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PII: S0023-6438(21)01550-4

DOI: https://doi.org/10.1016/j.lwt.2021.112397

Reference: YFSTL 112397

To appear in: *LWT - Food Science and Technology*

Received Date: 16 June 2021

Revised Date: 3 August 2021

Accepted Date: 30 August 2021

Please cite this article as: Adámek, R., Pachlová, V., Salek, R.N., Němečková, I., Buňka, Františ., Buňková, L., Reduction of biogenic amine content in Dutch-type cheese as affected by the applied adjunct culture, *LWT - Food Science and Technology* (2021), doi: https://doi.org/10.1016/j.lwt.2021.112397.

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Reduction of biogenic amine content in Dutch-type cheese as affected by the applied adjunct culture

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Abstract

The aim of the study was to reduce the concentration of biogenic amines (BAs) in Dutch-type cheese by the activity of the added culture. The reduction of BAs was caused by the use of the selected strains of *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* over a three-month period (at 12±1 °C). The results indicated that the use of different microbiological strains does not have a significant influence on the basic chemical parameters of the model cheese samples. The lowest BA concentrations were determined in model cheese with *Lacticaseibacillus casei* CCDM 198. These samples contained fewer total BA content than the control samples: after 28 days of ripening by 32%, after 56 days by 37% and after 84 days by 32%. The adjunct culture demonstrated high efficacy of reduction of putrescine, phenylethylamine and tyramine in real conditions of the model Dutch-type cheese samples.

Keywords Biogenic amine, Food safety, Dutch-type cheese; Ripening; Adjunct culture, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*

Chemical compounds studied in this article

2-phenylethylamine (PubChem CID: 1001); Putrescine (PubChem CID: 1045); Tyramine (PubChem CID: 5610)

1. Introduction

Cheese is one type of food characterised by its organoleptic properties, in particular flavour and aroma. These properties are the result of biochemical reactions in which sensory active substances are formed (McSweeney, 2017). Hence, the most important stage during cheesemaking is the ripening process, where the most intensive chemical and physical changes occur. During cheese ripening, casein decomposes leading to the accumulation of free amino acids (FAAs), which can be converted into a number of sensory active substances by the effect of the microflora present, but they might also be decarboxylated into biogenic amines (BAs) (Church & Widdowson, 2002).

BAs are low-molecular substances with a biological activity, and some of them (serotonin, histamine and tyramine) play an important role in human, animal and plant physiology (Medina, Urdiales, Rodríguez-Caso, Ramírez, & Sánchez-Jiménez, 2003). In addition, BAs affect acid tolerance (Romano, Ladero, Alvarez, & Lucas, 2014) and the regulation of human osmotic and oxidative stresses (Fernandez & Zuniga, 2006). However, a few BAs can also have a direct or indirect effect on the human cardiovascular and nervous system (Shalaby, 1996). High intake of BAs from food may cause food intoxication. Histamine and tyramine in particular are the most frequently accumulated BAs during cheese ripening. Thus, the consumption of large amounts of these BAs (in food) can lead to undesirable effects such as headache, nausea, hypotension (histamine) or hypertension (tyramine). Relevant issue of putrescine is the potentiation of the toxicity of other amines (EFSA, 2011). Furthermore, BAs could be produced in foods at higher concentrations by the activity of both contaminating microorganisms (mainly *Escherichia*, *Enterobacter*, *Salmonella*, *Shigella*) and starter or non-starter lactic acid bacteria (LAB; *Streptococcus*, *Lactobacillus* and derived genera, *Lactococcu*s, *Leuconostoc*) and may pose a health risk to the consumer (Halász, Barath, Simon-Sarkadi, & Holzapfel, 1994; EFSA,

2011). Due to the diversity of microorganisms in cheeses, BAs can be produced in high concentrations during cheese ripening (Pachlová et al., 2018; Combarros-Fuertes et al., 2016).

As mentioned above, cheeses can contain high concentration of BAs, and their consumption together with other BA-containing foods presents a significant risk to consumer health, for example in combination with fish products, meat, beers or wines where BAs have been created (Lorencová et al., 2020; Palomino-Vasco, Rodríguez-Cáceres, Mora-Diez, Pardo-Botello, & Acedo-Valenzuela, 2019; Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001; Shalaby, 1996). Nevertheless, concurrent consumption of food and beverages with a possible high content of BAs can significantly increase the probability of deterioration in the consumer's health and for this reason the content of BAs in food should be reduced for providing of food safety (García-Díez & Saraiva, 2021).

Reducing the number of microorganisms in the raw material (heat treatments, high pressure treatment etc.) is one of the possibilities to reduce BA production during product storage. On the other hand, this method may not be sufficient due to increasing number of microorganism (as well as decarboxylase-positive strains) during manufacturing and storage. Other possibility of increasing of quality of products is reduction of BA by microbial activity where amino oxidase in particular is a major factor in the decomposition of BAs. The previous research in this field has predominantly concentrated on the determination of BAs in Dutch-type cheese (Flasarová et al., 2016; Buňková et al., 2010). However, only few publications dealt with BA reduction by microorganisms capable of degrading BA for application during cheese ripening (Renes et al., 2019; Tittarelli, Perpetuini, Di Gianvito, & Tofalo, 2019; Herrero-Fresno et al., 2012). Due to the fact that microorganisms responsible for the production of BAs are present in different types of cheese, it is necessary to investigate ways to reduce BA content using adjunct strains which are suitable for the certain type of cheese. The aim of the present study was to reduce the concentration of BAs in real conditions during Dutch-type cheese ripening

(in Central Europe one of the most popular type of cheese) by using an adjunct cultures with *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum*.

2. Material and Methods

2.1. Model cheese samples

Altogether, five batches of model cheeses were produced with selected microorganisms (Table 1), specifically (i) a control cheese batch; (ii) a model cheese batch with the addition of the BA-degrading strain of *Lacticaseibacillus casei* CCDM 422 (hereinafter "Lb.c422"); (iii) a model cheese batch with the addition of the BA-degrading strain of *Lacticaseibacillus casei* CCDM 198 (hereinafter "Lb.c198"); (iv) a model cheese batch with the addition of the BA-degrading strain of *Lactiplantibacillus plantarum* CCDM 189 (hereinafter "Lb.p189"); and (v) a model cheese batch with the addition of the BA-degrading strain of *Lactiplantibacillus plantarum* CCDM 187 (hereinafter "Lb.p187"). All model cheese batches were produced with basic mesophilic culture (Laktoflora®, Milcom, Prague, Czech Republic) containing *Lactococus lactis* subsp. *lactis, Lactococus lactis* subsp. *cremoris* and *Lactococus lactis* subsp. *lactis* biovar *diacetylactis* and also with the addition of a BA-producing strain *Lactococcus lactis* subsp. *cremoris* CCDM 946 for comparison of intensity of BA reduction by selected strains during ripening. All BA-producing/degrading microbiological strains were obtained from the Culture Collection of Dairy Microorganisms (Laktoflora®, Milcom, Prague, Czech Republic).

2.2. Bulk starter preparation

For the preparation of the bulk starter for the control cheese samples with the addition of the BA-producing strain, a commercial lyophilised mesophilic culture (Laktoflora®, Milcom, Prague, Czech Republic) was used. For the preparation of the starter bulk, 120 mL

of heat-treated milk after cooling (at a temperature of 25 ± 1 °C) was inoculated with 0.45 g of the lyophilised commercial mesophilic culture. The bulk starter with the addition of the BA-producing strain was prepared by mixing 35 mL of heat-treated milk and 5 mL of overnight *Lactococcus lactis* subsp. *cremoris* CCDM 946 culture. Individual bulk starters were incubated at a temperature of 25 ± 1 °C for 20 h.

