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Research paper

Biogenic amine production and metabolic effects of *Latilactobacillus curvatus* in raclette-type cheese

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ABSTRACT

This study investigated biogenic amine production in Raclette cheese using *Latilactobacillus curvatus* strains capable of producing tyramine, β-phenylethylamine, putrescine, cadaverine, and tryptamine as adjunct cultures. Control cheeses without *L. curvatus* and with a non-amine-producing strain were included for comparison. During ripening, cheeses with tyramine-producing strains accumulated tyramine up to approximately 300 mg/kg after 120 days, along with strain-dependent levels of β-phenylethylamine. Strains capable of producing putrescine, cadaverine, and tryptamine also led to the accumulation of these amines. Analyses of free amino acids and carboxylic acids revealed strain-specific effects on amino acid metabolism (tyrosine, ornithine, arginine, aspartic acid, serine) and on D-lactate and formic acid levels. No effect on eye formation was detected by X-ray imaging. These findings demonstrate that *L. curvatus* is a notable producer of biogenic amines and modulates amino acid and organic acid metabolism in cheese.

1. Introduction

Biogenic amines are low-molecular-weight, nitrogen-containing basic compounds that can be aliphatic, alicyclic, or heterocyclic. In cheese, they can be formed during ripening through the activity of bacteria capable of amino acid decarboxylation. The main biogenic amines found in cheese—histamine, tyramine, β-phenylethylamine, cadaverine, putrescine, and tryptamine—originate from the corresponding amino acids histidine, tyrosine, phenylalanine, lysine, ornithine, and tryptophan (EFSA, 2011). Among these, histamine and tyramine pose great health concern, as consumption of high concentrations may trigger migraines, hot flashes, or blood pressure fluctuations, particularly in sensitive individuals (Dala-Paula et al., 2023; del Rio et al., 2023). The risk increases with alcohol consumption or certain medications. A well-known example is the “cheese reaction,” a hypertensive response that occurs in individuals taking monoamine oxidase inhibitors (MAOIs) after consuming tyramine-rich cheese. Limited data are available on the dose–response of cadaverine and putrescine, which are considered less toxic than histamine and tyramine. However, they may enhance the effects of histamine, since they slow down its breakdown (Sánchez-Pérez et al., 2022).

It is important to note that biogenic amines form through decarboxylation reactions, which release carbon dioxide. When produced in large amounts in cheese, they can cause an increase in eye numbers or the development of cracks (Decadt et al., 2024; Fröhlich-Wyder et al., 2013). The latter, in particular, lowers product quality and results in economic losses for the cheesemaker.

Therefore, it is desirable to prevent the formation of biogenic amines during cheese ripening. A highly effective approach is to identify and characterize amine-producing bacteria and restrict their entry into milk or cheese. This also enables the development of targeted detection methods for monitoring and tracing these bacteria to their sources.

Experimental cheese studies showed that *Lentilactobacillus parabuchneri* promotes histamine accumulation, *Enterococcus* spp. and *Latilactobacillus curvatus* contribute to tyramine formation, and *Paucilactobacillus wasatchensis* as well as enterobacteria drive cadaverine and putrescine production (Berthoud et al., 2022; Delbés-Paus et al., 2012; Pachlová et al., 2008; Rea et al., 2004, Ryser et al., 2022; Wechsler et al., 2021b). However, to our knowledge, no studies have yet investigated the formation of β-phenylethylamine and tryptamine in cheese. Since *Enterococcus* spp. can synthesize β-phenylethylamine in vitro, it is reasonable to assume they may do so in cheese as well (Bargossi et al., 2015; Marcobal et al., 2006). Regarding tryptamine, a tryptamine-producing *L. curvatus* strain was isolated from cheese, where tryptamine formation was linked to tryptophan decarboxylase activity (Irmler et al., 2023). Another study has isolated further

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tryptamine-producing species, such as *Loigolactobacillus coryniformis* and members of the *Latilactobacillus sakei* group, both of which exhibited strong tryptamine production under laboratory conditions (Arranz et al., 2024).

L. curvatus has frequently been isolated from cheese, and in vitro analyses showed that its strains can produce tyramine, β-phenylethylamine, cadaverine, putrescine, and tryptamine (Aymerich et al., 2006; Straub et al., 1995). Despite this, few studies have systematically examined its role in amine production in cheese environments.

The aim of the present study was to gain deeper insight into the influence of *L. curvatus* on amine formation in cheese. Raclette cheese was selected as the experimental matrix, as microbiome analyses of raw-milk Raclette have revealed the presence of *L. curvatus*, indicating that this cheese serves as a suitable model for studying its metabolism (Wechsler et al., 2021a). For this work, various *L. curvatus* strains were added during Raclette production. During ripening, bacterial growth and biogenic amine formation were monitored. At the end of ripening, the levels of free amino acids and carboxylic acids, as well as the extent of eye formation, were analyzed.

2. Materials and methods

2.1. Chemicals

All chemicals, unless otherwise stated, were obtained from Sigma-Aldrich (Merck & Cie, Switzerland).

2.2. Microbial strains

The mesophilic RSW 901 starter culture (Liebefeld Kulturen AG, Switzerland), grown overnight at 25 °C in skimmed UHT milk, was used as the starter culture. This undefined culture consists of *Lactococcus lactis*, *Lactococcus cremoris*, and *Lactococcus lactis* biovar *diacetylactis*.

L. curvatus strains were obtained from the Agroscope culture collection and cultured in MRS broth supplemented with Tween-80 (Biolife Italiana, Italy). For long-term storage, stock cultures were preserved in reconstituted milk at −80 °C. To determine the formation of biogenic amines, supernatants were collected from starter culture and *L. curvatus* strains grown in MRS broth at 30 °C for approximately 20 h.

2.3. Cheesemaking and sampling

Model Raclette-type cheeses were produced at the Agroscope cheese dairy, each from 50 L of pasteurized cow milk, following the procedure described by Wechsler et al. (2021b). Six cheese variants were manufactured: (i) a control without *L. curvatus* (2 cheeses), and (ii–vi) cheeses supplemented with one of five *L. curvatus* strains, namely FAM18731 (3 cheeses), FAM24637 (4 cheeses), FAM24959 (4 cheeses), FAM25164 (4 cheeses), and FAM25224 (4 cheeses). Each variant is hereafter referred to as control, FAM18731, FAM24637, FAM24959, FAM25164, and FAM25224, respectively.

