Scientific note

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Can frozen stored curd be used for making caprine white brined cheese?

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Abstract

This study explored the potential of overcoming the seasonal nature of caprine milk by using frozen curds from late lactation, frozen at two different pressing stages, to produce a 14-day ripened white brined cheese. Frozen curds present notable advantages over frozen milk as a raw material, including reduced storage space requirements, the elimination of whey production, and lower water consumption compared to powdered milk. Textural analysis of the resulting cheeses demonstrated significantly reduced firmness by the 14th day of ripening in brine. Microstructural examination using Scanning Electron Microscopy (SEM) on the 1st day of ripening revealed that the experimental cheeses exhibited a disordered and less compact structure compared to the control samples. By the 14th day, the experimental cheeses disintegrated in brine, due to increased water absorption associated primarily with structural changes of the cheese matrix. To address these challenges, the study proposes two viable strategies for successful white brined cheese production: achieving a lower pH through prolonged traditional salting methods or shortening the maturation period in brine.

Keywords: white brined cheese; caprine milk; microstructure; texture; frozen storage

Introduction

Producing cheese from caprine milk year-round is challenging. Caprine milk production follows a seasonal pattern, with kidding occurring in winter and early spring, and peak production happening during the grazing season in summer. To overcome this issue, several approaches have been tested. Freezing milk (Kljajevic et al., 2024; Yu et al., 2021), curd (Campos et al., 2011; Seçkin et al. 2011), or cheese (Park, 2013) has shown mixed results, with issues like softer texture, milder taste, reduced yield, and lower viable microorganism counts (Alichanidis et al., 1981; Campos et al., 2011).

Previous studies on milk from the same Saanen goat flock demonstrated that freezing milk-compared to freezing curds after various stages of pressing, followed by thawing in water or directly in whey-best preserves the quality of the resulting caprine white brine cheese (Kljajevic et al., 2017). However, concerns may arise regarding the practicality of the required frozen storage capacity. Apart from the aforementioned study, no research has specifically explored the production of white brined cheese exclusively from thawed caprine curd. However, studies such as those by Picon et al. (2010) and Sendra et al. (1999) on freezing bovine or mixed milk curds offer promise that similar methods could be applied to caprine milk, allowing thawed caprine curds to be used effectively. Moreover, frozen cheese curd offers the one additional advantage that no whey is produced, while the water requirement is less than when using milk powder, as a raw material (Schmidt and Hinrichs, 2024).

A few aims are set for this study. The cost of raw materials in cheese production can account for up to 87 % of total expenses (Schmidt and Hinrichs, 2024), making frozen curd a more cost-effective option compared to freezing milk, as it requires less storage space (Alichanidis et al., 1981). On the other hand, we hypothesized that frozen storage might lead to deterioration in the technological and functional properties of curd and aimed to investigate potential areas for improvement.

Considering that, we want to provide more insight into a more economically viable alternative to produce caprine white brine cheese year-round, beside the already investigated frozen stored caprine milk. Lastly, it could be hypothesized that cheese curd frozen at different pressing stages will exhibit varying technological properties upon thawing. By analysing the properties of each variant, the freezing and thawing methods can be optimized.

Materials and methods

Three separate batches of raw caprine milk (30 L) had been used for the cheese making process that was carried out in three consecutive weeks in November of 2015. Milk was transported from Saanen goat farm in Kukujevci (Serbia) to the pilot plant (Faculty of Agriculture, University of Belgrade) at a temperature of 4 °C.

Cheesemaking process, freezing and thawing of curds

In this study, we used the same control cheese data that were previously published by Kljajevic et al. (2024). These data were originally collected under the same experimental conditions as in the current study which is a parallel branch to the aforementioned study. Two branches of research were done simultaneously from the same batch of caprine milk, and all analyses were done in the same laboratory with identical methods and by the same researchers. This allowed for the usage of control group data for comparison in the present study.