For each model batch of cheeses with the BA-degrading strain, three bulk starters were prepared separately: (i) a basic bulk starter of 80 mL volume containing the commercial mesophilic culture; (ii) a second bulk starter of 40 mL volume containing the BA-producing strain and (iii) a third bulk starter of 40 mL volume containing the BA-degrading strain. The basic bulk starter was prepared by mixing 80 mL of heat-treated milk and 0.3 g of the lyophilised commercial culture (Laktoflora®, Milcom, Prague, Czech Republic). The second bulk starter (containing the BA-producing strain) was prepared by mixing 35 mL of heat-treated milk and 5 mL of broth inoculated with the BA-producing strain *Lactococcus lactis* subsp. *cremoris* CCDM 946. The third bulk starter (containing the BA-degrading strain) was prepared by mixing 35 mL of heat-treated milk and 5 mL of broth inoculated with the appropriate degrading strain (see Table 1). All bulk starters were separately incubated at a temperature of 25 ± 1 °C for 20 h. The broth which was used for the preparation of the bulk starter was prepared according to Buňková et al. (2011).

2.3. Cheese production

A schematic illustration of cheese manufacturing protocol is depicted in the Figure 1. Firstly, raw milk was skimmed (Disc Bowl Centrifuge FT15, Armfield Inc., UK) and standardised (the fat content to 3%). Pasteurisation with an FT75 laboratory pasteuriser (Armfield Inc., UK) was performed (74 ± 1 °C for 30 s). A total of 35 L of standardised and pasteurised milk was used for the production of the batch. Subsequently, 17.5 mL of $CaCl_2$

(36% (w/w) solution, Milcom a.s., Czech Republic) and microbiological culture were applied. Furthermore, coagulant (5.4 mL, Chymax M200, 190 IMCU/mL, Chr. Hansen, Denmark) was used for renneting (30 min. at 32 ± 1 °C). After this process, the curd was processed according to the steps shown in Figure 1. Subsequently, the pressed blocks of cheese were left at 12 ± 1 °C (16 h). The batches were brined (20% NaCl (w/w), pH 5.3 during 3 h) to a target NaCl concentration of 1.5% (w/w). Therefore, Delvocid (antimycoticum; DSM, Netherlands) was applied onto the surface of the cheese blocks (on the next day). Finally, the model cheese samples were packaged in shrinkage foil and stored in a controlled temperature ripening chamber (at 12 ± 1 °C).

The model cheese samples were analysed (physico-chemical analysis, microbiological analysis, determination of the free amino acids and biogenic amines) after 1, 14, 28, 56 and 84 days of ripening. Each type of cheese was produced three times (5 types of model cheese × 3 repetitions, 15 model batches produced in total).

2.4. Physico-chemical analysis

The physico-chemical analysis was focused on determining the dry matter content by the gravimetric method at a temperature of 102 ± 2 °C to constant mass according to ISO 5534: 2004, fat content by the Soxhlet method with hexan extraction (ISO 1211, 2010), NaCl content (ISO 5943, 2006). The pH was determined (at ambient temperature) by inserting the glass tip electrode of a calibrated pH-meter (pH Spear, Eutech Instruments, Oakton, Malaysia) directly into the samples at three randomly chosen locations. The physico-chemical analysis was performed from two blocks of cheese and each sample was subjected to physico-chemical analysis three times (3 repetitions of manufacture × 2 cheese blocks × 3 repetitions of determination; n = 18). The samples were subsequently lyophilised (Pachlová et al., 2011) to determine the free amino acid and biogenic amine content.

2.5. Microbiological analysis

The microbiological analysis was performed according to Buňková et al. (2010) and Flasarová et al. (2016). The total number of selected groups of microorganisms was determined: (i) mesophilic aerobic and facultative anaerobic microorganisms (total count of microorganisms – TCM; Plate Count Agar, PCA, cultivation at 37 ± 1 °C for 24 h, Merc, New Jersey, USA); (ii) lactic acid bacteria (M17 agar, cultivation at 37 ± 1 °C for 48 h, Merck, New Jersey, USA), microscopic preparations were performed to assess the number of cocci and rods; (iii) enterococci (Slanetz-Bartley agar, SB, cultivation at 37 ± 1 °C for 24 h, Merck, New Jersey, USA); enterobacteria (Endo agar, cultivation at 37 ± 1 °C for 24 h, Merc, New Jersey, USA). The microbiological analysis was performed from two blocks of cheese and each sample was subjected to microbiological analysis three times (3 repetitions of manufacture × 2 cheese blocks × 3 repetitions of analysis; n = 18).

2.6. Determination of free amino acids

The lyophilised cheese samples (Christ Alpha 1-4, Christ, Osterode, Germany) were used to determine the free amino acid content (FAA). The extraction of the FAAs was performed by triple extraction by lithium citrate buffer according to Pachlová et al. (2011). The lyophilised sample and the lithium citrate buffer were mixed in a ratio of 1:7 for 1 h at 22 ± 2 °C and centrifuged (15,000 g for 30 min at 4 ± 1 °C). The supernatant was filtered. The extraction of solid residue was repeated (a total of three extractions). All extraction was mixed and lithium citrate buffer was added up to 25 mL. The resulting extract was filtered by a 0.45μm filter and analysed by ion-exchange liquid chromatography (AAA400 Amino Acid Analyser, Ingos, Czech Republic) as described in Flasarová et al. (2016) and Buňková et al. (2009). The reagents for sample preparation and detection were obtained from Ingos

(Czech Republic). Standards were purchased from SigmaAldrich (St. Louis, USA). The extraction of free amino acids was performed from two blocks of cheese and each extract was subject to chromatographic analysis twice (3 repetitions of manufacture × 2 cheese blocks × 2 extractions × 2 separation and determination of eluents; n = 24).

2.7. Determination of biogenic amines

The lyophilised cheese samples were used to determine the biogenic amine content (histamine, tyramine, phenylethylamine, tryptamine, putrescine, cadaverine, spermidine and spermine). A triple extraction from the lyophilised cheeses was used by 0.6 mol/L perchloric acid (Merck, New Jersey, USA) according to Flasarová et al. (2016). Derivatisation and chromatographic separation (ZORBAX Eclipse Plus C18, 50 mm × 3.0 mm, 1.8 μm, Agilent Technologies, USA) were performed according to Dadáková, Křížek, & Pelikánová (2009) and Smělá, Pechová, Komprda, Klejdus, & Kubáň (2003). Standards and reagents was obtained from SigmaAldrich (St. Louis, USA). The extraction of biogenic amines was performed from two blocks of cheese and each extract was subject to chromatographic analysis twice (3 repetitions of manufacture × 2 cheese blocks × 2 extractions × 2 separation and determination of eluents; n = 24).

2.8. Statistical analysis

The obtained data was statistically evaluated by means of the Kruskal–Wallis test and the Wilcoxon test. Unistat® 5.5 software (Unistat, London, UK) was used for the statistical evaluation. A level of significance of P = 0.05 was used in the whole work.

3. Results and discussion

3.1. Physico-chemical analysis

It can be stated that the applied microbiological strains, the method of production and the ripening conditions did not have a significant effect on the basic chemical parameters of model cheese samples ($P \geq 0.05$). The average pH values recorded for all model cheese samples at the beginning of the ripening period were 5.19 ± 0.12. The pH gradually increased and was 5.34 ± 0.11 at the end of the ripening. The trend of increasing pH is consistent with proteolysis and subsequent production of basic substances (e.g. aldehydes, ketones). In addition, the lactic acid breaks down into products that are not so acidic, e.g. acetate, carbon dioxide (Fox, Guinee, Cogan, & McSweeney, 2017).

The dry matter content for the whole ripening period was in the range of 53.98–55.88% in the samples of natural cheeses with different adjunct culture. Furthermore, the fat and salt content was relatively constant throughout the experiment for all model samples. The fat content in the dry matter was about 44.2–45.2% and the salt content was about 1.37–1.45%.

3.2. Microbiological analysis

The development of groups of microorganisms in the model cheese sample is shown in Table 2.

The control samples, which were not inoculated with the BA-degrading strains, showed a gradual increase in the TCM until day 28 and then these values gradually decreased. The control samples contained the lowest TCM of all model cheese samples at the end of the ripening period. In the case of samples Lb.c198, Lb.p189 and Lb.p187, the TCM rose until day 28 and then the total count of microorganisms began to decrease. The TCM even started to decrease after 14 days for the Lb.c422 samples.

