



**PROCEEDINGS OF ABSTRACTS
VIII. INTERNATIONAL
AGRICULTURAL, BIOLOGICAL,
LIFE SCIENCE CONFERENCE
AGBIOL 2026**

06-09 SEPTEMBER 2026

ISTANBUL, TÜRKİYE



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**Organized by
Trakya University
İstanbul Beykent University
International Researchers Association**

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WELCOME NOTES

You are welcome to our VIII. AGBIOL Conference that is organized by Trakya University, Beykent University and International Researchers Association. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal was to bring three communities, namely science, research and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to get benefit from the interaction with each other.

In September 2018, we organized the first AGBIOL Conference with more than 700 scientists and researchers from all over the world with over 800 scientific papers. Due to COVID-19 situation, II. AGBIOL 2020 has organized fully on-line event which was one of the biggest online conferences in recent years in the world with 499 papers and 1133 authors with 333 oral and 166 e-poster presentations from 55 countries. Due to COVID-19 situation, AGBIOL 2021 was organized online again. AGBIOL 2022 conference was organized with a worldwide participation from 44 countries over 522 papers contributed by over 1300 authors. AGBIOL 2023 was organized with a record and worldwide participation from 33 countries 833 papers contributed by over 2000 authors with 522 oral and 311 poster presentations. AGBIOL 2024 consisted of 835 papers contributed by about 2000 authors with worldwide record participation again from 55 countries with 522 oral and 311 poster presentations. AGBIOL 2025 was organized with a worldwide record participation from 60 countries 988 papers contributed over 2300 authors with 400 oral and 588 poster presentations.

The AGBIOL 2026 is normal participation as well as with online participation in Beykent University in Istanbul, Turkey on 06-09 September, 2026. The program included oral talks by invited prominent scientists and oral and e poster presentations by participants in selected topics from the submitted abstracts focusing on Agriculture, Biology and Life Sciences topics.

There is a worldwide record participation again in AGBIOL 2026 from 61 countries 1057 papers contributed over 2500 authors then 800 of them were accepted and then the conference was organized with 390 oral and 410 poster presentations.

With care for our nature and environment, we aim the green congress, meaning that as little as possible papers will be used. Abstract book is published in electronic book and is distributed to the participants by e mail for online participants. All the e-posters are prepared in electronic form and then submit to via the conference e mail and exhibited in electronical poster boards as well as in online e poster hall in our web page during the conference.

The participants with paid conference fee accessed all the normal and virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided. The abstracts were published in the Conference Abstract and Proceedings Book. Participants might send us their full papers, which based on their preferences are published either in our Conference Abstract and Proceedings Book or in selected International Indexed Scientific Journals.

Conference Topics:

Agriculture, Forestry, Life Sciences, Agricultural Engineering, Aquaculture and Biosystems, Animal Science, Biomedical science, Biochemistry and Molecular Biology, Biology, Bioengineering, Biomaterials, Biomechanics, Biophysics, Bioscience, Biotechnology, Botany, Chemistry, Chemical Engineering, Earth Sciences, Environmental Science, Food Science, Genetics and Human Genetics, Medical Science, Machinery, Pharmaceutical Sciences, Physics, Soil Science.

We would like to thank all of you for joining this conference and we would like to give also special thanks to TUBITAK and collaborators for giving us a big support to organize this event.

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CHANGES IN THE CONTENT OF LACTOSE AND FREE AMINO ACIDS DURING THE RIPENING OF SALTED CHEESE

Dr. Cand. Eva Ceka¹Dr. Ida Kruti²

¹ Qiriazi University College, Professional Higher College in Food Technology, Tirana, 1001, Albania
eva.ceka@qiriazi.edu.al, <https://orcid.org/0009-0009-4719-0941>

² Qiriazi University College, Faculty of Education, Social Sciences and Sports, Tirana, 1001, Albania
ida.kruti@qiriazi.edu.al, <https://orcid.org/0009-0009-7929-9722>

Corresponding author e-mail: eva.ceka@qiriazi.edu.al

ABSTRACT

This study examined the changes in lactose content and free amino acid (FAA) levels during the ripening of cow's milk cheese treated with 20% brine (14–16 °C, 24 h). Two cheese variants were prepared from pasteurised cow's milk (3.8% fat): one coagulated with chymosin only (DLK) and one with chymosin supplemented with a microbial starter culture (DLK-S). Cheese samples were stored at 14–15 °C and analysed on days 12, 22 and 32 of ripening for moisture, pH, titratable acidity, sodium chloride, lactose and free amino acids.

