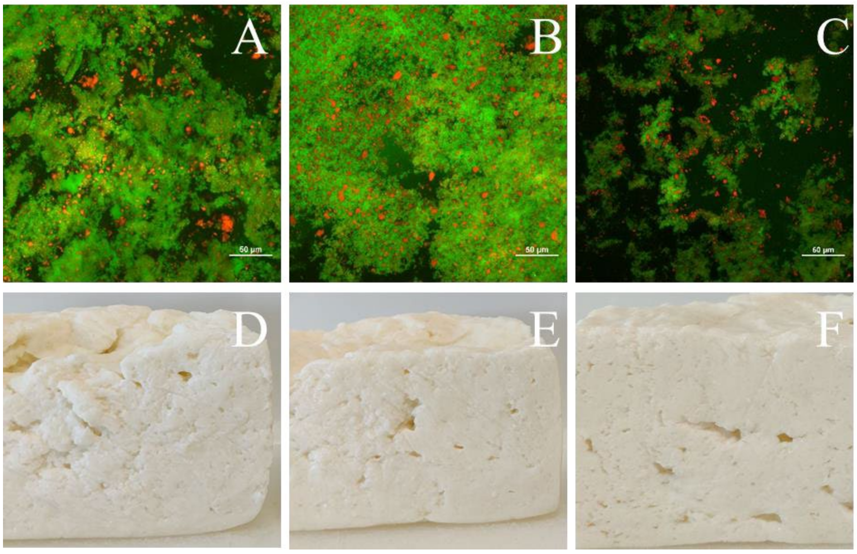

Figure 1. CLSM micrographs and images of heat and acid-induced milk gels produced from full-fat milk with 2% added micellar casein isolate (A, D), no addition (B, E), and 2 % added whey protein isolate (C, F)

In conclusion, altering CN:WP and protein concentration showed a very efficient approach to altering the microstructure and texture of heat and acid-induced milk gels. The addition of MCI resulted in increased calcium content, forming a dense and rigid gel. This observation was confirmed by LF-NMR, where relaxation times of MCI-added gels decreased, thus the water was more bound to the protein structure. Whereas the addition of WPI diluted the calcium resulting in a soft gel with high moisture content, with a larger contribution of free water.

The results of this work are published in:

Laursen, A. K., Rovers, T. A. M., Berg F. v. d., & Ahrné, L. (2023). Formulation of heat- and acid-induced milk gels – Effect on texture, microstructure, and water distribution, Food Structure, Volume 37, 100341, https://doi.org/10.1016/j.foostr.2023.100341.

WP 2: Understanding how processing affects microstructure in cookable milk gels

T2.1 Effect of acidification temperature on the texture of pressed heat and acid-induced milk gels
Objective: Understanding the effect of acidification temperature and final pH on the composition, and chemical protein interactions of the resulting gel.

Main results: Figure 2 shows the pH decrease caused by the addition of citric acid (0.01M) to milk at temperatures 60, 70, 80, and 90 °C. Until a pH of approximately 5.5 the rate of pH decrease is independent of the acidification temperature. After this pH, the pH of milk acidified at 90 °C decreases at the fastest rate, whereas increasingly slower rates are observed for 80-60 °C. At pH 5.5 the acid-induced gelation between β-lg aggregates and WP-covered CN micelles begins (Morand et al., 2011; Morand, Guyomarc'h, et al., 2012). The mineral solubility, e.g. CCP, decreases with increasing temperature. However, the pH decrease increases the mineral solubility, and calcium is released from the CN micelles to the serum phase (Schmitt et al., 1993; Wang & Ma, 2020). The ability of calcium to be released from the CN micelle is hindered by the protein aggregation and formation of coagulum (Horne, 1998). Thus, the coagulum formation at 90 °C is likely fast enough to retain calcium. Whereas for the lower acidification temperatures, more calcium (CCP) is solubilized from the CN micelles, and therefore more citric acid is needed to decrease the pH. Thus, less acid is needed to reach the wanted pH at 90 °C compared to 60 °C.