The *L. curvatus* strains were grown individually in MRS broth supplemented with Tween-80 at 30 °C for approximately 20 h prior to cheese manufacture and then added together with the starter culture to achieve an initial population of 10^4 colony-forming units (CFU)·mL^{-1} of vat milk.

Cheeses were ripened for 120 days, corresponding to the typical maturation period for Raclette-type cheese (Raclette Suisse, 2024), and allowing the monitoring of relevant biochemical changes. Samples were collected after 0, 1, 14, 45, 90, and 120 days of ripening for molecular and chemical analyses.

2.4. Quantitative PCR for L. curvatus (Lcurv-qPCR)

DNA was extracted from the curd and cheese samples as follows: 10 g cheese were mixed with 90 mL of peptone water (casein peptone 10 g L^{-1}, sodium chloride 5 g L^{-1}, trisodium citrate dihydrate 5 g L^{-1}, pH 7.0) and heated at 40 °C for 10 min. The sample was then homogenized for 3 min using a laboratory homogenizer (Masticator, IGZ Instruments AG, Switzerland). From the homogenate, 10 mL was mixed with 50 μL 10% (w/v) sodium dodecyl sulfate and centrifuged (4000×*g*, 30 min, room temperature) using a Hettich Rotanta 460R centrifuge equipped with a 4-place swing-out rotor (Andreas Hettich GmbH, Germany). The sediment was suspended in 400 μL G2 buffer of the Qiagen EZ1 DNA Tissue Kit (Qiagen, Germany), mixed with 100 mg 0.1 mm zirconia beads (OPS Diagnostics, Lebanon, USA), and shaken for 60 s at medium speed using an Omni Bead Ruptor (Omni International Inc., Kennesaw, USA) to lyse the bacterial cells. After centrifugation (16000×*g*, 10 min) using an Eppendorf 5418R centrifuge (Huberlab, Switzerland), 200 μL of the supernatant was mixed with 10 μL of proteinase K solution from the Qiagen EZ1 Tissue Kit and incubated for 1–2 h at 56 °C. The DNA of the sample was then extracted using a Qiagen BioRobot EZ1 Workstation. The final elution volume was 100 μL.

For the quantification of *L. curvatus*, a species-specific primer system from Dreier et al. (2021) was adopted and slightly adapted. The sequences of the primers and the hydrolysis probe for the qPCR assay were as follows: Lcurv_g1171_bdheF (5′-CAA CTT TGA TGC GTG AGT TAG AAG A-3′), Lcurv_g1171_bdheR (5′-TCG TTC GTA TTC AGC CTG TCC T-3′), and Lcurv_g1171_bdheFAM (5′-ACG GGT CAA TCA AAT TGG CAT GCA A-3′). The PCR was performed with a volume of 12 μL. The reaction mixture contained 6 μL Takyon No Rox Probe 2x MasterMix UNG (Eurogentec, Seraing, Belgium), 300 nmol/L forward and reverse primers, 100 nmol/L hydrolysis probe, and 2 μL 10-fold diluted DNA. PCR was performed using the Corbett Rotor-Gene 3000, 6000, or 5Plex (Qiagen). The PCR conditions were as follows: 50 °C for 2 min, 95 °C for 3 min, followed by 40 cycles at 95 °C for 3 s and 60 °C for 20 s. The Cq values of the PCR reactions were calculated using Rotor-Gene Q Series Software v2.3.5. Calibration curves for *L. curvatus* were generated using the HT-qPCR standard plasmid described by Dreier et al. (2021).

2.5. Chemical analysis

The pH value in the cheeses was determined using a pH puncture electrode, LoT 406-M6-DXK-S7/25 (Mettler Toledo, Switzerland). To assess the extent of proteolysis, the total nitrogen content and the nitrogen content of the water-soluble fraction (WSN) and non-protein fraction (NPN) were determined using the Kjeldahl method (ISO 8968-3:2004, IDF 20-3:2004; ISO, 2004a). For WSN fraction, cheese samples were homogenized in distilled water and filtered. The NPN fraction was obtained by adding trichloroacetic acid to the water-soluble nitrogen filtrate (ISO 8968-4:2016, IDF 20-4:2016; ISO, 2016). The total amount of free primary amine compounds in the cheeses was determined photometrically using o-phthaldialdehyde (OPA) (Rohm et al., 1996). A dilution series of a glutamic acid was used to create a calibration curve.

The water and fat content were determined gravimetrically in accordance with existing ISO standards (ISO 3433:2008, IDF 222:2008; ISO, 2008; ISO 5534:2004, IDF 4:2004; ISO, 2004b) or using Fourier-transform near-infrared spectroscopy (FT-NIR) validated for Raclette cheese. D- and L-lactate concentrations were quantified using enzymatic assay kits (ThermoFisher Scientific) on an automated Gallery™ enzyme analyzer (ThermoFisher Scientific). Free volatile carboxylic acids were determined by gas chromatography using an Agilent 8890 gas chromatograph (Agilent Technologies, Switzerland) equipped with an Agilent HP-5 capillary column (50 m × 0.32 mm × 0.52 μm) and a flame-ionization detector (FID), following extraction from cheese and subsequent esterification (Badertscher et al., 2023).

2.6. Determination of biogenic amines

The following biogenic amines were determined: tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, and tyramine.

The analytical procedure was based on the original method for biogenic amine determination in cheese (Bütikofer et al., 1990). Briefly, a piece of cheese without rind was grated, and 2.5 g were mixed with 500 μL internal standard solution (20 mM 1,7-diaminoheptane in 10 mM H_2SO_4) and 25 mL extraction solution (0.1 M perchloric acid in 50% acetonitrile). Samples were homogenized with ceramic beads using an Omni Bead Ruptor (Labforce AG, Switzerland) and centrifuged (900×*g*, 10 min, 10 °C) using an Eppendorf 5804R (Huberlab). Two hundred μL of supernatant was mixed with 800 μL acetonitrile, 700 μL H_2O, 200 μL 1.75 M sodium carbonate, and 100 μL dansyl chloride solution (50 mg mL^{-1} in acetone), followed by incubation at 40 °C for 30 min at 200 rpm. For bacterial cultures, 200 μL of the culture supernatant was used instead of the cheese extract. Residual dansyl chloride was quenched by adding 200 μL 270 mM L-glutamic acid and incubating at 40 °C for 60 min. After addition of 1 mL ethyl acetate and centrifugation (800×*g*, 10 min, 10 °C) using an Eppendorf 5804R (Huberlab), the organic phase (1 mL) was collected, dried at 40 °C, re-dissolved in 200 μL acetonitrile: H_2O (3:1), and filtered (0.45 μm).