In the chymosin-only variant (DLK), the lactose content decreased gradually on days 12, 22 and 32. The FAA content remained low and essentially unchanged indicating that lactic acid bacteria originating from the milk alone exerted limited lactose-metabolising and proteolytic activity during the 32-day ripening period. By contrast, the starter-supplemented variant (DLK-S) showed a marked acceleration in both processes: lactose declined on day 12 and fell below the detection limit by day 32, while FAA levels increased steadily. A positive correlation between lactic acid production and lactose consumption was observed in both variants.

These results confirm that the addition of a microbial starter culture to brine-salted cheese markedly enhances lactose catabolism and proteolysis during ripening. While the indigenous lactic acid bacteria in DLK maintained only a basal level of metabolic activity, the defined starter in DLK-S drove lactose to exhaustion by day 32 and produced FAA levels approximately five times higher than those in the chymosin-only cheese. The findings highlight the essential role of starter cultures in the development of flavour and aroma compounds in salted cheese during extended ripening.

Keywords: cheese ripening, salted cheese, brine treatment, lactose metabolism, free amino acids, proteolysis, starter culture, chymosin, cow's milk

INTRODUCTION

The application of salt to cheese curd is one of the most universal steps in cheese manufacture. Salt contributes directly to flavour, inhibits spoilage and pathogenic organisms, influences texture, and modulates the activity of ripening enzymes (Fox et al., 2004). This review examines how brine salting affects the metabolism of residual lactose and the proteolytic

degradation of casein during cheese ripening, with particular attention to the interaction between salt concentration, starter culture activity and FAA accumulation.

The aim of this study was to investigate the changes in the content of lactose and free amino acids (FAA) during cheese ripening, with particular attention to the effects of two factors: (i) the addition of a microbial starter culture (commercial yogurt) versus coagulation with chymosin alone omission of a salting treatment. Two cheese variants were produced from pasteurised cow's milk and monitored over their respective ripening periods for a suite of physicochemical indices, including moisture, pH, titratable acidity, sodium chloride, lactose and free amino acid content.

Salting methods and their effect on cheese composition

Brine salting involves immersing pressed cheese in a concentrated NaCl solution, typically 18–22% (w/v), at 10–16 °C for periods ranging from hours to days (Fox et al., 2004). Salt uptake is a diffusion-driven process: NaCl migrates inward while moisture migrates outward, creating a characteristic salt-in-moisture gradient that is highest at the surface and lowest at the centre (Kleter et al., 1984). This gradient has important implications for the spatial distribution of microbial activity and enzymatic ripening within the cheese.

In brine-salted cheeses, the time required for NaCl to reach inhibitory concentrations throughout the cheese is longer than in dry-salted varieties, which has consequences for controlling undesirable fermentations in the interior.

Effect of salt on LAB and lactose fermentation

Sodium chloride inhibits LAB primarily through the reduction of water activity (a_w). As NaCl concentration increases, the a_w decreases, creating osmotic stress that impairs bacterial growth, reduces sugar transport across the cell membrane, and inhibits glycolytic enzyme activity (Turner and Thomas, 1980). Sensitivity to NaCl varies among LAB species and strains.

At the salt-in-moisture levels typical of semi-hard cheeses (approximately 4–6% S/M), starter LAB growth is slowed but not arrested, and residual lactose is typically fermented to completion within the first weeks of ripening. At higher S/M levels, lactose fermentation may be significantly delayed, providing a potential substrate for late gas-producing organisms (McSweeney and Fox, 2004).

The anaerobic metabolism of lactate by *Clostridium tyrobutyricum* to butyrate and hydrogen gas known as late gas blowing is particularly associated with brine-salted cheeses in which NaCl has not yet penetrated to inhibitory concentrations (Kleter et al., 1984; Fox et al., 1990). Complete and timely fermentation of residual lactose by the starter culture is therefore an important quality parameter.