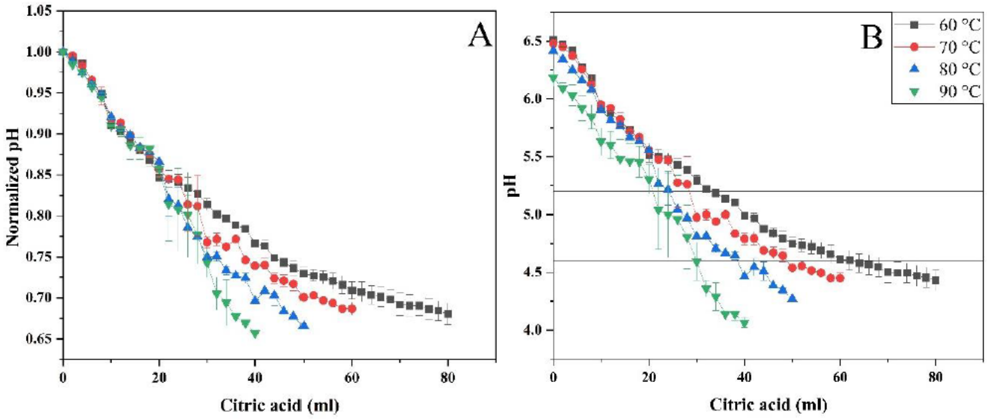

Figure 2. (A) Normalized pH and (B) pH decrease in pre-heated (90 °C, 12 min) milk during acid addition at a rate of 20 ml/min, at milk temperatures 60, 70, 80, and 90 °C.

These differences in acidification temperature affect the gel formation and composition of the gels during direct acidification of pre-heated CM. A decrease of roughly 10 % in moisture content between the gels acidified at 60 and 90 °C both pHs (5.2 and 4.6). This decrease reflects enhanced aggregate and gel density with rising acidification temperature, as the aggregation speed increases. For acidification temperatures of 70 and 80 °C, a significant difference in moisture was also observed between the two pHs. Yield followed the moisture decrease and the inverse trend was therefore observed for protein and fat as they were measured on wet basis. Protein content and calcium content are inherently connected as CCP is present in the CN micelle. Therefore, a significant increase in calcium pr. g of protein in the gels acidified at temperatures 60 to 80 °C, was observed.

The microstructure of the gel was assessed using LF NMR and the distribution of protein bonds stabilizing the gels. Figure 3 shows that the higher calcium content for gels acidified at 80 and 90 °C was reflected in the increasing contribution of CaB to the chemical stabilization of the protein gel. For pH 5.2 and 4.6, the increase in CaB occurred from 60 to 80 °C (6 and 54 % respectively), then stagnating as also observed for calcium pr. g of protein. EB and HB dominated at pH 4.6 and Hy dominated at pH 5.2.

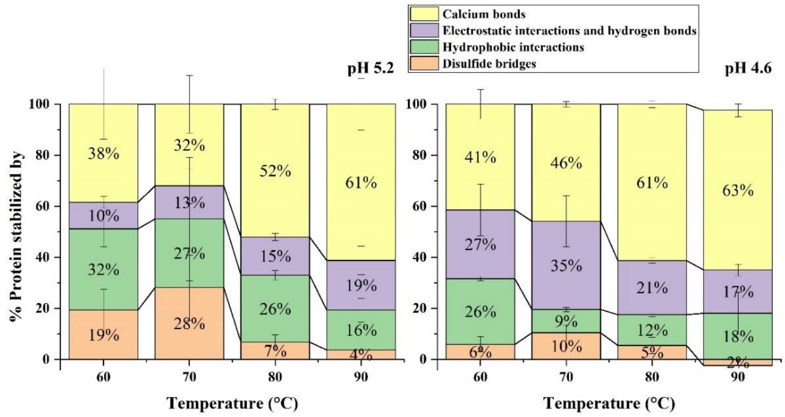

Figure 3. Distribution of protein bonds stabilizing (%) heat and acid-induced milk gels acidified to pH 5.2 (left) and 4.6 (right). The bonds described are calcium bonds (yellow), electrostatic interactions and hydrogen bonds (purple), hydrophobic interactions (green), and disulfide bridges (orange). Data presented is the mean value ± SD (n = 3)

With increasing acidification temperature, a general decrease in relaxation times observed by LF MNR indicated a protein structure growing denser. The bond types underpin this as they changed, for pH 5.2, with increasing acidification temperature, toward stronger bonds i.e. from Hy to HB, EB, and CaB. The deprotonation of the functional sidechains, e.g. cysteine, and asparagine, as pH decrease drives the change toward the stronger bonds (Amaro-Hernández et al., 2022). When the gels were acidified to pH 4.6, the κ-casein layer of the CN micelle collapsed allowing Hy between the micelles. Hy is however not stable at high temperatures, so the contribution decreases with the temperature. Stronger bonds will, in turn, result in an increasingly dense gel, holding less moisture and therefore decreasing the amount of free water.