As for lactic acid bacteria (LAB) cocci, some differences were noticed. It is assumed that they are mainly starter bacteria from the basic mesophilic culture. The LAB cocci content

values of the control samples gradually declined throughout the ripening. In terms of samples with the BA-degrading strain, the number of LAB cocci CFU increased. However, a decrease was recorded at the end of the ripening period. This decrease is due to the lysis of their cells (Lortal & Chapot-Chartier, 2005) and this trend in the number of total LAB cocci was also observed by other authors (Combarros-Fuertes et al., 2016; Porcellato, Østlie, Brede, Martinovic, & Skeie, 2013).

Regarding the development of LAB rods, it can be seen in Table 2 that the number of these bacteria showed an increase in all samples throughout the ripening period. Higher numbers of LAB rods were observed at the beginning of ripening in the batches with the BA-degrading strain compared to the control sample ($P < 0.05$). In the case of samples Lb.c198, Lb.p189 and Lb.p187, there was a steeper increase in LAB rods by the 28th day of ripening.

This increasing trend was also noticed in the case of bacteria of the genus *Enterococcus*. The greatest increase was recorded after 14 days and subsequently the number of colonies grew until the end of storage. The presence of enterococci could be caused by natural thermoresistant milk microflora that survived pasteurisation temperatures. However, the occurrence of enterococci in cheeses is not unique due to that they are able to face the conditions of cheese manufacture and ripening (Tofalo et al., 2019). Broadbent, Budinich, & Steele (2011) reported that after 3-4 months of ripening, the number of enterococci, together with lactobacilli, can reach up to 8.00 log CFU/g. Generally, cheeses may contain non-starter bacteria, which can create the largest content of BAs. These bacteria survive in advanced stages of cheese ripening even at low lactose concentrations and they use amino acids to gain energy, which can lead to the formation of BAs (Zuljan et al., 2016). Moreover, non-starter lactic acid bacteria, especially enterococci, are tolerant of environmental changes during ripening of the cheese (Montel et al., 2014). For this reason, it is necessary

to reduce BA content by using the appropriate adjunct culture to ensure food safety.

Bacteria of the family *Enterobacteriaceae* were not detected throughout the whole ripening period. From these results, it can be stated that the cheeses were produced with good hygienic manufacturing practices, because the presence of *Enterobacteriaceae* is considered to be an indicator of hygienic conditions (Tofalo et al., 2019).

3.3. Determination of free amino acids

Differences between model batches were observed in the concentration of free amino acids during ripening ($P < 0.05$).

As can be seen in Figure 1, the total number of free amino acids had an increasing character. An increasing proteolysis was noticed in all the model cheese samples. However, it was always higher for samples with the adjunct cultures compared to the control sample (Figure 1). Development of individual free amino acids during ripening in the model cheese is shown in Table 3. The major free amino acids were proline, valine, leucine, lysine and ornithine in the model samples. After cheese production (the 1st day), the FAA concentration was similar for all examined samples (Figure 1). Nevertheless, other authors did not note a different intensity of proteolysis at the beginning of ripening between cheeses that ripen under different conditions either (Pachlová et al., 2018; Pinho, Ferreira, Mendes, Oliveira, & Ferreira, 2001). This similar course in FAA development was observed by day 28 for all samples. The first major differences were noted on day 56 of ripening. The total content of FAAs for the Lb.c422, Lb.c198 and Lb.p189 samples increased by an average of 37% compared to the control. At the end of the ripening period (the 84th day), there was a significant increase in the FAA content for all model cheese samples. The total concentration of FAAs even sharply increased for samples with a protective strain compared to the control samples after 84 days. The highest concentration of FAAs was recorded in

288 batches Lb.c198 and Lb.p187. Specifically, in the case of the Lb.c198 sample, the increase
289 was 39%, and in the case of the Lb.p187 sample, the increase was 27% compared to the
290 control cheese. The results are in accordance with those previously reported by Pachlová et
291 al. (2018) and Flasarová et al. (2016).

292 The release of amino acids from the protein matrix of the cheese is mainly due to the
293 enzymatic activity of the microflora present. However, the resulting concentration of FAAs
294 is also affected by their conversion to secondary products, which may contribute to the
295 development of the flavour of the cheese (Battelli et al., 2019). The intensity of proteolysis
296 depends predominantly on the proteolytic activity of the present microflora. Subsequently,
297 after cell lysis, the proteolytic enzymes which are inside the cell are released into the cheese
298 matrix. The above-mentioned enzymes can intensively hydrolyse peptides and proteins to
299 form free amino acids (Fenelon & Guinee, 2000). On the other hand, FAAs can also be
300 decarboxylated to potentially dangerous biogenic amines (Diaz et al., 2016).

301

302 ***3.4. Determination of biogenic amines***

303 The total content of BAs in the samples (Table 4) increased with the ripening period due
304 to the progress of proteolysis of the casein network, more precisely by releasing their
305 precursors - FAAs (Halász et al., 1994), and activity of BA-producing strain *Lc. lactis*
306 subsp. *cremoris* CCDM 946, which was added during manufacturing of cheese for
307 comparison of degradation intensity of BA by observed adjunct culture. As can be seen in
308 Table 4, the total BA content in the control batch at the end of ripening was 585.3 ± 20.2
309 mg/kg. In the case of samples with adjunct culture, the values were lower compared to
310 control batch at the end of ripening. The lowest concentration was determined in the
311 Lb.c198 samples (394.0 ± 16.4 mg/kg) where was determined almost a third lower total BA
312 content. Even though the BA content increased in all model batches (due to addition of BA-

313 producing strain), demonstrably lower concentrations were detected in batches with added
314 adjunct cultures *Lacticaseibacillus casei* CCDM 198 and *Lactiplantibacillus plantarum*
315 CCDM 187. In the case of the Lb.c198 samples, the recorded values showed preferable
316 results. Although the Lb.c198 samples showed the highest FAA content (as precursors for
317 BA) at the end of ripening, the lowest total BA concentrations were determined. Lb.c198
318 samples contained also significantly less total BA content than the control samples during
319 the whole ripening period: after 28 days of ripening by 32%, after 56 days by 37% and after
320 84 days by 32% ($P < 0.05$). Moreover, the total BA content in Lb.p.187 samples was lower
321 compared to the control samples: by 34 % after 28 days, resp. by 27 % after 56 days of
322 ripening. However, the intensity of degradation activity in the Lb.p 187 samples slowed
323 down and at the end of the ripening time the total BA content was detected almost 17%
324 lower compared to the control. The decrease in degradation efficiency of BAs during
325 ripening was probably due to loss in viability and subsequently autolysis of the used adjunct
326 culture (Wilkinson & LaPointe, 2020).

327 Although histamine is very often present in matured cheeses, similar to the study by
328 Tofalo et al. (2019) histamine was not detected during the ripening period. The main BAs
329 detected in cheese samples were tyramine, putrescine and phenylethylamine. Other BAs
330 were reported at very low concentrations (<5 mg/kg; below the detection limit).
331 Additionally, the development of the content of selected BAs in the model cheese during
332 ripening is shown in Table 4. The content of precursor of detected BAs (tyrosine, ornithine
333 and phenylethylalanine; Table 3) increased during ripening, since the conditions for the
334 creating of BA were ensured.

335 All adjunct strains demonstrated high efficacy of reduction of putrescine in real
336 conditions of the model Dutch-type cheese samples which were produced with BA
337 producing strain. Even the Lb.c198 sample contained a 92% lower concentration of

putrescine at the end of storage (the 84th day) in comparison with the control sample. In general, putrescine does not have a significant toxicological effect on humans, it may intensify the negative effects of tyramine and histamine, which are abundant in cheeses (EFSA, 2011). In addition to that, a significantly lower content of phenylethylamine was determined in the Lb.c198 samples during ripening ($P < 0.05$).