A 5 μL aliquot was injected into a UPLC system (UltiMate 3000 RS, Thermo Fisher) equipped with a C18 column (Accucore C18, 2.6 μm, 150 × 4.6 mm) at 35 °C. Separation was performed at 1.3 mL min 1 using buffer A (300 mL acetonitrile, 470 mL H_2O, 200 mL ethanol, 30 mL 40 mM Tris(hydroxymethyl)aminomethane) and buffer B (450 mL acetonitrile, 100 mL H_2O, 450 mL ethanol, 2 mL 40 mM Tris(hydroxymethyl)aminomethane). The gradient increased from 15% B to 100% B over 6 min, was held for 1 min, and the column was re-equilibrated for 3 min at 15%B. Biogenic amines were detected at 254 nm and quantified using external standards (20 μg mL 1, Sigma Aldrich). The lower limit of quantification (LOQ) for the biogenic amines analyzed was 5 mg kg^{-1} cheese (Bütikofer et al., 1990).

2.7. *Free amino acids*

Free amino acids were quantified after extraction, protein precipitation, derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reagents, and ultrahigh-performance liquid chromatography (UPLC) analysis. A piece of cold cheese without rind was first grated. Samples weighing 800 mg were then taken and 9 mL 0.1 M HCl and L-norvaline (end concentration 100 pmol/μL) as internal standard were added, together with approximately 5 g ceramic beads. Samples were homogenized using an Omni Bead Ruptor (5 m s^{-1}, 2 × 30 s with a 5-s pause). Protein precipitation was achieved by adding 10 mL of 20% trichloroacetic acid (final concentration 10%) followed by thorough mixing. The samples were centrifuged (4000×*g*, 10 min, 10 °C) using an Eppendorf 5804R. From the aqueous phase, 1 mL was collected carefully through the fat layer using a Pasteur pipette.

For derivatization, 10 μL of the collected extract were mixed with 70 μL borate buffer and 20 μL AQC derivatization reagent (3.0–4.0 mg AQC dissolved in 1 mL LC grade acetonitrile). Derivatized samples were analyzed by reversed-phase UPLC with UV detection as described by Jaudzems et al. (2019). Amino acids were quantified using a derivatized external standard mixture containing all amino acids plus L-norvaline, and all concentrations were normalized to the internal standard (L-norvaline) to correct for extraction and derivatization efficiency.

2.8. *X-ray imaging*

X-ray imaging was performed using an Esotron compact X-ray instrument (Schweizer Röntgen, Switzerland). The X-ray tube was operated at 65 kV and 20 mAs. The X-ray system was located adjacent to the ripening cellar, allowing immediate transfer and imaging of the cheeses. The acquired images were processed using the Musica acquisition workstation NX (Agfa NV, Belgium).

2.9. *Statistical analyses*

Exploratory data analysis and statistics were performed using R (v4.3.3) and the tidyverse packages (v2.0.0, Wickham et al., 2019). Variance between groups was analyzed using one-way ANOVA followed by Tukey post-hoc with robust multiple comparisons (Herberich et al., 2010), accounting for unequal group sizes and heteroscedasticity.

3. Results

3.1. *Strain selection*

To select *L. curvatus* strains for the cheese experiment, 32 isolates from the Agroscope culture collection were initially screened for biogenic amine production (data not shown). Based on the screening results and strain origin, five strains were selected: FAM25224, which did not produce biogenic amines; FAM24637 and FAM24959, which produced tyramine and β-phenylethylamine; and FAM18731 and FAM25164, which also produced putrescine and tryptamine, respectively. Biogenic amine formation was subsequently quantified in MRS broth (Table 1). No biogenic amines were detected when the starter culture was grown in MRS broth.

3.2. *Enumeration of L. curvatus*

Growth of *L. curvatus* was determined in the curd and at different stages of ripening using a species-specific qPCR. *L. curvatus* was not detected in the control variants with one exception (Table 2). After 45 days of ripening a sample of the control variants yielded a signal of 2.3 log copies per reaction. After 90 and 120 days of ripening, the samples from the control variants were tested negative again. The low value and the fact that no signal was detected in later samples indicated a one-time contamination.

In the variants produced with *L. curvatus*, the species was clearly detected using the Lcurv-qPCR. The gene copy numbers of curd and cheese sample taken one day after production ranged from 4.2 to 4.8 log copies per reaction in the variants with added *L. curvatus* (Table 2). After 14 days, the values increased to between 5.7 and 6.3 log copies per reaction in the cheeses with *L. curvatus*. These values remained stable for all variants up to 120 days of ripening, except for variant FAM24959, which showed a slight increase from day 14 to day 45 and exhibited the highest values at the end of the ripening. Notably, variant FAM25224, containing the non-amine forming strain, showed the lowest values.

3.3. *Acidification*

The pH was measured in all cheeses at 4 and 24 h after production to monitor acidification. Acidification occurred as expected, and no significant differences were observed between the variants.

Table 1
Origin and biogenic amine production of *L. curvatus* strains used in this study.

Strain	Origin	BA production in vitro[a]
FAM18731	Salami	Tyra: 36.3 ± 1.0, PEA: 8.1 ± 4.6, Cad: <LOQ, Put: 8.2 ± 3.0
FAM24637	Vacherin Mont d'Or	Tyra: 33.2 ± 4.1, PEA: <LOQ
FAM24959	Cheddar	Tyra: 32.8 ± 2.3, PEA: <LOQ
FAM25164	Raclette	Tyra: 33.7 ± 0.2.5, PEA: <LOQ, Tryp: 29.4 ± 2.1
FAM25224	Raclette	not detected

[a] Concentrations are presented as mean ± standard deviation of two independent experiments in mg L^{-1}. Tyra: tyramine, PEA: β-phenylethylamine, Cad: cadaverine, Put: putrescine, Tryp: tryptamine. <LOQ compound was identified as a distinct peak in the HPLC chromatograms but concentration was below the limit of quantification.

Table 2
Enumeration of *L. curvatus* in model Raclette-type cheese variants by qPCR during ripening. Values are presented as the mean ± standard deviation (log gene copies per reaction).