To conclude, the results show that gel formation and chemical cohesion are highly dependent on the temperature at acidification, as the temperature influences the coagulation speed and mineral partitioning. The increasingly dense aggregates formed at higher acidification temperatures (> 80 °C), observed by LF-MNR as shorter relaxation times, also showed stronger chemical bonds resulting in denser aggregates. Therefore, it is relevant to assess how these effects influence the texture of the pressed gels, in the next study (T2.2) the effect of acidification temperature on the gels after pressing is reported.

The results of this work are published in:

Laursen, A. K., Czaja, T. P., Rovers, T. A. M., Ipsen, R., Barone, G., & Ahrné, L. (2023). The effect of acidification temperature and pH on intermolecular protein bonds and water mobility in heat and acid-induced milk gels. International Dairy Journal, 105611. https://doi.org/10.1016/j.idairyj.2023.105611.

T2.2. Effect of acidification temperature on the texture of pressed heat and acid-induced milk gels

Objective: Understanding the effect of acidification temperature on the composition and texture of pressed heat and acid-induced milk gels.

Main results: The composition and yield of gels are presented in Table 1. The differences observed occurred mainly above and below acidification temperature 75 °C. Thus, the acidification temperature 60 °C resulted in a more moist gel and it was therefore the gel with the highest yield, as was also observed for the un-pressed gels. From that followed a lower protein and fat content in gels acidified at 60 °C. Despite the lower protein content, the acidification temperature of 60 °C, resulted in the second-highest calcium content pr. g. of protein. This was not observed for the un-pressed gels reported in T2.1, but may be a consequence of a high amount of calcium-containing serum retained by the gel. The compositional observations of the pressed gels are otherwise in good alignment with the unpressed, which showed the main differences occurred above and below an acidification temperature of 80 °C.

Table 1. Composition in terms of moisture (w/w %), protein (w/w %), fat (%), total calcium (mg/g protein), and yield (w/w %), of heat and acid-induced milk gels acidified at 60, 75, and 90 °C.

Temperature °C	Moisture (w/w %)	Protein (w/w %)	Fat (%)	Total calcium (mg/g protein)	Yield (w/w %)
60	64.51 ± 3.14^{a}	14.86 ± 0.41^{a}	16.25 ± 0.52^{a}	18.32 ± 0.17^{a}	25.49 ± 1.29^{a}
75	53.59 ± 2.33^{b}	20.71 ± 1.81^{b}	19.17 ± 1.63^{ab}	17.61 ± 0.17^{b}	18.62 ± 0.84^{b}
90	53.01 ± 3.97^{b}	19.98 ± 1.37^{b}	20.25 ± 2.07^{b}	20.93 ± 0.18^{c}	17.11 ± 0.43^{b}

Superscripts$^{a\text{-}c}$ denotes a significant difference ($P < 0.05$) between temperatures.

The hardness is negatively correlated to moisture, meaning that the harder gels formed at 75 and 90 °C contain less moisture (Figure 4). Counterintuitive fat is positively correlated with hardness, thus more fat results in a harder gel which implies that fat is present in the gel as an active filler (Aguilera & Kessler, 1989, Scholten, 2017).

In terms of calcium content, we observed a significant difference between all three acidification temperatures (Table 1). More calcium pr. g of protein results in a harder gel (Figure 1C).

Agreeing with the observations for the un-pressed gels the relaxation times obtained by LF NMR showed a decrease with the increasing acidification temperature as the gel grows denser. Only a minor difference was observed between acidification temperatures 75 and 90 °C.

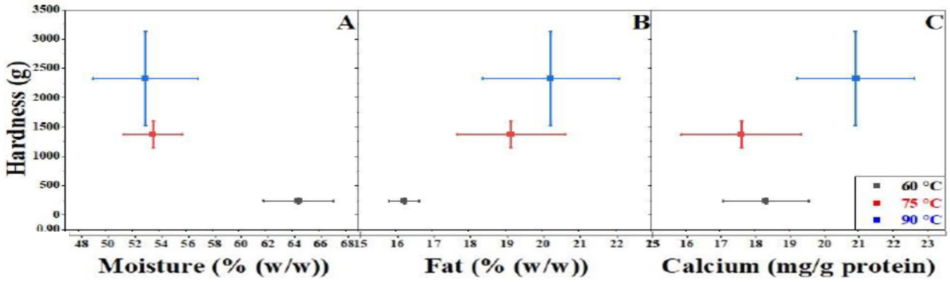

Figure 4. Textural attributes expressed as hardness (g) (A, B, C) of heat and acid-induced milk gels formed at temperatures 60 (grey), 75 (red), and 90 °C (blue) related to the composition in terms of moisture (%) (A), fat (% w/w) (B), and calcium (g/g protein) (C).