The same production procedure was used for control and experimental cheeses. Briefly, milk was pasteurized at 65 °C/30 min, cooled to 31 °C, then calf chymosin powder (94 % of chymosin, 6 % pepsin) (Clerici-Sacco Group, Cadorago, Italy), commercial starter culture MFC-4 (Biochem s.r.l., Montelibretti, Italy) and 0.02 % (w/v) of $CaCl_2$ were added. The coagulation lasted 45 min at 31 °C, the gel was cut in ≈5 cm cubes and left to rest for another 15 min. The cheese mass was then carefully transferred from the cheese vat into the mold. The curd was drained for 20 min and then pressed in two stages: (i) 2 kg per 1 kg of curd; (ii) 4 kg per 1 kg of curd. Each stage lasted 1 h at room temperature (≈ 25 °C). After pressing, the cheese was cut into blocks, the surface was dry-salted and it was left over night at room temperature. Cheese blocks were matured in a brine solution containing 6 % NaCl and a pH of 4.6, adjusted using 80 % lactic acid (Sigma Aldrich, Steinheim, Germany), for a period of 56 days (8 weeks).

Experimental cheeses followed the same procedure until coagulation and draining. After coagulation and draining, two parts of curd were taken out of the initial curd mass and pressed separately. After the first stage of pressing, the first part of the curd was frozen. Cheese made from this curd was labeled FC (Frozen Curd). The second part of the curd was frozen after the second stage of pressing. Cheese made from this curd was labeled FPC (Frozen Pressed Curd). Frozen storage of curds lasted 60 days at -27 °C.

Curd samples were thawed in the refrigerator at 4 °C for 36 h. After thawing, curds were processed, as described for the control cheese (Kljajevic et al., 2024). The ripening of experimental cheeses lasted 14 days at 12-14 °C. After 14 days, both experimental cheeses disintegrated in brine.

Physico-chemical properties of cheeses

Chemical composition was determined with the following methods: fat content (FIL-IDF 1986); nitrogen content (AOAC, 1990); dry matter content (FIL-IDF, 1982); NaCl content (FIL-IDF, 1988). The following parameters were calculated: protein content as nitrogen content multiplied by 6.38 and moisture in non-fat solids (WnFS). To measure pH value of cheeses, 10 g of cheese was thoroughly mixed with 10 mL of distilled water using a mortar and pestle (Kljajevic et al., 2024). The pH value was measured using a digital pH-meter (Consort, Turnhout, Belgium). Cheese samples were analysed on the

1st and 14th day after production. Measurements were done in triplicate.

Texture profile analysis

Sample preparation and methodology was as described by Kljajevic et al. (2024) and Romeih et al. (2002). Briefly, Samples were prepared by cutting the cheese into cylindrical shapes with a height and diameter of 15 mm. In this experiment, the cylindrical probe (P/25 mm) of Texture Analyzer TA.XT Plus (Stable Micro System, Surrey, UK), with compression cell in capacity of 5 kg. Texture profile analysis (TPA) of cheeses was done at 1 and 14 day of ripening. Attributes obtained from TPA test were: cohesiveness (dimensionless), springiness (dimensionless), hardness (g) and chewiness (g).

Microstructure of cheeses

Sample preparation was the same as described by Kljajevic et al. (2024):

1. Cheese samples were cut into 1×1×10 mm cubes and fixed with 2.8 % glutaraldehyde in phosphate buffer (pH 6.00/0.05 M sodium phosphate) for 48 h at a temperature of 7 °C.
2. Samples were dehydrated in rising concentrations of ethanol solution, 1 h for each concentration. Dehydrated samples were defatted with chloroform.
3. Lastly, samples were dried with liquid CO_2 in critical point dryer CPD 030 (BAL-TEC, Scotia, NY, USA). Coating of samples with gold was done with sputter coater SCD 005 (BAL-TEC) at a current of 30 mA and a duration of 100 s.

Scanning electron microscopy was done with JEOL JSM-6390 LV (USA) at 10kV and magnifications: 1000, 2000, 5000 and 10 000, at 1st day of ripening.

Statistical analysis

Analysis of variance (ANOVA) was used to investigate the effects of freezing in different stages of pressing and the maturation period on physicochemical properties and textural characteristics. Tukey test was used as a post hoc. Conclusions were drawn at a significance level of p=0.05. Analysis was conducted with STATISTICA 10 (Stat Sof. Inc., Tulsa, USA).