Day	Cheese variant					
	control (n = 2)	FAM18731 (n = 3)	FAM24637 (n = 4)	FAM24959 (n = 4)	FAM25164 (n = 4)	FAM25224 (n = 4)
0 [1]	0^{a}	4.2 ± 0.3^{b}	4.3 ± 0.1^{b}	4.3 ± 0.1^{b}	4.5 ± 0.2^{b}	4.2 ± 0^{b}
1	0^{a}	4.5 ± 0.1^{b}	4.6 ± 0.2^{bcd}	4.5 ± 0.2^{bcd}	4.8 ± 0.1^{c}	4.3 ± 0.1^{d}
14	0^{a}	6.3 ± 0.1^{bc}	6.0 ± 0.1^{bc}	6.3 ± 0.2^{b}	6.3 ± 0.2^{bc}	5.7 ± 0.3^{c}
45	1.1 ± 1.6^{a}	6.3 ± 0^{ab}	6.0 ± 0.2^{ac}	6.7 ± 0.3^{b}	6.4 ± 0.2^{bc}	5.7 ± 0.1^{a}
90	0^{a}	6.0 ± 0.3^{bc}	5.9 ± 0.3^{bc}	6.6 ± 0.1^{d}	6.2 ± 0.1^{b}	5.6 ± 0.1^{c}
120	0^{a}	6.1 ± 0.1^{b}	5.9 ± 0.2^{b}	6.6 ± 0.2^{c}	6.2 ± 0.1^{b}	5.5 ± 0.1^{d}

1 DNA extracted from curd.
Variants that differed significantly in the multiple-comparison test ($p < 0.05$) are indicated by different letters within each row.

3.4. Changes in lactate and OPA values during ripening

The mean value of L-lactate on the first day after cheese production was 162 mmol/kg (Fig. 1). D-lactate was not present. After 45 days of maturation, the concentration of L-lactate in the control variant had decreased and averaged 89 mmol/kg. With a mean of 41 mmol/kg, lower concentrations of L-lactate were determined in the variants with *L. curvatus,* but D-lactate was present with a mean of 32 mmol/kg. After 120 days of ripening, the picture remained unchanged with slightly lower concentrations. The control variants contained only L-lactate, while both lactate isomers were present in the variants with *L. curvatus*.

The OPA method was used to study proteolysis during ripening. Plotting the mean values against the age of the cheese variants shows a steady increase in glutamic acid equivalent (Fig. 2). No significant difference in the slopes was detected between variants with and without *L. curvatus.*

3.5. Chemical parameters of the 120-day ripened cheeses

The comparison of the water, protein, and fat content of the 120-day ripened variants showed no significant differences (Table 3). Regarding NPN and WSN as indicators of proteolysis, no significant differences were found for NPN. In contrast, WSN values were significantly higher in all variants produced with *L. curvatus* than the control, with variant FAM24959 exhibiting the highest WSN values.

For volatile carboxylic acids, formic acid levels were significantly higher in variants FAM18731, FAM24637, FAM24959, and FAM25164, all produced with amine-forming strains, compared to the control (Table 3). As with WSN, variant FAM24959 showed the highest formic acid concentrations, which were significantly higher than those of all other variants. No significant differences were found for other volatile carboxylic acids such as acetic acid and propionic acid (Table 3).

3.6. Formation of biogenic amines

The levels of biogenic amines all model cheeses were determined after 45, 90, and 120 days of ripening. Tyramine, β-phenylethylamine, putrescine, cadaverine, and tryptamine were already present after 45 days of ripening and their concentrations increased during ripening.

Tyramine accumulated in variants FAM18731, FAM24637, FAM24959, and FAM25164, all produced with tyramine-forming *L. curvatus* strains, during ripening and reached a mean value of 308 (±37) mg/kg at the end of ripening (Fig. 3A). No tyramine was detected in the control. In variant FAM25224, the tyramine levels were 21 (±25)

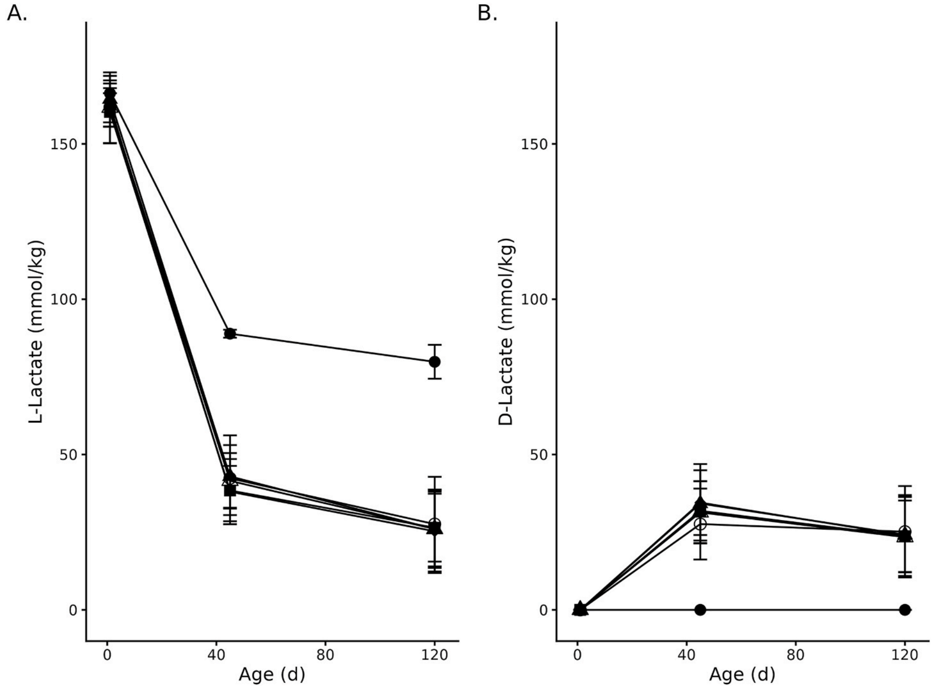

Fig. 1. Concentration of L- (A) and D-lactate (B) in model Raclette-type cheese variants during ripening (1, 45, and 120 days). Control (●), FAM18731 (▲), FAM24637 (■), FAM24959 (◆), FAM25164 (○), FAM25224 (△). Data are presented as means ± standard deviation.

