



**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 UNSALTED CHEESE

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ABSTRACT

This study investigated the changes in lactose content and free amino acid (FAA) levels during the ripening of cow's milk cheese produced without salt treatment. Two cheese variants were prepared from pasteurised cow's milk (3.8% fat): one coagulated with chymosin only (DLKP) and one with chymosin supplemented with a microbial starter culture (DLK-SP). Cheese samples were stored at 14–15 °C and analysed on days 1, 3 (or 5) and 7 of ripening for moisture, pH, titratable acidity, lactose and free amino acids.

In the chymosin-only variant (DLKP), lactose decreased from day 5 and 7, and FAA levels fell over the same period, reflecting limited proteolytic activity in the absence of a defined starter culture. The starter-supplemented variant (DLK-SP) exhibited an accelerated pattern: lactose declined on day 1 to day 3 and fell below the detection limit by day 7, while FAA levels were high, rising on days 1, 3 and 7. The pH of DLK-SP dropped accompanied by an increase in titratable acidity. The results demonstrate that in the absence of salt, the addition of a microbial starter culture stimulates both lactose catabolism and proteolysis from the earliest stages of ripening. The FAA content in the starter-supplemented unsalted cheese (DLK-SP) was higher than in any of the salted variants, indicating that the omission of salt with starter inoculation, creates conditions highly favourable for the enzymatic degradation of caseins and the release of flavour precursors. These findings suggest that unsalted cheese produced with a starter culture develops flavour and aroma compounds more rapidly than its salted counterparts.

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

INTRODUCTION

Cheese is among the most ancient and diverse fermented food products. While many commercial cheeses undergo a salting step, a number of traditional and artisanal varieties are produced with reduced salt or without salt entirely. The biochemical changes that occur during the ripening of unsalted cheese are of particular interest because the absence of sodium chloride removes a major regulatory factor that normally modulates microbial growth and enzymatic activity in the cheese matrix (Fox et al., 2004). This review examines the current

understanding of the two principal biochemical events during the ripening of unsalted cheese: the catabolism of residual lactose by lactic acid bacteria (LAB) and the proteolytic degradation of casein to peptides and free amino acids (FAA). These processes are the primary determinants of flavour and texture development (McSweeney, 2004). 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 without adding salt.

Residual lactose and its fermentation

During cheese manufacture, most of the lactose in milk is lost in the whey. Nevertheless, a small but significant amount remains in the curd at the end of production, quantified at approximately 0.8–1.0% for Cheddar-type cheeses (Huffman and Kristoffersen, 1984). The complete fermentation of this residual lactose during the early stages of ripening is essential for cheese quality, as unfermented lactose can serve as a carbon source for undesirable organisms such as *Clostridium tyrobutyricum*, which causes late gas blowing (Fox et al., 1990).

The rate and completeness of lactose fermentation depend primarily on the metabolic activity of the LAB population. In cheeses made with defined starters, organisms such as *Lactococcus lactis* ferment lactose rapidly to L-lactate via the homofermentative pathway (Parente and Cogan, 2004). Without a defined starter, indigenous non-starter lactic acid bacteria (NSLAB) ferment lactose more slowly and may produce both L- and D-lactate (Turner and Thomas, 1980).

In unsalted cheese, the absence of NaCl-induced osmotic stress allows LAB to grow more rapidly and ferment lactose more completely than in salted counterparts. Salt-in-moisture levels normally exert a significant inhibitory effect on LAB growth rates and metabolic activity (Turner and Thomas, 1980). When this inhibitory factor is removed, residual lactose may be exhausted within days rather than weeks.

Proteolysis: agents and mechanisms

Proteolysis is the most important primary biochemical event during the ripening of most rennet-coagulated cheeses (McSweeney, 2004). It contributes to flavour through the release of short peptides and free amino acids, influences texture through hydrolysis of the casein matrix, and provides substrates for secondary catabolic reactions (Upadhyay et al., 2004).

The residual coagulant most commonly chymosin is responsible for the initial hydrolysis of intact caseins into large and medium-sized peptides, with its primary cleavage sites well characterised (McSweeney et al., 1993). Plasmin, an indigenous serine proteinase associated with the casein micelle, also contributes to primary proteolysis (Bastian and Brown, 1996). The proteinases and peptidases of starter LAB and NSLAB are then responsible for degrading these peptides into short peptides and free amino acids (Kunji et al., 1996; Christensen et al., 1999). The proteolytic system of *Lactococcus* comprises the cell envelope-associated proteinase lactocepin, which degrades caseins at the cell surface, and a diverse array of intracellular peptidases aminopeptidases, dipeptidases, tripeptidases and proline-specific peptidases released into the cheese matrix upon cell lysis (Law and Haandrikman, 1997; Upadhyay et al., 2004). These intracellular peptidases are essential for the terminal stages of proteolysis.