Furthermore, interesting data were also obtained in the case of tyramine. The Lb.c198 samples reached a significantly lower level of tyramine compared to the control samples until the 56^{th} day of ripening ($P < 0.05$). For consumers who are under classical monoamino oxidase (MAO) medication, tyramine may cause serious health problems (EFSA, 2011). For this reason, it may be recommended that for sensitive individuals to consume cheeses with a shorter maturation period, or cheeses with an adjunct culture capable of reducing BA content. In addition, an effective reduction of tyramine during the ripening time of two months may be sufficient for Dutch-type cheese intended for further processing, such as the production of processed cheese, where a highly mature raw material is not usually used for economic reasons (Talbot-Wash, Kannar, & Selomulya, 2018). Due to the fact that BA are thermostable, further processing of food will not eliminate them if they are already present (Ruiz-Capillas & Herrero, 2019). From this point of view, it is important to ensure low concentrations of BA in the cheese when is applied as raw material. The use of adjunct cultures able of reducing BA together with high quality raw materials and good hygienic manufacturing practices might be the best way of making products with reduced BA associated health risks (Tittarelli, Perpetuini, Di Gianvito, & Tofalo, 2019).

Pištěková et al. (2020) demonstrated the intensity of the degradation of putrescine, tyramine, histamine and cadaverine by the *Lacticaseibacillus casei* CCDM 198 strain in a simple growth system (MRS broth and milk). As a result of the reduction of BAs in real conditions of model cheese, it can be stated that the *Lacticaseibacillus casei* CCDM 198

strain could be used in dairy cultures to decrease the concentration of BAs during cheese ripening in order to reduce the risk of adverse effects on humans.

4. Conclusion

From the results obtained, it is clear that the use of different microbiological strains does not have a significant influence on the tested physico-chemical parameters of the model cheese samples. On the other hand, differences in the total FAA content during ripening between model batches were determined. In addition, more intensive proteolysis was observed in the cheese with adjunct cultures able reducing BA content. The increasing trend of BA content was also observed during ripening of model batches due to the activity of added BA-producing strain. Degradation of putrescine, phenylethylamine and tyramine was reported because of strain *Lacticaseibacillus casei* CCDM 198 addition, resulting in the highest intensity of reduction of BA in real conditions during cheese ripening. At the end of the ripening period, the values of BAs in cheese with *Lacticaseibacillus casei* CCDM 198 were significantly lower in comparison with the control cheese (by 32%). Provided information can be used for further research on the decrease of BA content in cheeses with the aim of limiting the negative impact on human health.

Acknowledgements

This work was supported by the Ministry of Agriculture of the Czech Republic, the National Agency for Agriculture Research, project No. QK1710156 in the ZEMĚ programme and the Internal Grant Agency of Tomas Bata University in Zlín (project IGA/FT/2021/004).

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Table 1: Applied microbiological strains during the manufacture of the model Dutch-type cheese samples.

Sample	The used microbiological strains
Control	mesophilic culture of Laktoflora + *Lactococcus lactis* subsp. *cremoris* CCDM 946 (producer of biogenic amines)
Lb.c422	mesophilic culture of Laktoflora + *Lactococcus lactis* subsp. *cremoris* CCDM 946 (producer of biogenic amines) + *Lacticaseibacillus casei* CCDM 422 (degrader of biogenic amines)
Lb.c198	mesophilic culture of Laktoflora + *Lactococcus lactis* subsp. *cremoris* CCDM 946 (producer of biogenic amines) + *Lacticaseibacillus casei* CCDM 198 (degrader of biogenic amines)
Lb.p189	mesophilic culture of Laktoflora + *Lactococcus lactis* subsp. *cremoris* CCDM 946 (producer of biogenic amines) + *Lactiplantibacillus plantarum* CCDM 189 (degrader of biogenic amines)
Lb.p187	mesophilic culture of Laktoflora + *Lactococcus lactis* subsp. *cremoris* CCDM 946 (producer of biogenic amines) + *Lactiplantibacillus plantarum* CCDM 187 (degrader of biogenic amines)

Table 2: Counts of microorganisms (log CFU/g) in model Dutch-type cheese samples (n = 18) produced with different strains of *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* during a 84-day storage period (at 12±1 °C). *

Sample	Time of ripening (days)	Group of microorganisms (log CFU/g)			
		TCM[a]	LAB cocci[b]	LAB rods[c]	Enterococci
Control	1	7.16 ± 0.44 [a] A	8.67 ± 0.43 [a] A	6.26 ± 0.33 [a] A	1.20 ± 0.14 [a] A
	14	8.08 ± 0.40 [b] A	8.22 ± 0.50 [a] A,B	8.07 ± 0.38 [b] A	2.30 ± 0.09 [b] A
	28	8.21 ± 0.36 [b] A	8.20 ± 0.46 [a] A	8.21 ± 0.40 [b] A	3.2 ± 0.10 [c] A
	56	8.19 ± 0.43 [b] A	8.15 ± 0.33 [b] A	8.18 ± 0.46 [b] A	3.16 ± 0.08 [c] A
	84	8.04 ± 0.49 [b] A	8.16 ± 0.33 [b] A	8.29 ± 0.46 [b] A	3.19 ± 0.17 [c] A
Lb.c422	1	7.39 ± 0.44 [a] A	8.78 ± 0.38 [a] A	7.46 ± 0.38 [a] B	1.10 ± 0.07 [a] A
	14	8.29 ± 0.45 [b] A	8.67 ± 0.35 [a,b] A,C	7.96 ± 0.37 [b] A	2.86 ± 0.15 [b] B
	28	8.26 ± 0.37 [b] A	8.40 ± 0.37 [b] A	8.18 ± 0.33 [b,c] A	3.40 ± 0.13 [c] B
	56	8.17 ± 0.42 [b] A	8.54 ± 0.31 [a,b] A	8.36 ± 0.45 [c] A	3.87 ± 0.12 [d] B
	84	8.13 ± 0.49 [b] A	8.27 ± 0.38 [b] A	8.43 ± 0.40 [c] A	3.74 ± 0.15 [d] B
Lb.c198	1	7.39 ± 0.51 [a] A	8.32 ± 0.38 [a,b] B	7.27 ± 0.39 [a] B	1.38 ± 0.14 [a] B
	14	8.06 ± 0.35 [b] A	8.20 ± 0.32 [a] B,C	8.07 ± 0.35 [b] A	2.48 ± 0.14 [b] A
	28	8.38 ± 0.46 [b] A	8.59 ± 0.37 [b] A	8.49 ± 0.31 [c] A,B	3.2 ± 0.11 [c] A
	56	8.35 ± 0.33 [b] A	8.58 ± 0.33 [b] A	8.41 ± 0.33 [c] A	3.70 ± 0.10 [d] B,C
	84	8.24 ± 0.38 [b] A	8.33 ± 0.49 [a,b] A	8.50 ± 0.45 [c] A	3.76 ± 0.07 [d] B
Lb.p189	1	8.07 ± 0.41 [b] B	8.21 ± 0.43 [a] B	7.32 ± 0.37 [a] B	1.48 ± 0.14 [a] B
	14	8.34 ± 0.32 [b] A	8.43 ± 0.41 [a] A,B,C	7.89 ± 0.40 [b] A	2.16 ± 0.12 [b] C
	28	8.45 ± 0.35 [b] A	8.55 ± 0.41 [a] A	8.58 ± 0.33 [c] B	3.76 ± 0.11 [c] C
	56	8.36 ± 0.40 [b] A	8.40 ± 0.48 [a] A	8.34 ± 0.38 [c] A	3.85 ± 0.08 [c] B
	84	8.28 ± 0.36 [b] A	8.33 ± 0.44 [a] A	8.60 ± 0.44 [c] A	3.81 ± 0.07 [c] B
Lb.p187	1	8.01 ± 0.47 [b] B	8.07 ± 0.32 [a] B	7.10 ± 0.31 [a] B	1.48 ± 0.18 [a] B
	14	8.26 ± 0.50 [b] A	8.78 ± 0.49 [b] C	7.87 ± 0.47 [b] A	2.53 ± 0.14 [b] A
	28	8.31 ± 0.41 [b] A	8.83 ± 0.31 [b] A	8.34 ± 0.35 [c] A,B	3.68 ± 0.10 [c] C
	56	8.22 ± 0.40 [b] A	8.60 ± 0.48 [b] A	8.36 ± 0.42 [c] A	3.50 ± 0.12 [d] C
	84	8.21 ± 0.32 [b] A	8.39 ± 0.45 [a,b] A	8.42 ± 0.44 [c] A	3.67 ± 0.18 [c, d] B

[a] The total count of microorganisms (TCM) − determination of mesophilic aerobic and facultative anaerobic microorganisms.