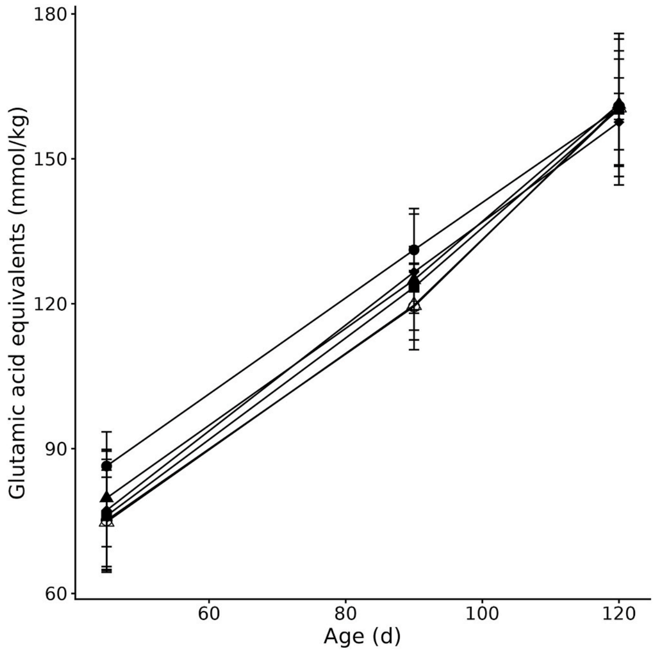

Fig. 2. Concentration of low-molecular-weight primary amines determined by the OPA method in model Raclette-type cheese variants during ripening (45, 90, and 120 days). Control (●), FAM18731 (▲), FAM24637 (■), FAM24959 (◆), FAM25164 (○), FAM25224 (△). Data are presented as means ± standard deviation.

mg/kg after 45 days, which then slightly decreased during further ripening.

β-Phenylethylamine was only detected in variants produced with the tyramine-forming strains of *L. curvatus* (Fig. 3B). Two groups could be distinguished based on the strains used. One group (variant FAM18731 and FAM24637) had less than 60 mg/kg at the end of ripening, while the other group (variant FAM24959 and FAM25164) contained approximately 150 mg/kg at this time point.

Putrescine and tryptamine were detected only in variants FAM18731 and FAM25164, respectively (Fig. 3C and E). At the end of ripening, mean concentrations reached 240 (±20) mg/kg for putrescine and 57 (±15) mg/kg for tryptamine. Cadaverine also formed in variant FAM18731, reaching 12 (±10) mg/kg after 45 days and 23 (±5) mg/kg after 120 days (Fig. 3D). In the other variants, only minor amounts of cadaverine (7 ± 10 mg/kg) were detected after 120 days.

3.7. *Free amino acids*

The mean values of the free amino acid concentrations are listed in Table 4. Comparison of the variants revealed significant differences in arginine, aspartic acid, ornithine, serine, and tyrosine. Tyrosine levels were significantly lower in all variants produced with tyramine-producing *L. curvatus* compared to the control and variant FAM25224. Ornithine was almost depleted in variant FAM18731, while more than 3 mmol kg^{-1} was still present in the other cheeses. Notably, no significant differences were found for phenylalanine and tryptophan, the precursors for β-phenylethylamine and tryptamine, respectively. For phenylalanine, variants FAM24959 and FAM25164, which exhibited the highest β-phenylethylamine levels, tended to show the lowest phenylalanine concentrations, although this was not statistically significant. Arginine concentrations were comparable across all variants except variant FAM24959, in which they were significantly lower. Serine and aspartic acid concentrations were also significantly lower in variant FAM24959 than in all other variants.

3.8. *X-ray analysis*

X-rays images of all model cheeses were taken after 120 days of ripening to visualize holes and openings. There were eyes in all variants, which is not unusual for Raclette cheeses (Fig. S1). No noticeable differences were observed in the number or size of the eyes among the cheese variants.

4. Discussion

Proteolysis during cheese ripening is one of the most important biochemical processes. During this process, amino acids are released and metabolized, with bacteria playing an important role in these reactions. However, bacterial-induced decarboxylation of amino acids in cheeses is undesirable because it yields biogenic amines and gas.

One of the most effective measures for reducing the formation of biogenic amines in cheese is to prevent amino acid decarboxylase-positive bacteria from entering and growing in or on cheese. This can be achieved by identifying the microbiological causes, which requires the development of diagnostic tools for the detection and quantification of undesired bacteria. Known biogenic amine formers in cheese are *Lentilactobacillus parabuchneri* (histamine), enterococci (tyramine and β-phenylethylamine), enterobacteria, and *Morganella morganii* (putrescine and cadaverine) (Berthoud et al., 2017; del Rio et al., 2019; Ladero et al., 2010; Marino et al., 2000; Pircher et al., 2007; Rea et al., 2004; Ryser et al., 2022). Putrescine and cadaverine are also formed by certain lactic acid bacteria, such as *Paucilactobacillus wasatchensis*, which are

Table 3
Composition of model Raclette-type cheese variants after 120 days of ripening. Values are presented as mean ± standard deviation.

Parameter	Cheese variant					
	control (n = 2)	FAM18731 (n = 3)	FAM24637 (n = 4)	FAM24959 (n = 4)	FAM25164 (n = 4)	FAM25224 (n = 4)
Fat (g/kg)	266.5 ± 0.7	268.3 ± 8.6	265.8 ± 6.8	265.5 ± 8.7	270.2 ± 5.9	265.0 ± 9.8
FDM (g/kg)	471.0 ± 1.4	474.7 ± 3.8	471.8 ± 4.3	470.2 ± 9.3	469.5 ± 5.7	466.5 ± 9.9
TN (g/kg)	40.9 ± 0.1	40.8 ± 1.0	40.9 ± 1.2	40.9 ± 0.3	41.5 ± 0.7	41.4 ± 1.5
NPN (g/kg)	6.2 ± 0.1	6.5 ± 0.4	6.6 ± 0.4	6.6 ± 0.5	6.5 ± 0.4	6.6 ± 0.4
WSN (g/kg)	21.6 ± 2.1^{a}	31.2 ± 5.9^{ab}	31.3 ± 4.8^{ab}	33.6 ± 4.6^{b}	29.7 ± 8.7^{ab}	30.4 ± 6.3^{ab}
Moisture (g/kg)	434.5 ± 3.5	434.7 ± 14.4	436.8 ± 14.0	435.2 ± 7.9	424.8 ± 7.7	432.2 ± 14.4
WFFC (g/kg)	592.0 ± 4.2	594.3 ± 12.7	594.8 ± 13.8	592.8 ± 4.9	582.2 ± 6.9	588.2 ± 13.8
Formic acid (mg/kg)	21.2 ± 1.3^{a}	51.1 ± 12.0^{b}	54.0 ± 16.9^{b}	96.9 ± 14.6^{c}	39.5 ± 6.6^{b}	41.4 ± 14.3^{ab}
Acetic acid (mg/kg)	332.7 ± 11.0	396.2 ± 85.7	405.1 ± 93.0	460.1 ± 125.0	329.4 ± 72.4	355.9 ± 80.3
Propionic acid (mg/kg)	7.8 ± 2.6	45.4 ± 24.9	38.0 ± 26.8	34.8 ± 16.1	12.4 ± 6.1	33.9 ± 24.1
Butyric acid (mg/kg)	23.3 ± 6.9	22.9 ± 3.1	22.0 ± 4.7	25.6 ± 8.5	18.1 ± 2.7	22.9 ± 4.9
Caproic acid (mg/kg)	6.4 ± 0.8	6.2 ± 0.7	6.4 ± 0.7	7.0 ± 2.8	4.9 ± 1.1	6.1 ± 0.6
Isobutyric acid (mg/kg)	1.3 ± 1.9	5.6 ± 1.3	6.2 ± 1.2	9.3 ± 6.0	2.4 ± 1.8	4.8 ± 0.5
Isovaleric acid (mg/kg)	6.1 ± 2.9	14.3 ± 1.8	15.1 ± 1.7	23.2 ± 13.0	8.2 ± 3.8	13.5 ± 1.7