Free amino acids as flavour precursors

Free amino acids serve as substrates for secondary catabolic reactions transamination, decarboxylation, deamination and desulfurylation that generate many volatile compounds responsible for cheese aroma (Yvon and Rijnen, 2001; Curtin and McSweeney, 2004). The catabolism of branched-chain amino acids yields aldehydes and alcohols; aromatic amino acid catabolism produces phenolic compounds; and sulfur-containing amino acid catabolism generates methanethiol and related volatiles that are important to cheese aroma even at very low concentrations (Smit et al., 2002; McSweeney and Sousa, 2000).

Implications for unsalted cheese ripening

In unsalted cheese, uninhibited LAB growth leads to faster lactose fermentation, more vigorous acid production, and more rapid accumulation of proteolytic enzymes. When a defined starter culture is used, this can produce FAA levels within days that exceed those in salted cheese after weeks of ripening (McSweeney, 2004). This accelerated biochemistry establishes the pool of amino acid substrates for secondary catabolic reactions earlier in the ripening process.

However, the absence of salt also removes an important hurdle against spoilage organisms, and the high water activity of unsalted cheese renders it more susceptible to microbiological deterioration during extended storage. The balance between accelerated flavour development and microbiological stability is therefore a critical consideration (Fox et al., 2004).

MATERIAL AND METHOD

Raw materials

Fresh cow's milk (10 liters) 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*)

Experimental design

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

- DLKP – cheese coagulated with chymosin only, without salt treatment;
- DLK-SP – cheese coagulated with chymosin + microbial starter culture, without salt treatment.

DLKP and DLK-SP were analysed at days 1, 3 (or 5) and 7.

Cheese manufacture

The 10 litres of milk were divided into two equal batches of 5 litres each. Each batch was used to produce unsalted variant of feta cheese as described below.

Unsalted variants (DLKP and DLK-SP)

The second batch of milk (5 litres, 3.8% fat) was pasteurised, cooled and tempered identically to the first batch (63–65 °C for 30 min, rapid cooling to 1–2 °C, tempering to 30 °C). Coagulation was carried out as described above: one portion with chymosin only (DLKP) and one with chymosin + 30 g yogurt (DLK-SP), both at 30 °C for 50–60 minutes. Syneresis and pressing under the curd's own weight were performed as for the salted variants. However, no brine treatment was applied. The unsalted cheese samples were stored at 14–15 °C and sampled for analysis on days 1, 3 (or 5) and 7.

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).

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 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).

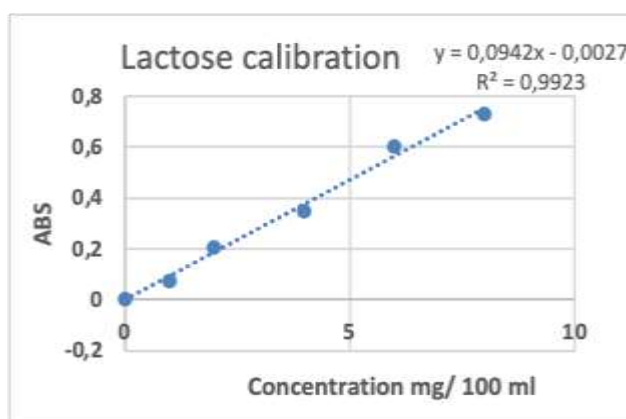


Chart 1. Lactose curve calibration

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 ($\mu\text{g}/100 \mu\text{l}$) as shown on chart 2. The FAA content of each cheese sample was calculated from the calibration curve and expressed as mg FAA per 100 g cheese.

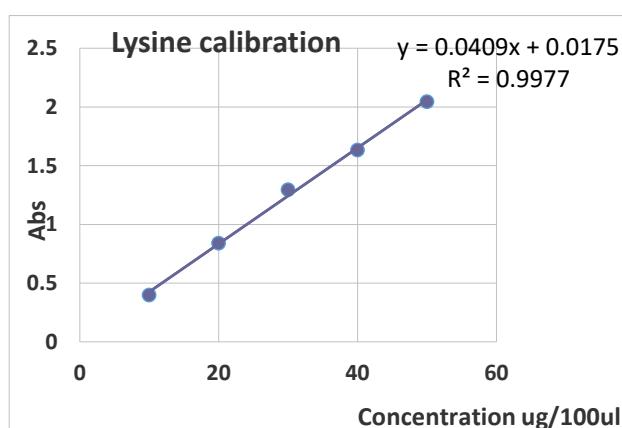


Chart 2. Lysine curve calibration

RESULTS AND DISCUSSION

This section presents the results of the physicochemical analysis of two unsalted cheese variants produced from pasteurised cow's milk (3.8% fat): DLKP (coagulated with chymosin only) and DLK-SP (coagulated with chymosin supplemented with a microbial starter culture). Both variants were stored at 14–15 °C without brine treatment and analysed at defined time points during the first 7 days of ripening Fig 2 and Fig 3.