[b] Lactic acid bacteria (LAB) including *Lactococcus* and *Leuconostoc* strains.

[c] Lactic acid bacteria including *Lactobacillus*, *Lacticaseibacillus* and *Lactiplantibacillus*

* Values are expressed as the mean (n = 18) ± standard deviation. The means within a column (the difference between samples with various times of ripening) followed by different superscript letters differ ($P < 0.05$); samples with each strain added were evaluated separately. Mean

values within a column with different capital letters indicate statistically significant ($P < 0.05$) differences between sample types (the difference between samples with different adjunct culture added).

Table 3: Development of free amino acid content (mg/kg) in model Dutch-type cheese samples (n = 24) during a 84-day storage period (at 12±1°C). *

Model sample	Free amino acid	Time of ripening (days)				
		1	14	28	56	84
control	asparagic acid	ND	ND	ND	ND	ND
	glutamic acid	ND	ND	ND	ND	ND
	serine	14.2 ± 0.4 [a]	132.2 ± 1.2 [b]	231.3 ± 7.9 [c]	507.8 ± 23.4 [d]	751.5 ± 1.5 [e]
	glycine	16.3 ± 0.1 [a]	34.4 ± 0.3 [b]	78.7 ± 0.4 [c]	175.1 ± 7.7 [d]	277.3 ± 1.4 [e]
	histidine	31.6 ± 0.5 [a]	54.3 ± 2.1 [b]	93.0 ± 0.4 [c]	191.4 ± 1.4 [d]	306.4 ± 1.6 [e]
	arginine	6.54 ± 0.23 [a]	26.7 ± 1.2 [b]	76.3 ± 1.8 [c]	102.2 ± 2.1 [e]	89.1 ± 0.9 [d]
	threonine	14.1 ± 0.2 [a]	113.1 ±1.8 [c]	47.6 ± 0.4 [b]	465.4 ± 10.2 [d]	714.3 ± 3.6 [e]
	γ-aminobutyric acid	2.63 ± 0.11 [a]	45.1 ± 1.4 [b]	59.2 ± 1.6 [c]	88.7 ± 2.7 [e]	69.1 ± 0.5 [d]
	alanine	5.81 ± 0.21 [a]	13.3 ± 0.1 [b]	46.3 ± 1.8 [c]	ND	379.1 ± 1.8 [d]
	asparagine	ND	ND	ND	ND	ND
	proline	109.9 ± 1.6 [a]	176.1 ± 1.5 [b]	326.1 ± 9.1 [c]	596.6 ± 16.7 [d]	936.2 ± 1.9 [e]
	tyrosine	39.95 ± 0.88 [a]	95.3 ± 1.2 [b]	137.3 ± 4.0 [c]	254.2 ± 3.1 [d]	356.2 ± 1.1 [e]
	cysteine	8.12 ± 0.33 [a]	ND	ND	ND	6.33 ± 0.18 [b]
	valine	16.9 ± 0.4 [a]	156.8 ± 6.7 [b]	346.6 ± 6.9 [c]	749.8 ± 6.1 [d]	1160.4 ± 5.8 [e]
	methionine	11.1 ± 0.3 [a]	53.7 ± 1.3 [b]	106.9 ± 1.3 [c]	235.4 ± 2.8 [d]	441.7 ± 1.3 [e]
	phenylalanine	45.3 ± 1.4 [a]	216.3 ± 8.3 [b]	383.1 ± 7.3 [c]	751.5 ± 6.1 [d]	978.6 ± 2.9 [e]
	isoleucine	9.96 ± 0.38 [a]	61.6 ± 0.7 [b]	143.2 ± 3.1 [c]	351.8 ± 3.7 [d]	539.2 ± 2.7 [e]
	lysine	115.8 ± 11.6 [a]	232.5 ± 14.9 [b]	393.9 ± 12.8 [c]	771.8 ± 37.8 [d]	1130.8 ± 65.7 [e]
	leucine	54.84 ± 2.23 [a]	300.6 ± 22.4 [b]	929.6 ± 40.9 [c]	1132.5 ± 43.1 [d]	2566.2 ± 77.3 [e]
	ornithine	42.1 ± 0.7 [a]	164.5 ± 5.9 [b]	419.5 ± 5.0 [c]	968.4 ± 28.1 [d]	1454.2 ± 4.4 [e]
	tryptophan	ND	7.08 ± 0.33 [a]	11.2 ± 0.1 [b]	50.9 ± 1.3 [c]	71.3 ± 0.8 [d]

Table 3 (*continued*)

Model sample	Free amino acid	Time of ripening (days)				
		1	14	28	56	84
Lb.c422	asparagic acid	ND	ND	ND	ND	ND
	glutamic acid	ND	ND	ND	ND	ND
	serine	16.5 ± 0.2 [a]	39.2 ± 0.1 [b]	132.4 ± 0.5 [c]	359.3 ± 16.5 [d]	514.6 ± 11.2 [e]
	glycine	17.2 ± 0.3 [a]	40.6 ± 0.7 [b]	96.7 ± 2.4 [c]	221.1 ± 3.8 [d]	326.7 ± 1.3 [e]
	histidine	32.6 ± 0.8 [a]	195.5 ± 8.2 [d]	138.7 ± 5.3 [b]	169.1 ± 4.2 [c]	397.1 ± 11.6 [e]
	arginine	12.3 ± 0.4 [a]	53.5 ± 1.8 [b]	135.1 ± 5.1 [c]	146.9 ± 3.2 [d]	156.2 ± 6.1[e]
	threonine	11.6 ± 0.3 [a]	134.1 ± 4.3 [b]	306.2 ± 11.3 [c]	594.7 ± 11.9 [d]	803.9 ± 2.4 [e]
	γ-aminobutyric acid	4.81 ± 0.18 [a]	23.8 ± 0.8 [b]	45.6 ± 0.5 [c]	68.9 ± 0.6 [e]	50.9 ± 0.2 [d]
	alanine	ND	ND	33.9 ± 0.9 [a]	117.7 ± 3.8 [b]	131.2 ± 0.5 [c]
	asparagine	ND	ND	ND	ND	ND
	proline	40.9 ± 1.0 [a]	200.5 ± 7.5 [b]	445.1 ± 15.3 [c]	834.3 ± 21.1 [d]	1181.3 ± 47.7 [e]
	tyrosine	30.7 ± 0.6 [a]	45.2 ± 0.7 [b]	74.5 ± 0.3 [c]	84.7 ± 1.6 [d]	161.1 ± 3.3 [e]
	cysteine	ND	ND	ND	ND	ND
	valine	21.6 ± 0.2 [a]	193.1 ± 3.7 [b]	507.3 ± 23.3 [c]	1045.5 ± 29.3 [d]	1504.9 ± 57.3 [e]
	methionine	6.61 ± 0.22 [a]	73.3 ± 2.6 [b]	162.2 ± 5.8 [c]	416.9 ± 6.7 [d]	549.5 ± 1.1 [e]
	phenylalanine	30.4 ± 0.2 [a]	152.9 ± 5.7 [b]	339.7 ± 7.8 [c]	578.7 ± 17.4 [d]	647.1 ± 6.9 [e]
	isoleucine	7.18 ± 0.11 [a]	63.4 ± 2.3 [b]	177.6 ± 4.8 [c]	421.8 ± 9.3 [d]	643.4 ± 1.9 [e]
	lysine	107.1 ± 1.4 [a]	200.1 ± 1.9 [b]	447.6 ± 7.2 [c]	853.3 ± 18.8 [d]	1249.1 ± 5.2 [e]
	leucine	42.4 ± 0.9 [a]	449.6 ± 4.3 [b]	1174.7 ± 18.8 [c]	2343.3 ± 93.7 [d]	2918.3 ± 5.8 [e]
	ornithine	40.2 ± 0.8 [a]	222.5 ± 8.9 [b]	618.7 ± 6.2 [c]	1349.1 ± 51.3 [d]	1893.9 ± 83.8 [e]
	tryptophan	6.73 ± 0.13 [a]	10.5 ± 0.4 [b]	52.6 ± 1.7 [c]	78.1 ± 2.4 [d]	110.2 ± 3.4 [e]