Proteolysis in salted cheese

The proteolytic agents in salted cheese are the same as in other cheese types: residual coagulant (chymosin), indigenous milk proteinases (plasmin), and LAB proteinases and peptidases (McSweeney, 2004; Upadhyay et al., 2004). However, their rates are modulated by NaCl.

Chymosin activity is relatively insensitive to NaCl at typical cheese concentrations, although high salt levels may alter the conformation of casein substrates (Fox and McSweeney, 1996b). Plasmin activity has been reported to be stimulated by moderate NaCl in some studies (Bastian and Brown, 1996). LAB peptidases are indirectly affected through salt's impact on cell viability

and lysis kinetics, as these intracellular enzymes are released into the cheese matrix upon cell death (Kunji et al., 1996; Upadhyay et al., 2004).

The practical consequence is that salted cheeses exhibit slower FAA accumulation than unsalted equivalents. However, this slower rate allows for a more gradual and balanced development of flavour compounds over extended ripening, which is characteristic of many valued aged varieties (McSweeney, 2004).

FAA and flavour development in salted cheese

FAA accumulation in salted cheese follows a characteristic pattern: an initial lag phase during which primary proteolysis generates large peptides, followed by a progressive increase as LAB peptidases hydrolyse these intermediates to individual amino acids (McSweeney, 2004). FAA levels may range from a few milligrams to several hundred milligrams per 100 g cheese, depending on the variety and ripening duration (McSweeney and Sousa, 2000).

These free amino acids serve as substrates for secondary catabolic reactions transamination, decarboxylation, deamination that produce volatile flavour compounds (Yvon and Rijnen, 2001; Curtin and McSweeney, 2004). In cheeses without a defined starter, FAA accumulation is typically much lower, reflecting the limited proteolytic capacity of indigenous NSLAB and the dominance of primary proteolysis by chymosin and plasmin, which generate large peptides but do not efficiently release individual amino acids (Christensen et al., 1999).

MATERIAL AND METHOD

Raw materials

Fresh cow's milk (10 litres) was obtained from a local dairy farm on the day of cheese manufacture. The milk had a fat content of 3.8% (w/w). The coagulant used was commercial bovine chymosin with a clotting strength of 1:10,000. A commercial plain yogurt was used as the source of microbial starter culture, providing a mixed population of lactic acid bacteria (principally *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). All reagents for physicochemical analyses were of analytical grade.

Experimental design

The study employed a 2×2 factorial design with two factors: coagulation method (chymosin only vs. chymosin + starter culture) and salting treatment (salted vs. unsalted). This yielded four cheese variants, each designated by a sample code as follows:

- DLK – cheese coagulated with chymosin only, with salt treatment;
- DLK-S – cheese coagulated with chymosin + microbial starter culture, with salt treatment;

The salted variants (DLK and DLK-S) were analysed at three time points during ripening: days 12, 22 and 32.

Cheese manufacture

The 10 liters of milk were divided into two equal batches of 5 liters each. Each batch was used to produce salted variant as described below. The production protocol was identical for both batches up to the pressing step to produce feta cheese, after which the salting treatment was applied.

Salted variants (DLK and DLK-S)

The milk (5 litres, 3.8% fat) was pasteurised using a low-temperature, long-time (LTLT) protocol at 63–65 °C for 30 minutes, followed by rapid cooling to 1–2 °C. After tempering to 30 °C, the milk was divided into two portions. The first portion was coagulated by the addition of chymosin at a dose of 150 mg per 5 litres (strength 1:10,000) at 30 °C for 50–60 minutes to produce the DLK variant. The second portion received both chymosin (same dose) and 30 g of plain yogurt as a source of starter culture, and was coagulated under the same conditions (30 °C, 50–60 minutes) to produce the DLK-S variant.

Following coagulation, syneresis was allowed to proceed at 30 °C. The curd was then pressed under its own weight to facilitate whey expulsion. The pressed curd was immersed in a 20% (w/v) NaCl brine solution at 14–16 °C for 24 hours. After brining, the cheese samples were stored for ripening at 14–15 °C and sampled for analysis on days 12, 22 and 32.