Results and discussion

The chemical composition and textural properties of the cheeses were analysed over the 14-day maturation period, revealing significant changes. Protein content, pH values, milk fat, and dry matter content decreased significantly by the 14th day compared to the 1st day, while moisture in non-fat substances (WnFS) showed a statistically significant increase (Table 1). No compositional differences were observed between the experimental cheeses (FC and FPC) and the control variant, indicating uniformity in baseline composition. However, analysis of variance identified interaction between pressing stages and the maturation period, influencing protein, milk fat, dry matter content, and WnFS.

Concurrently, textural analysis demonstrated that all textural attributes, including hardness, and chewiness,

Table 1. *Physico-chemical characteristics of control and experimental cheeses (P - protein %; MF - milk fat %; DM - dry matter %; WnFS - water in non-fat solids; FC - cheese made from curd frozen after first stage of pressing; FPC – cheese made from curd frozen after second stage of pressing)*

	Maturation day	Control*	FC	FPC
pH	1	5.49±0.47^{aA}	5.73±0.29^{aA}	5.40±0.12^{aA}
	14	5.24±0.10^{aA}	5.48±0.11^{aA}	5.16±0.05^{aA}
P	1	20.99±0.80^{aA}	22.85±1.50^{aA}	24.30±2.06^{aA}
	14	17.36±1.85^{aA}	14.84±1.20^{aB}	13.48±2.60^{aB}
MF	1	19.50±0.43^{aA}	21.25±0.43^{aA}	20.92±0.38^{aA}
	14	16.83±2.03^{aA}	13.42±0.88^{bB}	13.42±0.52^{bB}
DM	1	45.23±0.77^{aA}	49.27±2.19^{aA}	49.30±2.03^{aA}
	14	39.31±2.99^{aB}	33.06±1.40^{bB}	31.58±1.89^{bB}
WnFS	1	68.04±1.30^{aA}	64.41±2.58^{aA}	64.10±2.53^{aA}
	14	73.32±1.41^{aB}	77.30±0.88^{abB}	79.02±1.86^{bB}
Salt content in the water phase	1	2.62±1.30^{aA}	2.78±0.50^{aA}	3.08±0.67^{aA}
	14	5.57±1.08^{aB}	5,34±0.53^{aB}	5.01±0,52^{aB}

**Results for control cheese were taken from Kljajevic et al. (2024).*
Different lowercase letters in rows indicate statistically significant difference between cheese variants (at a significance level of p=0.05).
Different uppercase letters in columns indicate statistically significant difference between 1st and 14th day of maturation (at a significance level of p=0.05).

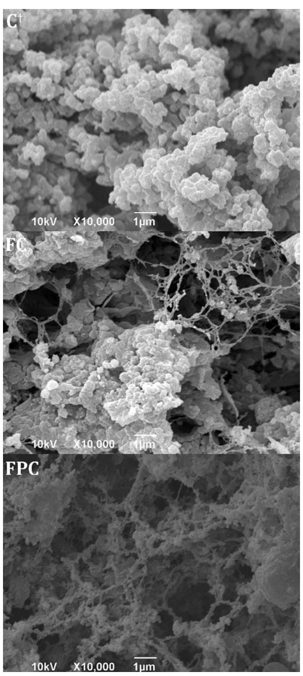
***Table 2.** Textural properties of control and experimental cheeses (FC - cheese made from curd frozen after first stage of pressing; FPC - cheese made from curd frozen after second stage of pressing)*

	Maturation day	Control*	FC	FPC
Hardness (g)	1	1496.42±410.43^{aA}	1775.38±57.00^{aA}	1519.06±423.58^{aA}
	14	1371.39±282.41^{aA}	183.02±53.44^{bB}	237.05±1.93^{bB}
Springiness (dimensionless)	1	0.97±0.13^{aA}	0.88±0.02^{aA}	0.87±0.01^{aA}
	14	0.75±0.09^{aB}	0.82±0.04^{aA}	0.80±0.01^{aA}
Cohesiveness (dimensionless)	1	0.77±0.16^{aA}	0.77±0.07^{aA}	0.72±0.04^{aA}
	14	0.42±0.05^{aB}	0.669±0.02^{bA}	0.54±0.09^{abA}
Chewiness (g)	1	1517.40±83.21^{aA}	1202.53±149.82^{abA}	958.5±292.71^{bA}
	14	501.24±81.88^{aB}	96.64±23.59^{bB}	97.17±10.83^{bB}

**Results for control cheese were taken from Kljajevic et al. (2024).*
Different lowercase letters in rows indicate statistically significant difference between cheese variants (at a significance level of p=0.05).
Different uppercase letters in columns indicate statistically significant difference between 1st and 14th day of maturation (at a significance level of p=0.05).