Table 3 (*continued*)

Model sample	Free amino acid	Time of ripening (days)				
		1	14	28	56	84
Lb.c198	asparagic acid	ND	ND	ND	ND	ND
	glutamic acid	ND	ND	ND	ND	ND
	serine	21.1 ± 0.2 [a]	112.2 ± 0.9 [b]	195.5 ± 8.4 [c]	415.5 ± 8.7 [d]	478.3 ± 1.9 [e]
	glycine	22.6 ± 0.6 [a]	851.5 ± 0.3 [b]	99.3 ± 3.5 [c]	212.2 ± 4.5 [d]	402.3 ± 0.8 [e]
	histidine	25.4 ± 0.5 [a]	122.2 ± 4.2 [b]	125.1 ± 1.2 [b]	176.1 ± 4.2 [c]	416.5 ± 2.1 [e]
	arginine	29.6 ± 0.6 [a]	155.3 ± 3.3 [b]	260.6 ± 11.5 [c]	292.8 ± 12.7 [d]	587.3 ± 1.8 [e]
	threonine	33.4 ± 1.1 [a]	164.1 ± 4.2 [b]	325.2 ± 3.3 [c]	583.1 ± 23.5 [d]	1020.6 ± 38.1 [e]
	γ-aminobutyric acid	68.7 ± 1.5 [a]	93.4 ± 4.1 [b]	110.6 ± 3.5 [c]	141.6 ± 5.1 [d]	154.7 ± 4.9 [e]
	alanine	ND	ND	ND	ND	ND
	asparagine	ND	ND	ND	ND	ND
	proline	83.3 ± 1.2 [a]	282.7 ± 11.3 [b]	419.2 ± 5.1 [c]	638.9 ± 13.4 [d]	1300.7 ± 2.6 [e]
	tyrosine	20.6 ± 0.7 [a]	123.1 ± 2.2 [b]	132.2 ± 3.8 [c]	194.3 ± 2.3 [d]	388.7 ± 9.8 [e]
	cysteine	ND	ND	ND	ND	ND
	valine	34.1 ± 0.8 [a]	254.2 ± 1.8 [b]	514.3 ± 17.1 [c]	938.8 ± 26.3 [d]	1800.7 ± 36.6 [e]
	methionine	6.72 ± 0.23 [a]	89.3 ± 0.6 [b]	171.4 ± 6.9 [c]	313.2 ± 14.4 [d]	628.3 ± 22.5 [e]
	phenylalanine	10.3 ± 0.2 [a]	187.6 ± 7.1 [b]	331.9 ± 11.1 [c]	682.5 ± 10.2 [d]	896.5 ± 41.5 [e]
	isoleucine	28.2 ± 1.1 [a]	90.1 ± 0.8 [b]	179.3 ± 7.2 [c]	435.2 ± 18.3 [d]	807.4 ± 24.6 [e]
	lysine	19.7 ± 0.3 [a]	319.6 ± 13.2 [b]	536.4 ± 17.7 [c]	915.8 ± 39.4 [d]	1552.8 ± 47.9 [e]
	leucine	31.3 ± 0.8 [a]	638.2 ± 21.1 [b]	1283.0 ± 60.3 [c]	2593.5 ± 85.6 [d]	4055.1 ± 61.2 [e]
	ornithine	52.4 ± 2.3 [a]	337.5 ± 11.5 [b]	692.4 ± 9.1 [c]	1387.5 ± 18.1 [d]	2360.3 ± 46.3 [e]
	tryptophan	ND	37.2 ± 0.6 [a]	57.1 ± 1.2 [b]	124.4 ± 3.9 [c]	165.2 ± 8.6 [e]

Table 3 (*continued*)

Model sample	Free amino acid	Time of ripening (days)				
		1	14	28	56	84
Lb.p189	asparagic acid	ND	ND	ND	ND	ND
	glutamic acid	ND	ND	ND	ND	ND
	serine	ND	10.2 ± 0.2 [a]	103.9 ± 4.9 [b]	350.6 ± 15.1 [c]	453.8 ± 18.2 [d]
	glycine	ND	52.1 ± 1.3 [a]	67.8 ± 2.4 [b]	252.3 ± 7.1 [c]	302.9 ± 12.6 [d]
	histidine	251.7 ± 15.1 [b]	269.5 ± 11.7 [b]	130.5 ± 4.5 [a]	129.1 ± 2.3 [a]	346.1 ± 8.4 [c]
	arginine	16.1 ± 0.3 [a]	132.0 ± 2.6 [b]	254.8 ± 2.6 [c]	301.1 ± 3.6 [d]	454.3 ± 18.3 [e]
	threonine	8.13 ± 0.11 [e]	175.8 ± 1.4 [a]	287.7 ± 12.7 [b]	204.7 ± 4.3 [c]	877.5 ± 15.6 [d]
	γ-aminobutyric acid	ND	57.5 ± 2.2 [a]	65.5 ± 1.5 [b]	70.7 ± 3.1 [c]	85.7 ± 2.4 [e]
	alanine	ND	ND	22.6 ± 0.9 [a]	135.5 ± 6.9 [b]	ND
	asparagine	ND	ND	ND	ND	ND
	proline	107.1 ± 3.7 [a]	237.7 ± 4.8 [b]	318.2 ± 11.3 [c]	782.4 ± 23.5 [d]	1147.9 ± 53.4 [e]
	tyrosine	23.6 ± 0.8 [a]	28.1 ± 1.2 [b]	23.6 ± 0.7 [a]	69.1 ± 1.5 [c]	117.6 ± 3.5 [d]
	cysteine	ND	ND	ND	ND	ND
	valine	17.2 ± 0.3 [a]	229.3 ± 2.1 [b]	409.8 ± 12.7 [c]	1235.1 ± 28.4 [d]	1589.9 ± 64.1 [e]
	methionine	6.02 ± 0.15 [a]	83.4 ± 1.2 [b]	143.7 ± 3.9 [c]	636.7 ± 21.2 [d]	628.9 ± 13.1 [d]
	phenylalanine	20.2 ± 2.2 [a]	123.6 ± 4.2 [b]	150.9 ± 1.7 [c]	580.0 ± 14.1 [d]	521.5 ± 12.6 [e]
	isoleucine	5.85 ± 0.10 [a]	89.9 ± 3.9 [b]	137.7 ± 3.7 [c]	470.7 ± 12.7 [d]	705.2 ± 15.1 [e]
	lysine	103.7 ± 3.2 [a]	320.5 ± 5.1 [b]	346.7 ± 5.5 [c]	959.2 ± 15.5 [e]	1322.6 ± 60.6 [e]
	leucine	33.9 ± 0.2 [a]	567.1 ± 9.1 [b]	993.4 ± 38.7 [c]	2739.3 ± 80.9 [d]	3537.8 ± 112.7 [e]
	ornithine	28.5 ± 1.1 [a]	302.8 ± 11.2 [b]	547.9 ± 13.7 [c]	1575.9 ± 72.5 [d]	2007.5 ± 82.4 [e]
	tryptophan	6.79 ± 0.17 [a]	28.7 ± 0.5 [b]	55.8 ± 1.7 [c]	162.3 ± 4.9 [d]	226.8 ± 11.1 [e]

Table 3 (*continued*)