Analytical methods

All physicochemical analyses were performed in duplicate on each cheese sample at the designated ripening time points. The following parameters were determined:

Moisture content

Moisture content was determined gravimetrically by drying a weighed sample in a thermostat oven at 105 °C until a constant weight was achieved (Ardö and Polychroniadou, Cheese Analysis Manual). The result was expressed as a percentage (% w/w).

pH

The pH of each cheese sample was measured potentiometrically by inserting a calibrated glass electrode directly into the cheese mass at ambient temperature (Ardö and Polychroniadou, Cheese Analysis Manual).

Titrateable acidity

Titrateable acidity was determined by the potentiometric titration method (Ardö and Polychroniadou, Cheese Analysis Manual) and expressed as a percentage of lactic acid (% w/w).

Sodium chloride (NaCl)

The NaCl content of the salted cheese samples (DLK and DLK-S) was determined by the Volhard method (Ardö and Polychroniadou, Cheese Analysis Manual) and expressed as a percentage (% w/w). NaCl analysis was not performed on the unsalted variants (DLKP and DLK-SP), as no salt was added during their manufacture.

Lactose content

Lactose content was determined by the iodimetric method of Fine (1932), as described by James (1998, Analytical Chemistry of Food, Springer Science & Business). The result was expressed as mg lactose per 100 g cheese as shown in chart 1. Where the measured value fell

below the sensitivity of the method, the result was reported as below the limit of detection (> LD).

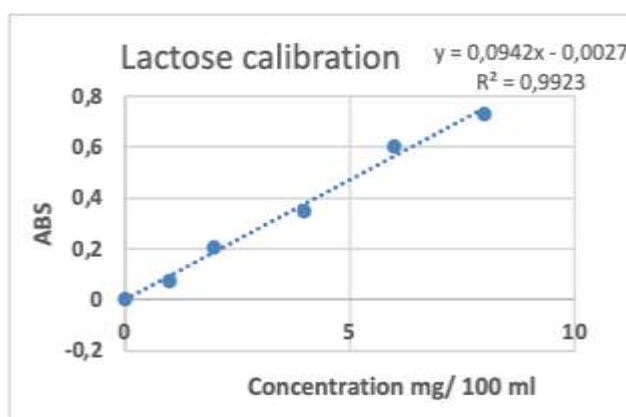


Chart 1. Lactose curve calibration

Free amino acid (FAA) content

The content of free amino acids was determined spectrophotometrically using a ninhydrin-based colorimetric assay, with L-lysine as the calibration standard (Ardö and Polychroniadou, Cheese Analysis Manual). A calibration curve was constructed by plotting absorbance at 570 nm against known concentrations of L-lysine ($\mu\text{g}/100 \mu\text{l}$). The FAA content of each cheese sample was calculated from the calibration curve and expressed as mg FAA per 100 g cheese as shown on chart 2.

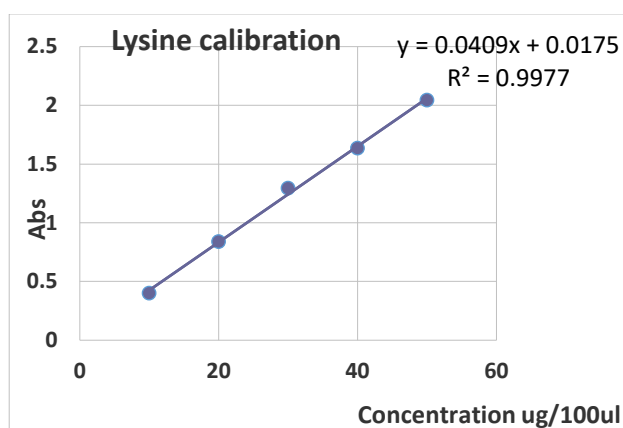


Chart 2. Lysine curve calibration

RESULTS AND DISCUSSION

This section presents the results of the physicochemical analysis of two salted cheese variants: DLK (chymosin only) and DLK-S (chymosin + starter culture). Both were treated with 20% NaCl brine at 14–16 °C for 24 hours, then stored at 14–15 °C and analysed on days 12, 22 and 32 of ripening.

The chymosin-only salted variant (DLK), the lactose content decreased gradually on days 12, 22 and 32 of ripening, Fig 2. The FAA content remained low throughout. The pH declined slowly, while titratable acidity decreased. Moisture fell, and NaCl in brine stabilised at approximately 19–21%.