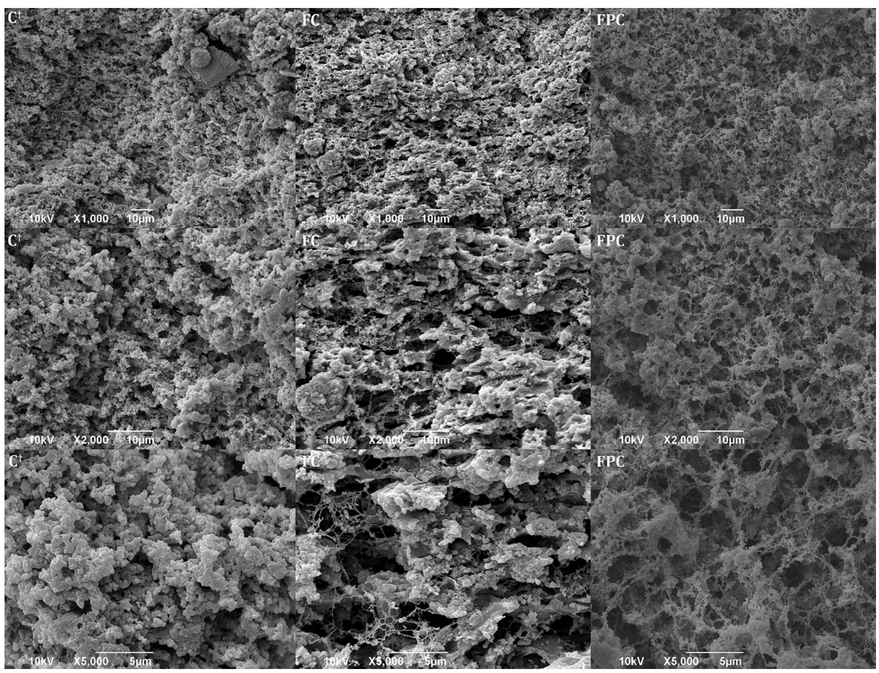
were significantly lower on the 14th day compared to the 1st day (Table 2). The FC and FPC cheese variants exhibited significantly reduced hardness and chewiness relative to the control cheese, while no differences were observed among the variants for other textural attributes. ANOVA results showed significant interaction between pressing stages and the maturation period for hardness and chewiness, underscoring the influence of processing conditions on textural evolution. These findings collectively highlight the dynamic interplay between compositional changes and textural modifications during cheese maturation, providing insight into the structural transformations occurring over time as a result of different processing conditions.

Microstructure of cheeses is presented in Figure 1 at a magnification of 10000. Additional magnifications can be seen in Figure 2. Notably, both FC and FPC variants exhibit a high prevalence of slender protein chains within the matrix, and no microorganisms can be seen in the matrix. Some bacterial cells can be seen in micrographs of the control cheese. The structure is disordered and not compact, compared to the control cheese.



**Micrograph of control cheese was taken from Kljajevic et al. (2024).*

***Figure 1.** Micrographs of control (C) and experimental (FC and FPC) cheeses on the 1st day of ripening (magnification of 10000)*



**Micrographs of control cheese were taken from Kljajevic et al. (2024).*

***Figure 2.** Micrographs of control (C) and experimental (FC and FPC) cheeses on the 1st day of ripening (magnifications of 1000, 2000 and 5000)*