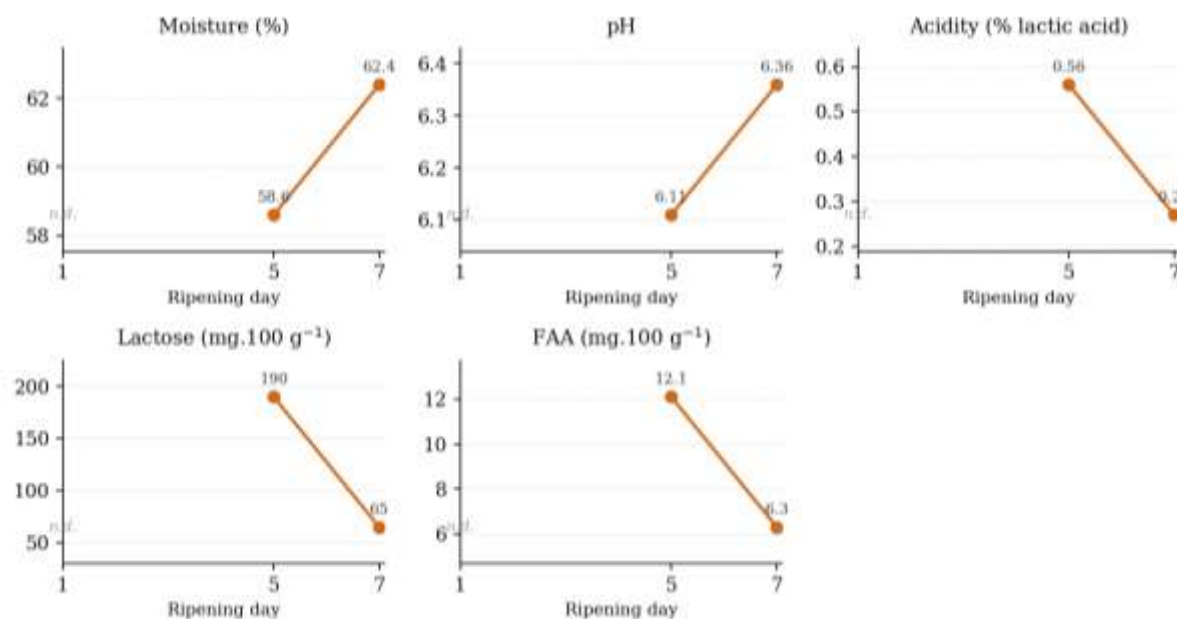


Fig 2. DLKP cheese chymosin only, unsalted during ripening days.