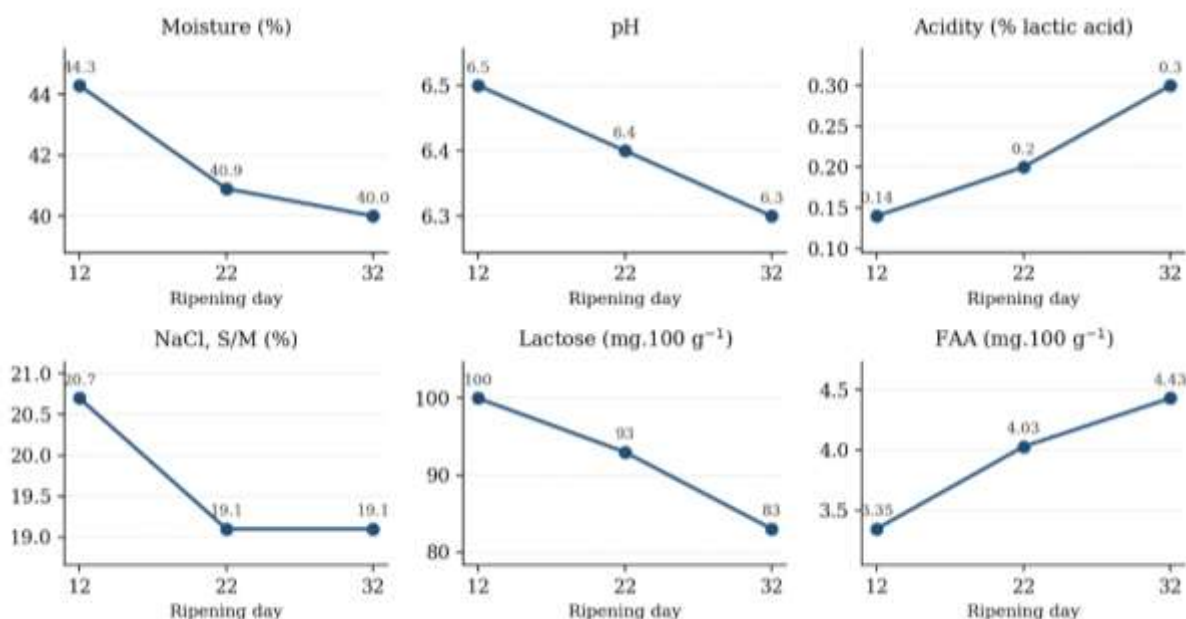


Fig 2. DLK cheese chymosin only, salted, changes during ripening from day 12 to day 32.

These results indicate that the indigenous lactic acid bacteria originating from the pasteurised milk exert limited metabolic activity throughout the 32-day ripening period in the presence of high salt concentrations and without a defined starter culture. The slow decline in lactose and the near-constant FAA levels suggest that the proteolytic activity observed is attributable primarily to the residual chymosin retained in the curd, which hydrolyses α 1-casein at the Phe₂₃–Phe₂₄ bond as its primary site of action, and to a lesser extent to indigenous plasmin activity. The low FAA accumulation indicates that the peptidases necessary for the terminal stages of proteolysis are present in insufficient quantities without a starter-derived enzyme complement.

The starter-supplemented salted variant (DLK-S) exhibited a markedly different metabolic profile. Lactose was consumed rapidly and falling below the detection limit by day 32. The pH was substantially lower than in DLK at all time points, reflecting vigorous acid production by the starter LAB. Titratable acidity rose on day 12 to day 22 before stabilising on day 32, consistent with the near-exhaustion of lactose as a fermentable substrate Fig 3.