This research found that the cheese mass in the FC and FPC variants disintegrated after 14 days of ripening. Alichanidis (2007) noted that brined cheese softens when pH exceeds 5.0 or water content surpasses 58 %, with salt content in the water phase below 2.5 %, after 24 hours from production. In this study water content was below 58 %, salt content above 2.5 %, but the pH remained above 5.0. Given the observation of a more compact and denser microstructure of caprine cheese made from thawed milk (Kljajevic et al., 2024), it can be inferred that the freezing of curds had more adverse effects on microstructure and played a more pivotal role in the diffusion of water into the cheese matrix and subsequent softening compared to the effect of pH value alone. Also, control cheese, which had a higher pH value on the first day of maturation, did not exhibit disintegration during the 56-day maturation period in the same conditions, which is another pinpoint showing that the diffusion of water into cheese matrix was more dependent on the structural changes in the matrix than higher pH values. Still, lower pH values of curd could have a positive effect on controlling water absorption during the maturation of white brined cheese (Alichanidis, 2007). Additionally, freezing curds shortly after milk coagulation, while the cheese matrix has not yet stabilized into a firm structure, appears to exert a more detrimental effect on microstructure than freezing milk with coagulation occurring subsequently.

It is noteworthy that no microorganisms were observed in the microstructure of the FC and FPC variants in contrast to the control cheese, suggesting that the potential for these cheeses to lower their pH values needs to be further investigated by quantifying starter culture microorganisms. It is advisable to consider potential modifications in the cheesemaking process to produce curds with a pH value below 5.0 prior to freezing as a means to address the issue of softening. One possibility might be to revert to the more traditional method of initial salting, to apply dry salting for 4-5 days before putting cheese into brine for ripening (Alichanidis et al., 1981), thus possibly allowing the pH value to drop below 5.0. Also, an alternative strategy could involve removing the cheese from the brine after the maturation period of 7-14 days, vacuum packaging it, and allowing it to ripen in the vacuum thereafter. And finally, the study used milk from the late lactation stage, which has higher minerals, proteins, and titratable acidity, resulting in high buffering capacity (Kljajevic et al., 2018). To lower pH faster, freezing caprine milk from mid-lactation may be considered.

Conclusion

This study demonstrates that freezing curds at different pressing stages significantly impacts the technological and functional properties of cheese. After 14 days of ripening, both FC and FPC cheese variants exhibited disintegration, likely due to alterations in microstructure and an increased water absorption. To address this, it is recommended to modify the cheesemaking process, such as reducing pH before freezing through traditional salting in order to potentially lower the amount of water that could be absorbed by cheese matrix. Additionally, using milk from mid-lactation, which has lower buffering capacity, could help achieve faster pH reduction. Overall, while frozen curd presents challenges in terms of texture, it offers opportunities for optimizing the production of caprine white brined cheeses with further process adjustments.

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Može li se zamrznuti gruš koristiti u proizvodnji bijelih sireva u salamuri?

Sažetak

Ova je studija ispitivala rješavanje problema sezonske prirode kozjeg mlijeka za proizvodnju bijelog sira u salamuri s 14 dana zrenja upotrebom zamrznutih gruševa s kraja laktacije, zamrznutih u dvije različite faze prešanja. Zamrznuti gruš ima značajne prednosti u odnosu na zamrznuto mlijeko radi smanjenih zahtjeva za prostorom skladištenja, eliminaciju sirutke, kao i manju potrošnju vode u usporedbi s korištenjem mlijeka u prahu. Analiza teksture eksperimentalnih sireva pokazala je značajno smanjenu čvrstoću do 14. dana zrenja u salamuri. Skenirajućom elektronskom mikroskopijom (SEM) prvog dana zrenja otkriveno je da eksperimentalni sirevi imaju neuređenu i manje kompaktnu mikrostrukturu u odnosu na kontrolne uzorke. Do 14. dana, eksperimentalni sirevi su se dezintegrirali u salamuri uslijed povećane apsorpcije vode, pretežno zbog strukturnih promjena u sirnom matriksu. Kako bi se ovi izazovi svladali, studija predlaže dvije strategije za uspješnu proizvodnju bijelog sira u salamuri: postizanje niže inicijalne pH vrijednosti kroz produžene tradicionalne metode soljenja ili skraćenje perioda zrenja u salamuri.

