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ORIGINAL ARTICLE

Effect of somatic cells count in cow milk on the formation of biogenic amines in cheese

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Abstract Comparative studies on physicochemical characteristics of milk with different somatic cells count (SCC) (L—low < 400,000 cells/ml, M—medium between 500,000 and 600,000 cells/ml and H—high > 1,000,000 cells/ml) and obtained cheeses, were conducted. No significant differences between samples were found. The H SCC milk was characterized by the highest total viable count. Higher levels of proteolysis were established in cheeses made from milk with SCC exceeding 500,000 cells/ml. After 10 months of ripening and cold storage the water-soluble nitrogen in total nitrogen (WSN/TN), non-casein nitrogen in total nitrogen (NCN/TN), nonprotein nitrogen in total nitrogen (NPN/TN) and free amino groups values of the sample with the highest SCC reached 28.4 ± 0.8%, 24.8 ± 0.9%, 18.3 ± 0.9% and 83.6 ± 0.3 mg/kg respectively. The biogenic amine concentration in the cheese samples from the L and M batches remained below 10 mg/kg throughout the ripening and cold storage period. The present study established an increase in the biogenic amine content during the ripening period and the cold storage of the cheeses made from milks with high SCC (batch H). The main amines accumulated at the end of the storage period (10th month) were tyramine (31.7 ± 0.3 mg/kg), putrescine (20.5 ± 0.2 mg/kg) and cadaverine (14.6 ± 0.2 mg/kg). Histamine was not found in any of the studied cheese samples.

Keywords Somatic cells count · Cow milk · Biogenic amines · Cheese · Ripening · Cold storage

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Introduction

Biogenic amines (BA) are low molecular weight nitrogenous compounds naturally occurring in many foods in which they are mainly the result of the microbial decarboxylation of amino acids. The toxicological effects of biogenic amines and their potential influence on human health are not fully clarified. Some BA are also considered a quality index for different foods (Costa et al. 2018). High concentrations of BA are associated with poor manufacturing practices or food spoilage. Histamine and tyramine are the most studied BA due to their toxicological effect. Histamine and tyramine are degraded in the organism by oxidative deamination catalyzed by monoamine and/or diamine oxidase (Galanakis 2019). The presence of monoamine and diamine oxidase inhibitors or the presence of other BA such as putrescine and cadaverine can increase their toxicity. According to the scientific opinion on risk-based control of BA formation in fermented foods (Collins et al. 2011) no adverse health effects were observed after exposure to 50 mg histamine (per person per meal) for healthy individuals and 600 mg tyramine for healthy individuals not taking monoaminoxidase inhibitor drugs.

Spanier et al. (1991) suggested that the sum of histamine, tyramine, putrescine and cadaverine concentration in cheese should not exceed the level of 900 mg/kg.

Cheese is one of the food items frequently associated with BA poisoning (Barone et al. 2018). Formation of BA in cheese is promoted by the availability of Free amino groups, due to the proteolysis as well as by the growth of the decarboxylase-positive microorganisms (Loizzo et al. 2013). Many studies on the presence of BA in cheeses have been performed (Mercogliano et al. 2010; Schirone et al. 2011; Benkerroum 2016; Manca et al. 2020). Authors reported for a great variability in BA content among different types of cheese. The main factors influencing BA content of cheeses are heat treatment of milk (El-Desoki 2017), hygienic conditions of cheese production (Razavi Rohani et al. 2013), used starter culture (Linares et al. 2012), cheese ripening and storage time (Pleva et al. 2014) and packaging methods (Andiç et al. 2010). Several authors reported that higher ripening temperature, excessive proteolysis, high pH, and low salt concentration may increase biogenic amines formation in cheese (Gardini et al. 2016).