Variants that differed significantly in the multiple-comparison test ($p < 0.05$) are indicated by different letters within each row.
TN: total nitrogen, NPN: non-protein nitrogen, WSN: water-soluble nitrogen, FDM: fat in dry matter, WFFC: water in fat-free cheese, n.d.:not detected.

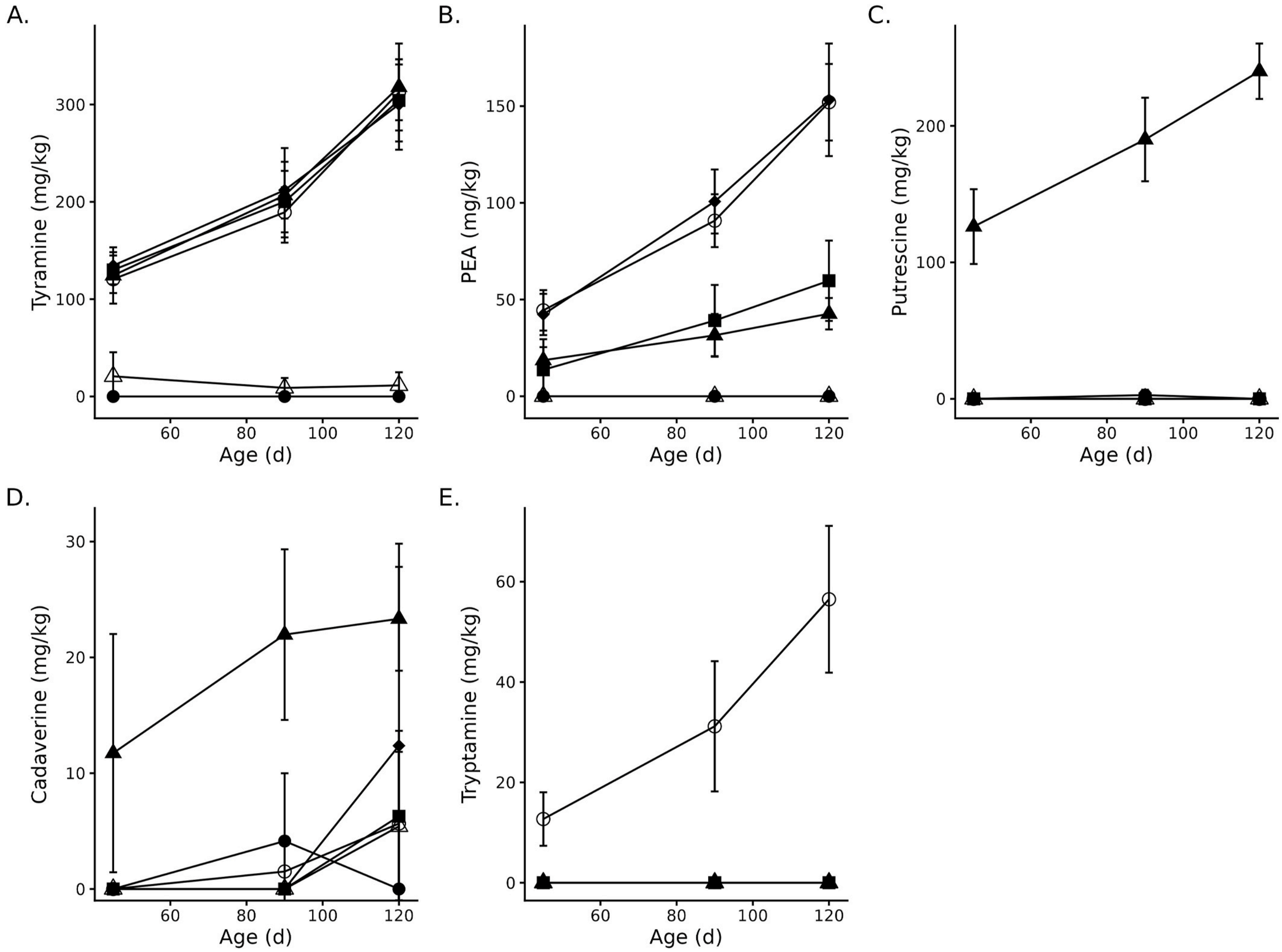

Fig. 3. Concentration of tyramine (A), β-phenylethylamine (PEA, B), putrescine (C), cadaverine (D), and tryptamine (E) in model Raclette-type cheese variants during ripening (45, 90, and 120 days). Control (●), FAM18731 (▲), FAM24637 (■), FAM24959 (◆), FAM25164 (○), FAM25224 (△). Data are presented as means ± standard deviation.

thought to be responsible for the formation of cracks and slits in Cheddar cheese (Berthoud et al., 2022; Ortakci et al., 2015). Recently, shotgun metagenomic analyses showed that *Loigolactobacillus rennini* encoding genes for decarboxylases, which form γ-aminobutyric acid, cadaverine, and putrescine, could be the bacterium causing defective Gouda cheeses (Decadt et al., 2024).