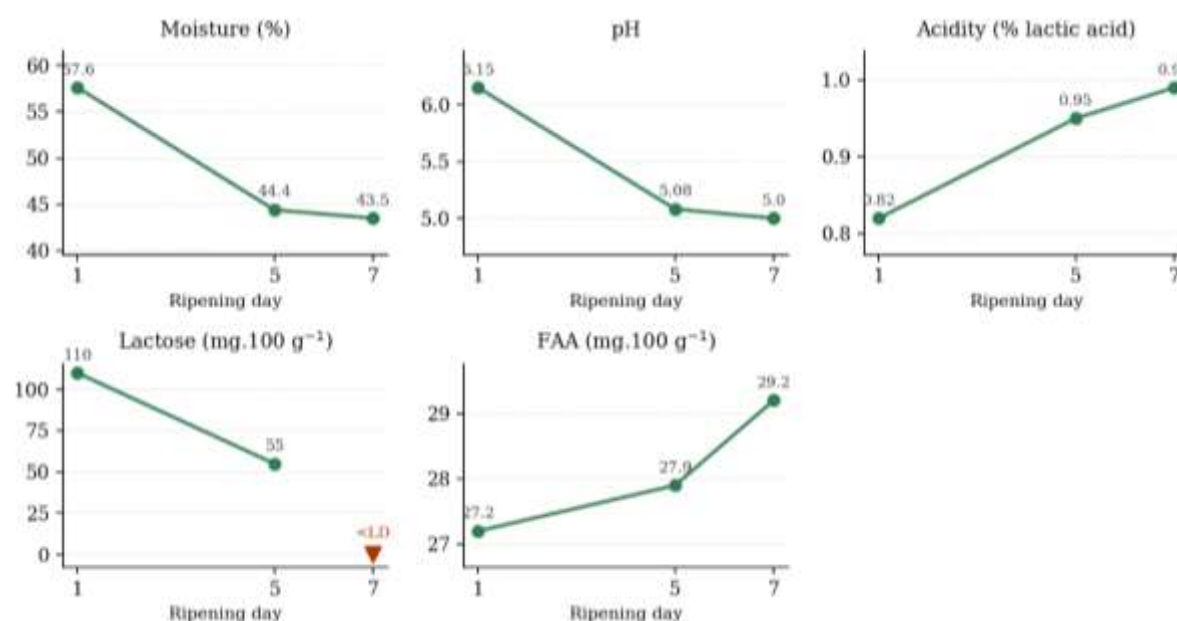


Fig 3. DLK-SP cheese chymosin + starter unsalted changes during ripening days.

The chymosin-only unsalted variant (DLKP), lactose content decreased on day 5 to day 7. Meanwhile, the free amino acid content fell over the same period. The pH declined from, and the titratable acidity dropped. Moisture increased likely reflecting ongoing whey redistribution in the absence of salt-driven osmotic dehydration.

These results indicate that in the absence of both a defined starter culture and salt, the indigenous lactic acid bacteria present in the pasteurised milk exhibit limited metabolic activity. The decrease in FAA between days 5 and 7 is noteworthy and may reflect the consumption of free amino acids by the adventitious microflora (non-starter lactic acid bacteria, NSLAB) as nitrogen sources for growth, rather than a continued release from casein hydrolysis. Proteolytic

activity in this variant appears to be driven primarily by the residual chymosin retained in the curd, with only a minor contribution from indigenous milk proteinases such as plasmin.

In marked contrast to DLKP, the starter-supplemented unsalted variant (DLK-SP) showed rapid and sustained biochemical changes during ripening. Lactose declined sharply on day 1 to day 3, indicating near-complete fermentation within one week. The pH dropped substantially consistent with the vigorous production of lactic acid.

The FAA content was markedly higher than in DLKP at every time point, rising steadily. This continuous accumulation demonstrates that the proteolytic enzymes released by the starter lactic acid bacteria including cell envelope-associated proteinases (lactocepine) and a range of intracellular peptidases are actively degrading the casein matrix from the first day of ripening. The absence of salt removes a key inhibitor of both LAB growth and enzymatic activity, explaining why FAA accumulation in this variant exceeded that of all other variants.

DISCUSSION

A comparison of DLKP and DLK-SP demonstrates the decisive role of the starter culture in driving both lactose catabolism and proteolysis in unsalted cheese. The starter-supplemented variant exhausted its lactose supply within 7 days and produced FAA levels higher than the peak value observed in the chymosin-only variant. The rapid pH drop in DLK-SP compared with the modest decline in DLKP confirms that the added LAB population dominates the fermentation.

The practical implication is that unsalted cheese produced with a starter culture develops flavour precursors particularly free amino acids that serve as substrates for secondary catabolic reactions producing volatile aroma compounds more rapidly than any of the salted variants examined in this study.

LIMITATIONS

Several limitations should be acknowledged. First, the unsalted variants were monitored for only 7 days due to cheese spoilage. Second, day 1 data are unavailable for DLKP, precluding a baseline comparison with DLK-SP. Third, the FAA assay employed a single calibration standard (L-lysine) and reports total FAA as a global index; it does not resolve the individual amino acid profile. Fourth, no sensory evaluation was performed. Fifth, the experiment was conducted with a single batch of milk without biological replicates, limiting statistical power. Finally, the microbial starter was introduced as commercial yogurt rather than as defined single-strain cultures, making it impossible to attribute the observed metabolic activities to specific bacterial species.

CONCLUSIONS

The literature demonstrates that in unsalted cheese, the removal of salt's inhibitory effect on LAB creates conditions that favour rapid lactose fermentation and enhanced proteolysis. Starter culture addition amplifies these effects, providing a large LAB population whose proteinases and peptidases release free amino acids from the earliest stages of ripening. Future research should focus on optimising the balance between accelerated biochemical ripening and microbiological safety in reduced-salt and unsalted cheese formulations.

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