Cheese quality is closely related to the quality of raw milk used for its production. The somatic cell count (SCC) is considered an important criterion for evaluating milk quality. The impact of SCC of raw milk on cheese quality is related mainly with the impairing milk coagulation properties, increasing moisture content in most cheeses and inducing cheese off-flavor development (Kochoski et al. 2011; Li et al. 2014). Ubaldo et al. (2015) reported that tyramine and tryptamine, which were not originally detected in the raw milk, were found in Mozzarella cheese from high SCC milk. The effect of SCC of milk on the BA formation in cheese is still not extensively studied.

Therefore, the aim of the present study was to evaluate the effect of SCC of cow milk on the biogenic amines formation in cheese during ripening and cold storage.

Material and methods

Milk samples

Raw bulk milk samples were collected from small-scale dairy farms affiliated with Dairy Producer Associations in Biga district of Çanakkale province, (Turkey). More than 100 samples were brought to the laboratory of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) at 4 °C every week. SCC, Total Viable Count (TVC) and composition of milk samples were measured. All analyses of raw milk were carried out in triplicate. For experimental cheese samples preparation were selected three different batches of raw milk with low (L) (< 400,000 cells/ml), medium (M) (between 500,000 and 600,000 cells/ml) and high (H) (1,000,000 cells/ml) SCC, respectively.

Cheese samples

Cheese samples were produced from cow milks with different somatic cells count according to the following procedure: the raw milks from three batches (L, M and H) with three different SCC were accepted into the pilot dairy processing plant of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) and the platform tests (total solids, fat, acidity and antibiotics) were carried out. Pasteurization procedure was realized at 68 °C for 15 min. Milk was cooled down to coagulation temperature of 35–36 °C. Starter culture consisting of 70% *Streptococcus thermophilus* and 30% *Lactobacillus delbrueckii* ssp. *bulgaricus* and cheese rennet were added. After 90 min of coagulation, the curd was sliced into nut sized curd grains and a portion of the whey was removed. After 5 min of curd stirring, curd and the remaining whey were heated at 41–42 °C for 15 min. At the end of the heating process, cheese was self-pressed for 2–3 h. Unripened cheeses were then removed from the moulds and salted in 16% NaCl solution at 15–18 °C for 24 h. After salting unripened cheese was taken out, dried and packed in polyamide/polyethylene foil under vacuum at 90–99.8 Pa. Ripening took place in these packages at 4 ± 1 °C and relative humidity of 75–80% for 3 months. After that the cheese samples were subjected to cold storage at 4 ± 1 °C for another 7 months (up to the 10th month after production).

Determination of SCC, TVC and chemical composition of raw milk

Bactocount IBCm (Bentley Instrument, USA) device was used for SCC determination.

Total viable count (TVC) was determined by using Plate Count Agar medium according to ISO 4833-2:2013. Inoculated petri dishes were subjected to incubation at 30 °C for 48 to 72 h and colony forming units (CFU) were counted on petri dishes.

The milk fat, protein, lactose and total solids content of studied milk samples were measured by using Infrared Milk Analyzer 150 (Bentley Instrument, USA). The instrument was calibrated with certified reference milk samples from Italy Accredited Dairy Laboratories A.I.

Physicochemical analysis of cheese

The cheese samples were prepared for chemical testing according to ISO 707:2008. The fat content of cheese samples was determined according to ISO 3433:2008. Determination of the total solids content was made