As described above, various studies on *L. curvatus* have shown that the species occurs in cheese and is able to form biogenic amines in the laboratory. Furthermore, *L. curvatus* has also been isolated from tyramine-containing cheeses (Beatrice et al., 2018; Bunkova et al., 2010; Burdychova & Komprda, 2007; Ladero et al., 2015; O'Sullivan et al., 2015). However, these studies do not explain any causalities, and to the best of our knowledge, only one study deliberately added *L. curvatus* to investigate the accumulation of tyramine in cheese (Pachlová et al., 2018). Unfortunately, the study showed no data on other biogenic amines, such as β-phenylethylamine or putrescine.

In the present study, the growth of *L. curvatus* in cheese was determined using species-specific qPCR, since there is no selective medium for the bacterial species that would allow selective microbial counting. After one day of ripening, the gene copy numbers did not increase significantly, but they rose rapidly by 1 to 2 logs within 14 days and then remained stable during ripening. It shows that *L. curvatus* can multiply rapidly in cheese ripened at 11 °C, and it is not surprising that it is often identified as a component of non-starter lactic acid bacteria (Beresford & Williams, 2004).

The total lactic acid content decreased during ripening, which was related to the rind microbiome. Raclette cheese is smeared, and the rind microbiome that develops during ripening contains yeast that metabolize lactic acid (Fröhlich-Wyder et al., 2019). In vitro studies describe that *L. curvatus* forms both L- and D-lactic acid from sugar (Hammes & Hertel, 2006). The present study shows that *L. curvatus* also converts L-lactic acid to D-lactic acid, as the latter formed in the cheeses with this bacterium during ripening.

One study in Cheddar cheese showed that proteolysis was affected by *L. curvatus* (Lynch et al., 1996). Various nitrogen fractions were analyzed and the OPA method was used to investigate whether *L. curvatus* can enhance proteolysis in Raclette cheese. Only WSN showed that the presence of *L. curvatus* led to the release of increased water-soluble, nitrogen-containing components. This was not observed with the OPA method, which primarily reacts with primary amines and di- and tri-peptides. The results suggest that *L. curvatus* can release more long-chain peptides from casein proteins. Since the study focused on the formation of biogenic amines, no in-depth peptide analyses were performed.

The presence of amine-forming *L. curvatus* led to the formation of biogenic amines in the cheeses. All cheeses that accumulated tyramine also contained β-phenylethylamine, and putrescine and tryptamine were formed depending on the strain used. The chemical structures of tyramine and β-phenylethylamine are similar, suggesting that both substances are formed via the same metabolic pathway. This is known from studies of enterococci, in which the tyrosine decarboxylase

Table 4

Concentration of free amino acids in model Raclette-type cheese variants after 120 days of ripening. Values are present as mean ± standard deviation (mmol kg^{-1}).

Amino acid	Cheese variant					
	control (n = 2)	FAM18731 (n = 3)	FAM24637 (n = 4)	FAM24959 (n = 4)	FAM25164 (n = 4)	FAM25224 (n = 4)
Ala	3.3 ± 0.2	2.8 ± 0.8	2.8 ± 1.2	5.1 ± 3.1	2.9 ± 1.4	2.9 ± 0.5
Arg	0.5 ± 0.1^{ab}	0.5 ± 0.1^{b}	0.5 ± 0.1^{b}	0.2 ± 0.1^{a}	0.6 ± 0.1^{b}	0.5 ± 0.1^{b}
Asn	6.9 ± 1.0	4.7 ± 2.4	4.2 ± 2.4	3.5 ± 2.5	4.6 ± 2.9	5.0 ± 2.1
Asp	3.1 ± 0.1^{a}	2.2 ± 0.8^{a}	2.3 ± 1.0^{a}	0.0 ± 0.1^{b}	2.4 ± 1.4^{ab}	2.5 ± 0.7^{a}
Cit	1.1 ± 0.4	1.0 ± 0.5	1.4 ± 0.6	0.5 ± 0.4	1.2 ± 0.3	1.7 ± 0.6
GABA	1.4 ± 0.4	0.7 ± 0.5	1.0 ± 1.1	0.7 ± 0.7	0.7 ± 0.7	0.8 ± 0.7
Gln	5.2 ± 0.7	4.1 ± 1.8	3.6 ± 2.5	3.5 ± 2.8	3.9 ± 2.7	3.9 ± 2.1
Glu	16.8 ± 0.8	13.1 ± 5.8	11.5 ± 5.2	12.3 ± 8.2	13.2 ± 7.8	13.7 ± 3.7
Gly	2.7 ± 0.1	2.3 ± 0.7	2.2 ± 0.8	2.1 ± 1.1	2.3 ± 1.1	2.5 ± 0.7
His	2.4 ± 0.1	2.6 ± 0.5	2.8 ± 0.3	2.7 ± 0.6	2.8 ± 0.3	3.3 ± 1.1
Ile	3.1 ± 0.4	2.6 ± 0.8	2.4 ± 1.1	2.5 ± 1.3	2.6 ± 1.3	2.7 ± 0.6
Leu	17.0 ± 0.0	12.6 ± 5.0	12.0 ± 5.9	12.0 ± 7.4	12.8 ± 7.3	13.5 ± 3.9
Lys	6.8 ± 0.7	6.2 ± 1.5	5.7 ± 2.1	5.7 ± 3.0	6.0 ± 2.8	6.5 ± 1.1
Met	2.5 ± 0.1	2.0 ± 0.7	1.7 ± 0.8	1.8 ± 1.1	1.9 ± 1.0	2.0 ± 0.5
Orn	5.4 ± 0.4^{a}	0.2 ± 0.4^{b}	3.6 ± 2.1^{ab}	4.3 ± 3.3^{ab}	3.9 ± 2.7^{ab}	4.0 ± 1.6^{a}
Phe	7.4 ± 0.2	5.5 ± 1.9	5.1 ± 2.2	4.4 ± 2.5	4.7 ± 2.4	6.2 ± 1.4
Pro	5.8 ± 0.5	5.1 ± 1.6	4.5 ± 2.1	4.8 ± 2.9	5.0 ± 2.6	4.9 ± 1.2
Ser	2.8 ± 0.4^{a}	1.8 ± 0.6^{ab}	1.6 ± 0.5^{a}	0.6 ± 0.2^{b}	1.7 ± 0.8^{ab}	2.3 ± 0.9^{a}
Thr	2.8 ± 0.1	2.1 ± 0.8	1.8 ± 0.7	1.7 ± 1.0	2.0 ± 1.0	2.3 ± 0.7
Trp	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.4	0.7 ± 0.4	0.5 ± 0.1	0.8 ± 0.1
Tyr	3.2 ± 0.5^{a}	0.9 ± 0.7^{bc}	0.5 ± 0.2^{c}	0.5 ± 0.3^{c}	0.5 ± 0.2^{c}	2.6 ± 0.7^{ab}
Val	7.8 ± 0.4	6.1 ± 2.1	5.7 ± 2.6	5.7 ± 3.3	6.1 ± 3.3	6.5 ± 1.6