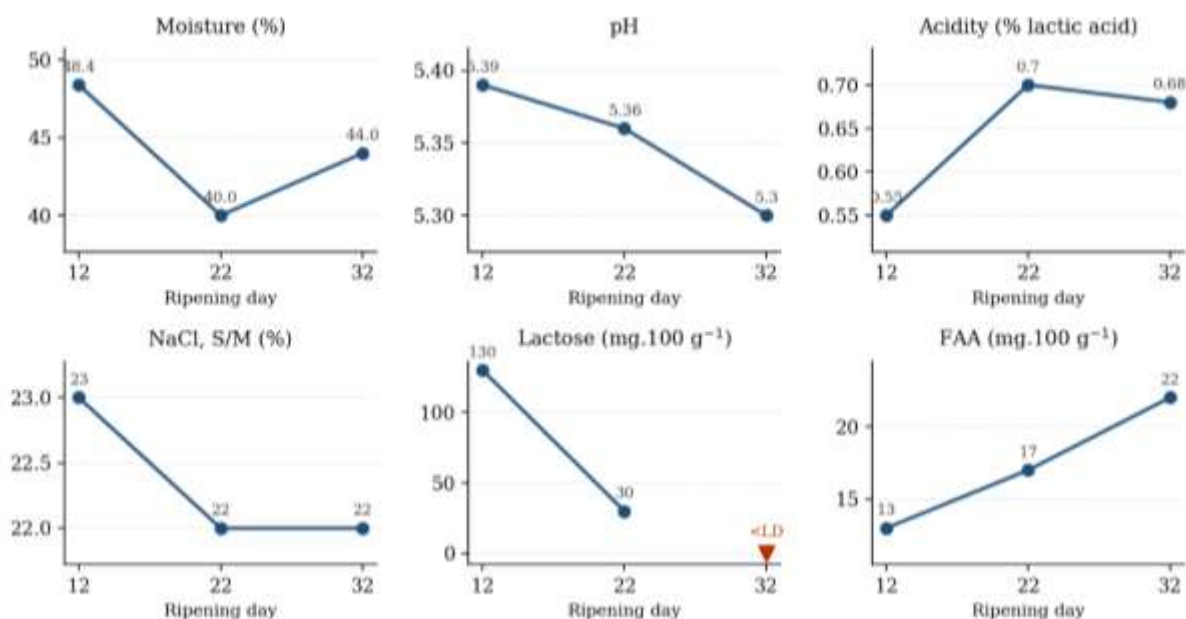


Fig 3. DLK-S cheese chymosin + starter, salted: changes during ripening from day 12 to day 32

The FAA content increased steadily approximately five times higher than in DLK at each corresponding time point. The continuous accumulation demonstrates that the proteolytic enzyme system of the starter LAB comprising cell envelope-associated proteinases and intracellular peptidases released upon cell lysis actively degrades the casein matrix throughout the ripening period.

A positive correlation was observed between lactic acid production and lactose consumption in both variants, confirming that lactose serves as the primary energy source for the LAB and that its fermentation to lactate drives the acidification of the cheese curd.

COMPARATIVE DISCUSSION

The comparison of DLK and DLK-S demonstrates that the addition of a microbial starter culture is the decisive factor in determining both the rate and extent of lactose metabolism and proteolysis in brine-salted cheese. While the indigenous microflora in DLK maintained only basal-level metabolic activity over 32 days, the defined starter in DLK-S drove lactose to exhaustion and produced FAA levels approximately five times higher.

The complete fermentation of residual lactose by day 32 in DLK-S is an important quality parameter, as unfermented lactose can serve as a carbon source for undesirable secondary microflora and contribute to defects during extended ripening. The elevated FAA levels represent a larger pool of substrates for secondary catabolic reactions that generate volatile flavour compounds.

LIMITATIONS

Several limitations should be considered. First, the earliest sampling point was day 12, so the initial phase of lactose fermentation (days 0–12) was not captured. Second, the NaCl values reported (19–23%) represent salt-in-moisture rather than salt-in-cheese; clarification is important as NaCl concentration strongly influences LAB activity. Third, the FAA assay reports

total free amino acids using a single method calibrated with L-lysine, without resolving individual amino acids. Fourth, no sensory evaluation was performed. Fifth, the experiment used a single production batch without biological replicates. Finally, the use of commercial yogurt as a starter precludes attribution of specific activities to defined bacterial strains.

CONCLUSIONS

Brine salting is a critical control point in cheese manufacture, simultaneously contributing to preservation, texture and flavour while modulating ripening biochemistry. Salt inhibits LAB growth and enzymatic activity in a concentration-dependent manner, slowing lactose fermentation and proteolysis. The use of a defined starter culture is essential for ensuring complete lactose fermentation and for providing the proteolytic enzyme complement necessary for sustained FAA release. The interplay between salt concentration, starter activity and ripening duration remains a key area for optimisation in consistent cheese quality production.

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