according to ISO 5534:2004. Sodium chloride content of cheese samples was determined according to ISO 5943:2006. pH values were measured potentiometrically by pH meter; Water soluble nitrogen (WSN), Noncasein nitrogen (NCN), nonprotein nitrogen (NPN) and total nitrogen (TN) content were determined by the Vakaleris and Price (1959) method modified to suit the specific conditions of the analysis. For water soluble nitrogen (WSN) and noncasein nitrogen (NCN) determination, approximately 5 g of cheese was extracted in 100 ml deionized water or sodium acetate buffer (pH 4.6), respectively. The homogenate was agitated at ambient temperature for 2 h and filtered. The nitrogen fraction soluble in 12% trichloracetic acid (TCA) was considered the nonprotein nitrogen (NPN). To determine the NPN content, approximately 5 g of cheese were homogenized in 40 ml sodium acetate buffer (pH = 4.6), the homogenate was agitated at ambient temperature for 2 h, then 10 ml of 60% TCA was added and the homogenate was filtered through Watman No 42 paper. Nitrogen determination was performed in duplicate by the Kjeldahl method using a Kjeltec Auto 1030 Analyzer (Tecator Sweden) combined with the Digestion System 20. Total protein (TP) was calculated as total nitrogen multiplied by coefficient of 6.38; free amino groups in cheese samples—water extracts of cheese samples were prepared according to the procedure described by Mayer et al. (1998). Free amino groups were determined by the reaction with ninhydrin with cadmium in cheese water extract according to the procedure described by Folkertsma and Fox (1992). 10 g of cheese were extracted as described by Vlaseva et al. (2014) in order to determine cheese lactose content by HPLC method as described by Hadjikinova et al. (2017); Cheese mineral content was determined according to AOAC official method for ash determination (2000).

Biogenic amines analysis

Sample treatments were according to fallowing procedure: 10 ml of 0.4 M $HClO_4$ were added to an amount of 5 g minced cheese. The samples were homogenized and then centrifuged for 10 min at 2500 rpm. The supernatant was pooled and diluted to 50 ml with 0.4 M $HClO_4$. The centrifuged acid extract was derived according to the following procedure: 200 µl of 2 N NaOH were added to 1 ml portions of the diluted supernatant, then buffered by adding 300 µl of saturated $NaHCO_3$ solution and then 2 ml of dansylchloride solution (10 mg/ml in acetone) were added. The dansylation reaction proceeds at room temperature (Ruggieri et al. 1995). 100 µl of NH_4OH were added after 15 min to stop the reaction and to remove residual dansylchloride. The final volume was adjusted to 5 ml by adding acetonitrile. The obtained dansylated solution was filtered and injected into the Liquid chromatograph (Agilent Technologies, Model 1260 Infinity) equipped with UV detector and Spherisorb ODS2 (C18) (4.6 × 150 mm, 5 µm, Waters, Milford, USA) column. The mobile phase was a mixture of acetonitrile and water (80:20). Chromatographic conditions: injected volume 20 µl, flow rate 0.5 ml/min; detection wavelength (λ) = 254 nm were used. After each run the column was conditioned for 10 min. Each HPLC run took about 18 min and afterwards the column must be conditioned again for 10 min.

The presence and abundance of biogenic amines (putrescine, cadaverine, histamine, tyramine – Sigma-Aldrich) were determinate by comparing sample peak retention time to standards. Additionally 1,7-diaminoheptane was used as internal standard. One milliliter of the standard solutions was derived as previously described for the acid extracts.

Statistical analysis

The data presented are the mean values ± SD (Standard Deviation) of the five replicates. The statistical significance of differences among samples was determined by LSD (Least Significant Difference) according to one-way ANOVA method for analysis of variance. Differences in mean values were considered significantly different when $p < 0.05$ (Donchev et al. 2002).

Results and discussion

Physicochemical and microbiological analysis of raw milk and cheese samples with different SCC

The results of the physicochemical and microbiological analysis of the raw milk used for obtaining the cheese test samples are presented in Table 1.

Data in Table 1 demonstrates that regardless of the differences in SCC, the content of milk fat, proteins and total solids in the batches L, M and H were similar ($p > 0.05$). The raw milk from batch H had slightly lower lactose content in comparison with the batches L and M. No statistically significant ($p > 0.05$) differences were established in the active acidity (pH) values between the three batches of raw milk used in the current study for obtaining cheese samples.

The results of the physiochemical analysis of the cheese samples in the end of the ripening period are presented in Table 2.