Ključne riječi: bijeli sirevi u salamuri; kozje mlijeko; mikrostruktura; tekstura; skladištenje u zamrznutom stanju

References

1. Alichanidis E. (2007): Cheeses ripened in brine. *In:* McSweeney, P.L.H. (ed.) Cheese problems solved. Woodhead Publishing, Cambridge England.
2. Alichanidis, E., Polychroniadou, A., Tzanetakis, N., Vafopoulou, A. (1981): Teleme cheese from deep-frozen curd. *Journal of Dairy Science* 64, 732-739. https://doi.org/10.3168/jds.S0022-0302(81)82641-0
3. AOAC (1990): Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists, Washington, DC.
4. Campos, G., Robles, L., Alonso, R., Nuñez, M., Picon, A. (2011): Microbial dynamics during the ripening of a mixed cow and goat milk cheese manufactured using frozen goat milk curd. *Journal of Dairy Science* 94, 4766-4776. https://doi.org/10.3168/jds.2010-4077
5. FIL-IDF (1982): Determination of the total solids content. Cheese and processed cheese. International Dairy Federation, Brusseles, Belgium.
6. FIL-IDF (1986): Determination of fat content. Cheese and processed cheese products. International Dairy Federation, Brusseles, Belgium.
7. FIL-IDF (1988): Determination of chloride content. Cheese and processed cheese. International Dairy Federation, Brussels Belgium.
8. Kljajevic, N.V., Miloradovic, Z.N., Miocinovic, J.B., Jovanovic, S.T. (2017): Textural and physico-chemical characteristics of white brined goat cheeses made from frozen milk and curd. The use of square I - distance statistics. *Mljekarstvo* 67, 130-137. https://doi.org/10.15567/mljekarstvo.2017.0205
9. Kljajevic, N.V., Miloradovic, Z.N., Miocinovic, J.B., Pavlovic, V.B., Tomic, N.S., Jovanovic, S.T. (2024): Insights into proteolysis, textural evolution, microstructural changes and sensory attributes of white brined cheese from frozen-stored caprine milk. *International Journal of Dairy Technology* 77, 313-323. https://doi.org/10.1111/1471-0307.13042
10. Kljajevic, N.V., Tomasevic, I.B., Miloradovic, Z.N., Nedeljkovic, A., Miocinovic, J.B., Jovanovic, S.T. (2018): Seasonal variations of Saanen goat milk composition and the impact of climatic conditions. *Journal of Food Science and Technology* 55, 299-303. https://doi.org/10.1007/s13197-017-2938-4
11. Park, Y.W. (2013): Effect of 5 years long-term frozen storage on sensory quality of Monterey Jack caprine milk cheese. *Small Ruminant Research* 109, 136-140. http://dx.doi.org/10.1016/j.smallrumres.2012.08.002

12. Picon, A., Alonso, R., Gaya, P., Fernández-García, E., Rodríguez, B., de Paz, M., Nuñez, M. (2010): Microbiological, chemical, textural and sensory characteristics of Hispánico cheese manufactured using frozen ovine milk curds scalded at different temperatures. *International Dairy Journal* 20**,** 344-351.
http://dx.doi.org/10.1016/j.idairyj.2009.12.008
13. Romeih, E.A., Michaelidou, A., Biliaderis, C.G., Zerfiridis, G.K. (2002): Low-fat white-brined cheese made from bovine milk and two commercial fat mimetics: chemical, physical and sensory attributes. *International Dairy Journal* 12, 525-540.
http://dx.doi.org/10.1016/S0958-6946(02)00043-2
14. Schmidt, F., Hinrichs, J. (2024): Frozen cheese curd as an intermediate for cheese making - homogenization as a tool to regain techno-functional properties. *Journal of Food Measurement and Characterization* 18,
https://doi.org/7124-7131. 10.1007/s11694-024-02723-3
15. Seçkin, A.K., Esmer, Ö.K., Balkir, P., Ergönül, P.G. (2011): Effect of curd freezing and packaging methods on the organic acid contents of goat cheeses during storage. *Mljekarstvo* 61, 234-243.
16. Sendra, E., Mor-Mur, M., Pla, R., Guamis, B. (1999): Evaluation of freezing pressed curd for delayed ripening of semi-hard ovine cheese. *Milchwissenschaft* 54, 550-553.
17. Yu Z., Qiao C., Zhang X., Yan L., Li L., Liu Y. (2021): Screening of frozen-thawed conditions for keeping nutritive compositions and physicochemical characteristics of goat milk. *Journal of Dairy Science* 104, 4108-4118.
https://doi.org/10.3168/jds.2020-19238