Model sample	Free amino acid	Time of ripening (days)				
		1	14	28	56	84
Lb.p187	asparagic acid	ND	ND	ND	ND	ND
	glutamic acid	ND	ND	ND	ND	ND
	serine	32.5 ± 1.1 [a]	21.5 ± 0.6 [b]	117.7 ± 5.1 [c]	235.4 ± 10.6 [d]	515.7 ± 2.6 [e]
	glycine	31.1 ± 0.8 [a]	34.2 ± 0.9 [b]	85.3 ± 3.8 [c]	153.9 ± 3.4 [d]	335.1 ± 5.3 [e]
	histidine	99.4 ± 3.2 [a]	89.2 ± 3.7[b]	143.9 ± 2.6 [c]	82.1 ± 3.2 [b]	413.7 ± 11.7 [d]
	arginine	30.4 ± 0.3 [a]	104.1 ± 1.4 [b]	164.8 ± 6.1 [c]	176.1 ± 2.7 [d]	311.6 ± 9.3 [e]
	threonine	14.8 ± 0.5 [a]	138.1 ± 4.6 [b]	259.8 ± 4.2 [c]	417.5 ± 12.1 [d]	893.6 ± 40.5 [e]
	γ-aminobutyric acid	ND	59.5 ± 2.4 [a]	90.3 ± 4.1 [c]	73.6 ± 1.5 [b]	102.6 ± 2.5 [d]
	alanine	ND	84.9 ± 1.5 [b]	126.6 ± 5.8 [c]	215.4 ± 8.0 [d]	49.1 ± 0.2 [a]
	asparagine	ND	ND	ND	ND	ND
	proline	239.8 ± 6.3 [b]	199.3 ± 5.2 [a]	304.9 ± 10.2 [c]	508.4 ± 22.6 [d]	1335.1 ± 37.2 [e]
	tyrosine	51.1 ± 1.9 [a]	65.9 ± 0.9 [b]	85.8 ± 0.6 [c]	149.7 ± 4.6 [d]	359.2 ± 1.1 [e]
	cysteine	ND	ND	ND	ND	ND
	valine	36.5 ± 1.5 [a]	225.8 ± 7.9 [b]	359.1 ± 16.9[c]	706.3 ± 7.1 [d]	1593.6 ± 51.6 [e]
	methionine	ND	100.3 ± 2.9 [a]	111.2 ± 4.6 [b]	240.1 ± 6.7 [c]	554.4 ± 11.7 [d]
	phenylalanine	56.7 ± 1.6 [a]	180.2 ± 5.4 [b]	281.8 ± 9.6 [c]	440.4 ± 16.7 [d]	846.3 ± 31.4 [e]
	isoleucine	16.6 ± 0.5 [a]	82.5 ± 2.1 [b]	151.2 ± 5.9 [c]	352.6 ± 6.7 [d]	821.6 ± 25.6 [e]
	lysine	222.1 ± 5.9 [a]	253.8 ± 10.7 [b]	336.4 ± 5.0 [c]	675.1 ± 23.3 [d]	1487.9 ± 45.7 [e]
	leucine	80.2 ± 2.3 [a]	612.1 ± 22.1 [b]	989.1 ± 32.6 [c]	1767.9 ± 30.6 [d]	3724.1 ± 71.2 [e]
	ornithine	65.2 ± 2.8 [a]	217.2 ± 1.1 [b]	434.3 ± 7.8 [c]	959.8 ± 1.9 [d]	2068.4 ± 41.9 [e]
	tryptophan	ND	9.6 ± 0.3 [a]	17.4 ± 0.1 [b]	77.3 ± 0.3 [c]	177.5 ± 2.9 [d]

* Values are expressed as the mean (n = 24) ± standard deviation. The means within a row (the difference between content of free amino acid with various times of ripening) followed by different superscript letters differ ($P < 0.05$)

Table 4: Development of biogenic amine content (mg/kg) in model Dutch-type cheese samples (n = 24) produced with different strains of *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* during a 84-day storage period (at 12±1 °C).*

Sample	Time of ripening (days)	Biogenic amine content (mg/kg)			
		Phenylethylamine	Putrescine	Tyramine	Total content
Control	1	35.8 ± 1.0 [a] D	35.8 ± 0.7 [a] E	114.1 ± 4.1 [a] E	185.7 ± 6.6 [a] E
	14	38.2 ± 0.6 [b] E	40.0 ± 1.0 [b] E	182.0 ± 5.9 [b] C	260.2 ± 9.2 [b] D
	28	23.4 ± 0.4 [c] C	106.7 ± 2.4 [c] E	154.8 ± 2.5 [c] B	284.9 ± 11.2 [c] D
	56	31.6 ± 0.8 [d] D	167.6 ± 2.6 [d] D	292.3 ± 9.1 [d] B	491.5 ± 15.8 [d] C
	84	37.9 ± 1.4 [b] D	198.1 ± 5.7 [e] E	349.3 ± 15.9 [e] A	585.3 ± 20.2 [e] D
Lb.c422	1	15.2 ± 0.4 [a] A	13.3 ± 0.4 [a] B	21.4 ± 0.1 [a] B	49.9 ± 1.5 [a] A
	14	20.5 ± 0.4 [b] A	23.3 ± 0.6 [b,d] B	166.1 ± 4.9 [b] B	209.9 ± 8.5 [b] B
	28	34.0 ± 0.6 [c] E	24.0 ± 0.7 [b] B	185.0 ± 4.0 [c] C	243.0 ± 10.9 [c] B
	56	26.7 ± 0.5 [d] C	25.7 ± 0.8 [c] A	429.4 ± 16.2 [d] C	481.8 ± 16.7 [d] C
	84	21.8 ± 0.8 [b] C	22.7 ± 0.3 [d] C	487.3 ± 18.5 [e] D	531.8 ± 19.1 [e] C
Lb.c198	1	29.0 ± 0.9 [a] C	6.3 ± 0.1 [a] A	19.6 ± 0.1 [a] A	54.9 ± 2.1 [a] B
	14	22.6 ± 0.6 [b] B	29.1 ± 0.9 [b] D	98.2 ± 0.7 [b] A	149.9 ± 5.3 [b] A
	28	15.2 ± 0.2 [c] A	36.1 ± 0.5 [c] D	141.6 ± 2.8 [c] A	192.9 ± 7.9 [c] A
	56	14.1 ± 0.1 [d] A	40.6 ± 1.6 [d] C	255.2 ± 6.3 [d] A	309.9 ± 12.4 [d] A
	84	13.0 ± 0.1 [e] A	16.0 ± 0.3 [e] A	365.0 ± 12.7 [e] A	394.0 ± 13.4 [e] A
Lb.p189	1	18.9 ± 0.8 [a] B	15.6 ± 0.5 [a] C	33.4 ± 0.7 [a] C	67.9 ± 2.9 [a] C
	14	29.8 ± 0.4 [b] C	26.0 ± 0.2 [b] C	165.1 ± 6.6 [b] B	220.9 ± 7.1 [b] C
	28	30.5 ± 0.9 [b] D	29.5 ± 0.7 [c] C	223.9 ± 8.7 [c] D	283.9 ± 10.1 [c] C
	56	34.1 ± 0.4 [c] E	33.1 ± 0.5 [d] B	433.7 ± 11.7 [d] C	500.9 ± 14.1 [d] C
	84	78.0 ± 2.8 [d] E	17.8 ± 0.2 [e] B	434.1 ± 15.6 [d] C	529.9 ± 16.4 [d] C
Lb.p187	1	39.6 ± 1.0 [a] E	22.4 ± 0.9 [a] D	75.9 ± 2.7 [a] D	137.9 ± 4.4 [a] D
	14	37.1 ± 0.6 [b] D	21.2 ± 0.2 [b] A	95.5 ± 3.3 [b] A	153.8 ± 6.5 [b] A
	28	20.4 ± 0.6 [c] B	20.4 ± 0.3 [c] A	146.0 ± 5.8 [c] A,B	186.8 ± 8.2 [c] A
	56	21.0 ± 0.7 [c] B	41.0 ± 1.0 [d] C	295.8 ± 11.5 [d] B	357.8 ± 12.5 [d] B
	84	15.6 ± 0.1 [d] B	68.4 ± 1.7 [e] D	402.9 ± 16.8 [e] B	486.9 ± 18.3 [e] B

* Values are expressed as the mean (n = 24) ± standard deviation. The means within a column (the difference between samples with various times of ripening) followed by different superscript letters differ ($P < 0.05$); samples with each strain added were evaluated separately. Mean values within a column with different capital letters indicate statistically significant ($P < 0.05$) differences between sample types with the same ripening time (the difference between samples with different adjunct culture added).