In conclusion, after pressing the gel, the curds merge and entrap both fat and serum in the protein network structure. The effect of the increasing aggregation speed as dictated by acidification temperature was confirmed by texture analysis (TPA). The increasingly dense aggregates formed a harder and denser, but not more cohesive gel. These results were further confirmed by the decreasing relaxation times indicating that the water present was more bound to the gel structure after pressing, determined by LF-NMR. Despite the harder texture, more fat was retained, actively reinforcing the gel network.

The results of this work are published in:

Laursen, A. K, Dyrnø S. B., Mikkelsen K. S., Czaja, T. P., Rovers, T. A. M., Ipsen, R., & Ahrné, L. (2023). Food Research International, Volume 169, 112846, https://doi.org/10.1016/j.foodres.2023.112846.

WP3: Cookability and sensory properties of milk gels made using heating combined with direct acidification

Objective: Understand the influence of acidification temperature on the cooking integrity of heat and acid-induced milk gels from cow milk. (Paper 4)

Main Results: The composition of the gels was affected by cooking depending on the acidification temperature of the gels. For the gel produced at acidification temperature 60 °C, moisture and therefore mass was lost during cooking indicating gel contraction rather than swelling. The reduced moisture content has the derived effect that the relative fat and protein content increases. However, the relative calcium content decreased, especially for the samples cooked w/NaCl, indicating an exchange of calcium-containing serum between the gel and the cooking water. For the gels produced at acidification temperatures of 75 and 90 °C there is moisture and mass loss for gels cooked in water, whereas cooking w/NaCl resulted in a significant mass increase.

The gel hardness was reduced significantly for all three acidification temperatures after cooking. The resilience and cohesiveness of the gel, on the other hand, increased. However, no effect of the salt content of the cooking water on the texture was observed. This increase in hardness was explained by LF-NMR that showed that the general increase in relaxation times, observed with cooking, indicated swelling of the protein network. The increase in relaxation times indicated that the structure was becoming more open as it was cooked.

In conclusion, the gels with the best cookability, i.e. gel stability and water uptake, were acidified at temperatures ≥ 75 °C. These gels swell and take up moisture, e.g. sauce in a curry. Whereas lower acidification temperatures (60 °C) resulted in weak gels which retained a lot of moisture during production but contracted upon cooking.

The results of this work are published in:

Laursen, A. K, Dyrnø S. B., Mikkelsen K. S., Czaja, T. P., Rovers, T. A. M., Ipsen, R., & Ahrné, L. (2023). Food Research International, Volume 169, 112846, https://doi.org/10.1016/j.foodres.2023.112846.

WP4: Upscaling of process for cookable milk gels (Months: 30-33)
Knowledge was transferred during the exchange of research environment of Anne Katrine Laursen at AIC and trials carried out at pilot scale, including the effect of cooling and sensorial evaluation

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

The project will generate tools and fundamental knowledge to understand and control microstructure formation in acid and heat induced gels, in order to create attractive cookable dairy gels. This knowledge will be beneficial for the Danish dairy industry to create a range of novel gelled dairy products with increased cooking functionality e.g. aimed at the dairy dinner sector (meat alternatives). In addition, the project will improve the quality of paneer type products made from cow milk. More specifically:

1. *Increased knowledge on the fundamentals of combined heat and acid induced milk gels.*
   The project has created knowledge enabling novel cookable products for various markets. There is an increasing consumer driven demand for novel meat alternatives. The fundamental knowledge on how to create novel cookable dairy products with increased functionality will be very beneficial for the Danish dairy industry and can be explored in development of new products and enabling products aimed at the dairy dinner sector.
2. *Achieving a more in-depth fundamental understanding of what governs cookability of milk gels using combined heating and direct acidification and how this can be controlled to produce attractive products.*
   Cookability in itself is a much sought after property and clarification of changes in the gels during cooking provided new opportunities for development of novel products on the market and improvement of exiting products
3. *Clarification of the differences between milk gels produced from cow and buffalo milk.*
   This knowledge provided an excellent base to understand how cow milk can be used to produce products that are similar to buffalo milk paneer. But also created the idea to produce these products using mixed milks, which generated new products as will be later discussed.
4. *Knowledge enabling production of cow milk meat analogues with high sensorial attributes, e.g. paneer with a quality equivalent to that made from buffalo milk by better processing or formulation.*
   This is relevant to the Danish dairy companies who wants to aim products at consumers wanting meat.