Variants that differed significantly in the multiple-comparison test ($p < 0.05$) are indicated by different letters within each row.
Cit: citrulline, GABA: γ-aminobutyric acid, Orn: ornithine.

decarboxylates tyrosine and phenylalanine (Liu et al., 2014; Marcobal et al., 2006). This could probably be the case with *L. curvatus*. However, there remain questions here. In vitro, no β-phenylethylamine formation was observed when the gene for tyrosine decarboxylase from *L. curvatus* was heterologously expressed in *Escherichia coli* (Irmler et al., 2023). Furthermore, strain-specific differences in the ratio of tyramine to β-phenylethylamine concentrations were observed in the present cheese study. A similar phenomenon was observed in *Enterococcus faecium* and *Enterococcus faecalis*, in which different β-phenylethylamine levels were formed under laboratory conditions depending on the strain and tyrosine availability (Bargossi et al., 2015). Post-translational modifications may alter the conformation of the decarboxylase and thereby influence substrate recognition (Pisithkul et al., 2015). However, this remains highly speculative, and further research is needed to better understand the metabolic pathways involved in the formation of β-phenylethylamine in *L. curvatus* and other bacteria.

Laboratory experiments showed that, at higher concentrations of ornithine in the medium, FAM18731 also produced larger amounts of putrescine (data not shown). Since ornithine was depleted in cheese variant FAM18731, putrescine was also formed from ornithine here. Ornithine is not a component of the milk proteins and must first be formed through the metabolic activity of bacteria. In this study, this was accomplished by the lactococci in the starter culture, which possesses an active arginine deiminase pathway in which arginine is converted to ornithine (data not shown). Since ornithine was depleted in cheese variant FAM18731 and arginine was still present, this indicates that the conversion of arginine to ornithine was limiting the formation of putrescine.

Cadaverine can be formed in Raclette cheese by the surface microbiome and then diffuses towards the core. Therefore, sampling Raclette cheese is critical when investigating whether cadaverine formation occurs in the cheese body. In the present study, it was found that cadaverine also accumulated in the cheese variant FAM18731, albeit in small quantities. This observation is consistent with the findings of Li et al. (2018). The researchers used PCR to detect ornithine decarboxylase in seven strains of *L. curvatus*. Compared to putrescine, all strains produced only low levels of cadaverine. It is assumed that *L. curvatus* possesses a strain-dependent, bifunctional ornithine decarboxylase, which can decarboxylate both ornithine and lysine. Genomic analyses of strain FAM18731 are planned to investigate this hypothesis.

Regarding tryptamine, it was recently shown that *L. curvatus* FAM25164 possesses a gene encoding tryptophan decarboxylase (Irmler et al., 2023). Since tryptamine was increasingly produced in the cheeses with this strain, it indicates that the corresponding gene was active in the cheese environment.

Analysis of the amino acid profiles revealed pronounced differences in variant FAM24959, which showed some of the lowest concentrations of arginine, aspartic acid, and serine. This suggests that *L. curvatus* FAM24959 possesses specific metabolic properties that influence cheese biochemistry. Arginine may have been consumed through the arginine deiminase pathway. Aspartic acid is hypothesized to be taken up and decarboxylated to alanine, a pathway described for *Tetragenoccus halophila* D10 (Abe et al., 2002). Serine could have been converted to pyruvate by aminotransferase or dehydrogenase activity and subsequently transformed into formic acid. This pathway would explain the elevated formic acid concentrations observed in cheeses containing FAM24959. Genomic analyses are planned to investigate whether the bacterium carries genes encoding these metabolic pathways.

The formation of biogenic amines is accompanied by gas release, which must diffuse through the cheese curd and rind. If gas cannot escape, internal pressure may build, potentially causing undesirable cracks and splits. In this study, X-ray imaging revealed no such openings in Raclette cheese, likely because its soft rind allows gas to diffuse easily.

The levels of tyramine, β-phenylethylamine, tryptamine, cadaverine and putrescine in cheese are not currently regulated by law. According to the European Food Safety Agency (EFSA, 2011), no adverse health effects were observed in healthy people consuming meals containing less than 600 mg of tyramine. Assuming a 200-g serving of Raclette, the corresponding tyramine intake would be 60 mg, based on a tyramine content of 300 mg/kg—well below the EFSA recommended limit. However, *L. curvatus* produces not only tyramine but also other biogenic amines, whose interactions remain poorly understood. Since biogenic amine levels increase with ripening, it can also be expected that in cheeses that are aged for longer periods, *L. curvatus* could produce amounts of toxicological concern. A thorough understanding of the prevalence and metabolic activity of *L. curvatus* in different cheese types is therefore essential to assess its impact on cheese quality and safety.

5. Conclusion

Biogenic amines can trigger allergy-like reactions in sensitive individuals and may cause undesirable gas formation, reducing cheese quality. Because these compounds are produced by microorganisms, identifying and characterizing the relevant bacterial species is essential. The results of this study show that *Latilactobacillus curvatus* can be a significant producer of biogenic amines—including tyramine, β-phenylethylamine, putrescine, and tryptamine—in Raclette, a surface-ripened, full-fat semi-hard cheese. It should be noted that using single strains in this study does not reflect the complex microbiota of some cheeses, which may include undefined thermophilic starter cultures and multiple non-starter lactic acid bacteria, thereby disregarding interactions, competition, and synergistic effects that influence the growth, metabolism, and amine formation of *L. curvatus*. Nevertheless, the species poses a risk of spoilage and quality deterioration. Testing for amine-producing *L. curvatus* is recommended in cheeses affected by biogenic amines or undesired gas formation. As no selective medium currently exists, the species-specific PCR method used here provides a useful tool for detection and quantification.

CRediT authorship contribution statement

Stefan Irmler: Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Charlotte Fleuti:** Writing – review & editing, Validation, Methodology. **Lotti Egger:** Writing – review & editing, Validation, Methodology. **Reto Portmann:** Writing – review & editing, Validation, Methodology. **Florian Loosli:** Writing – review & editing, Methodology, Conceptualization. **Hélène Berthoud:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2026.106679.

Data availability

Data will be made available on request.

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