Figure captions

Fig. 1

Model Dutch-type cheese production schema

Fig. 2

Total content of free amino acids (FAA; g/kg) development during ripening of model Dutch-type cheese samples (n = 24) produced with different strains of *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* during 84 days of storage (at 12±1 °C). A control sample without further lactobacillus addition was also developed, consisting of *Lactococcus lactis* subsp. *cremoris* CCDM 946. Model cheeses were sampled after 1 (black), 14 (silver), 28 (dark-gray), 56 (light-gray) and 84 (dim-gray) days of storage. The following abbreviations were utilized: Control: control sample; Lb.c422*:* *Lacticaseibacillus casei* CCDM 422; Lb.c198*:* *Lacticaseibacillus casei* CCDM 198; Lb.p189*:* *Lactiplantibacillus plantarum* CCDM 189; Lb.p187*:* *Lactiplantibacillus plantarum* CCDM 187. The results are expressed as means; the error bars represent standard deviation (n = 24).

Fig. 1

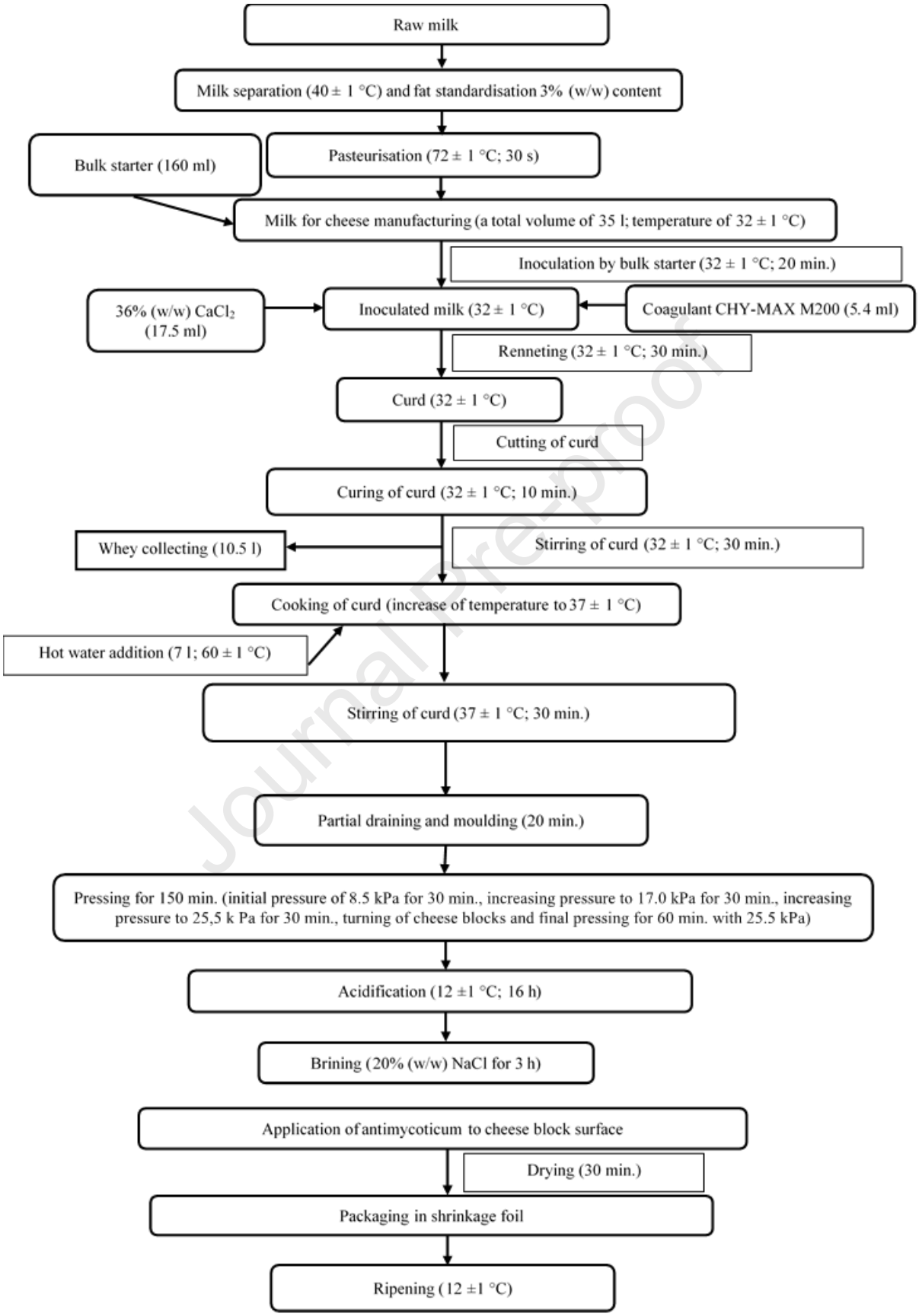
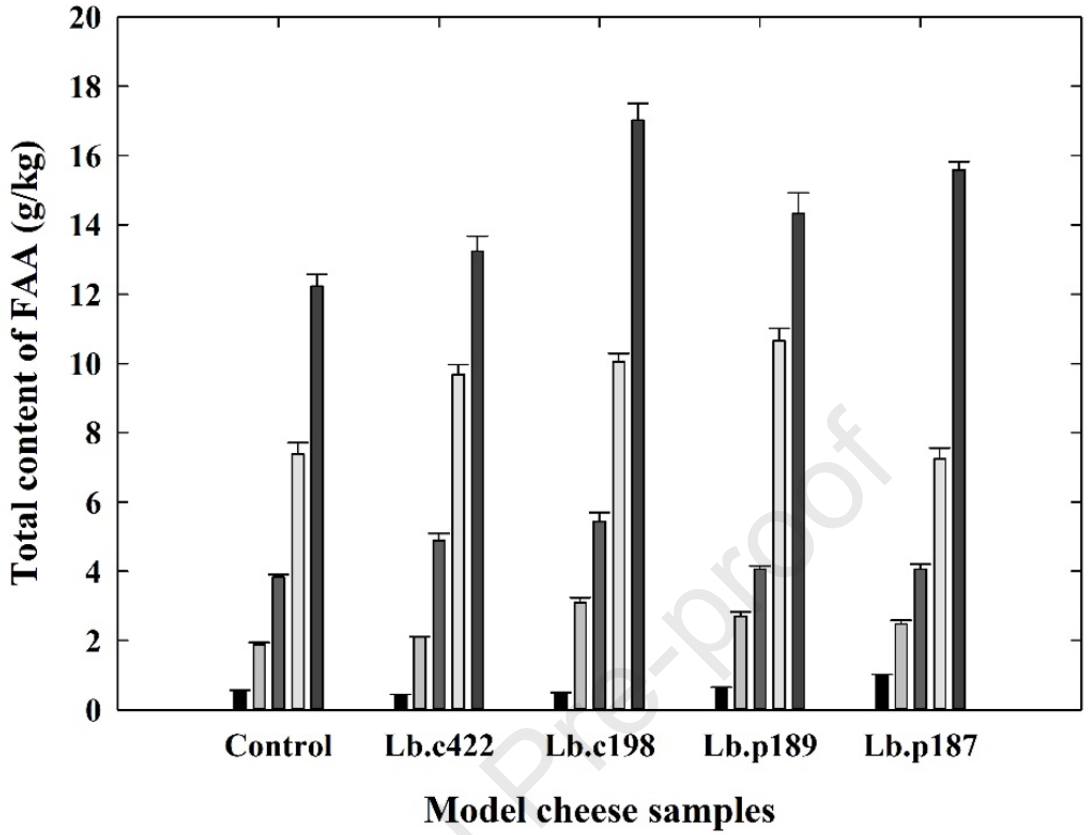


Fig. 2



Highlights

- Dutch type cheese samples were produced with different adjunct culture.
- The applied strains did not have an effect on the basic chemical parameters.
- Differences between batches in concentration of free amino acids were observed.
- The most effective BA reduction was caused by *Lacticaseibacillus casei* CCDM 198.
- Reduction of phenylethylamine, putrescine and tyramine was demonstrated in cheese.

Conflict of Interest Form

Dear Editors,

We would like to submit the enclosed manuscript entitled "*Reduction of biogenic amine content in Dutch type cheese as affected by the applied adjunct culture*", which we wish to be considered for publication in "LWT Food Science and Technology". Moreover, no conflict of interest exits in the submission of this manuscript, and the manuscript is approved by all authors for publication. I would like to declare on behalf of my coauthors that the work described was original research that has not been published previously, and not being under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Thank you and best regards.

Yours sincerely,

Vendula Pachlová