New projects: this project generated two projects, (i) one postdocs project about hybrid heat acid gels (type paneer) where plants and milk were combined with excellent results (see Popular cooking cheese made with peas yields same taste and texture – University of Copenhagen). (ii) a PhD project on going to further understand the effect of formulation of processing to modulate textural and sensorial properties.

13. Communication and knowledge sharing about the project

Papers in international journals:

Laursen, A. K., Czaja, T. P., Rovers, T. A. M., Ipsen, R., Barone, G., & Ahrné, L. (2023). The effect of acidification temperature and pH on intermolecular protein bonds and water mobility in heat and acid-induced milk gels. International Dairy Journal, 105611. https://doi.org/10.1016/j.idairyj.2023.105611.

Laursen, A. K., Rovers, T. A. M., Ipsen, R., & Ahrné, L. (2022). Texture and microstructure of heat and acid-induced gels from buffalo and cow milk: Effect of thermal treatment and fat content of milk. International Dairy Journal, 129, 105299. https://doi.org/10.1016/j.idairyj.2021.105299.

Laursen, A. K, Dyrnø S. B., Mikkelsen K. S., Czaja, T. P., Rovers, T. A. M., Ipsen, R., & Ahrné, L. (2023). Food Research International, Volume 169, 112846,https://doi.org/10.1016/j.foodres.2023.112846.

Laursen, A. K., Rovers, T. A. M., Berg F. v. d., & Ahrné, L. (2023) Formulation of heat- and acid-induced milk gels – Effect on texture, microstructure, and water distribution, Food Structure, Volume 37, 100341, https://doi.org/10.1016/j.foostr.2023.100341.

Easily read papers:

Mælkegeler som kød-alternativ, Mælkegeler som kød-alternativ (2021), Mælkeritidende, April 2021.

Student theses:

Methods development and application on fresh curds size distribution analysis of an Indian cheese – Paneer, Xuanrong Zhang (MSc thesis at University of Copenhagen, 2020).

Effect of Coagulation Temperature on the Textural Characteristics, Composition and Water Mobility of Raw and Cooked Indian, Kim Steven Mikkelsen and Steffan Bue Dyrnø ((MSc thesis at University of Copenhagen, 2021).

How is Heat and Acid Induced Gel Affected by Different Casein and Whey Protein Content, Ziqian Gao (MSc thesis at University of Copenhagen, 2022).

Small-Angle X-ray Scattering analysis in heat- and acid-induced skim milk gels when varying acid type and acidification temperature, Marius Birkemose and Julius Jessen Terp (BSc thesis at University of Copenhagen, 2022).

Training of students from India; Satya, Ziqian, Harshal, Sneha, and Richa.

Oral presentations at scientific conferences, symposiums etc.:
Structure formation in non-melting cheeses, Dairy research day in Herning 16th March 2023. (Lilia Ahrné).

Processing and formulation of cow milk for improved texture and cookability of Paneer, Cooking Cheese Symposium, Billund, 7 December 2023 (Lilia Ahrné).

Modification of texture and microstructure in cow and buffalo milk paneer, by thermal treatment and milk fat content, IDF International Cheese Science and Technology Symposium, Online, June 2021 (Anne Katrin Larsen).

Modification of texture in cow and buffalo milk paneer by thermal treatment and milk fat content, Dairy Science and technology symposium, Online, June 2021 (Anne Katrin Larsen).

Comparison of texture and microstructure of paneer from buffalo and cow milk: effect of thermal treatment and fat content of milk, NIZO Dairy Conference, Innovation in Dairy Ingredients, Online, October 2021 (Anne Katrin Larsen).

Poster presentations at scientific conferences, symposiums etc.:
Heat and acid-induced milk gels – Effect of coagulation temperature and pH on protein network structure, Food structure and functionality Symposium, Cork, Ireland, September 2022.

Non-melting cheese - understanding the effect of acidification temperature on the structure of heat and acid-induced milk gels, IUFoST Singapore, November 2022.

Oral presentations at meetings:
DDRF technology meeting September 2021.
DDRF technology meeting October 2023.

14. Contribution to master and PhD education

PhD thesis of Anne Katrine Laursen

Elucidating the formation of cookable milk gels, Copenhagen University, February 2023.

Teaching at MSc program – Dairy specialization

Dairy product technology 1 & 2, MSc course (2021, 2022).
Supervision of 3 MSc thesis projects (2020, 2021, 2022) and 4 BSc internships (2022).

15. New contacts/projects

Hybrid paneer – Postdoc project

Processing and Formulation of acid heat induced gels – PhD Zhe Cheng (CSC, Yili).

New applications under discussion to be submitted as a PhD project.

16. List of references

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