It was evident that the cheeses in all batches were characterized by similar values of the total solids, milk fat content, proteins, and NaCl ($p > 0.05$). Mazal et al. (2007) also reported that the SCC did not affect the protein and fat contents of the Prato cheese or the fat loss to the whey. No

Table 1 Somatic cells count and chemical composition of raw milk used for production of cheese samples

Sample	Characteristics						
	SCC, cells/ml	TVC, cfu/ml	Total solids, % (w/w)	Fat, % (w/w)	Proteins, % (w/w)	Lactose, % (w/w)	pH
L	106,000 ± 5300[a]	9.4 ± 0.5.10[5a]	12.45 ± 0.12[a]	3.67 ± 0.09[a]	3.21 ± 0.07[a]	4.62 ± 0.04[a]	6.57 ± 0.07[a]
M	556,000 ± 27,800[b]	9.7 ± 0.7.10[5a]	12.42 ± 0.16[a]	3.66 ± 0.08[a]	3.28 ± 0.06[a]	4.58 ± 0.05[a]	6.59 ± 0.05[a]
H	1 533,000 ± 76,650[c]	1.2 ± 0.3.10[6b]	12.41 ± 0.13[a]	3.73 ± 0.08[a]	3.30 ± 0.08[a]	4.47 ± 0.03[b]	6.61 ± 0.05[a]

[a, b] Means with different letters within a column are significantly different ($p < 0.05$)

L low somatic cells count < 400,000 cells/ml, *M* medium somatic cells count between 500,000 and 600,000 cells/ml, *H* high somatic cells count > 1,000,000 cells/ml, *SCC* somatic cells count, *TVC* total viable count

Table 2 Physicochemical characteristics of cheese samples at the end of ripening period

Sample	Characteristics						
	Total solids, %	TP, %	Lactose, %	Minerals, %	NaCl, %	Fat, %	pH
L	54.19 ± 0.46[a]	18.2 ± 0.27[a]	ND	3.82 ± 0.01[a]	2.15 ± 0.12[a]	32.12 ± 0.32[a]	5.84 ± 0.05[a]
M	54.20 ± 0.38[a]	18.6 ± 0.22[a]	ND	3.83 ± 0.02[a]	2.11 ± 0.09[a]	31.67 ± 0.28[a]	5.81 ± 0.05[a]
H	53.55 ± 0.41[a]	17.9 ± 0.31[a]	ND	3.82 ± 0.01[a]	2.08 ± 0.11[a]	32.13 ± 0.45[a]	5.85 ± 0.04[a]

[a, b] Means with different letters within a column are significantly different ($p < 0.05$)

L low somatic cells count < 400,000 cells/ml, *M* medium somatic cells count between 500,000 and 600,000 cells/ml, *H* high somatic cells count > 1,000,000 cells/ml, *TP* total protein, *ND* not detected

statistically significant ($p > 0.05$) differences were established in the pH values between the cheeses in batches L, M and H. This indicated a similar acid formation rate during ripening of the cheese samples.

Effect of SCC in milk on the proteolysis and biogenic amines formation during ageing and cold storage of cheese

Within the 3-month ageing period, no statistically significant ($p > 0.05$) differences were established in the WSN/TN, NCN/TN, NPN/TN and FAG values between the batch L and batch M cheeses (Table 3). In contrast, the batch H cheeses had significantly ($p < 0.05$) higher values of these two batches at the end of the 3rd month. More intensive process of proteolysis was found during the storage period in a comparison with the ripening period ($p < 0.05$). Greater was the SCC values more pronounced proteolysis was found. These results indicated more intensive proteolysis during the ageing of cheeses made from milk with a SCC exceeding 500,000 cfu/ml. Similar results were reported by other authors (Kochoski et al. 2011; Li et al. 2014). As a result of degradation of the small peptides, simple amine components were liberated, favoring a significant increase in NPN values ($p < 0.05$). Kalit et al. (2002) also observed a more intensive proteolysis during ageing in the cheese from milk with a high SCC. A higher proteolysis was observed in Swiss cheese (Cooney et al. 2000) and in Podravec cheese (Kalit et al. 2002) manufactured from milk with high levels of SCC.

An increase in the WSN/TN, NCN/TN, NPN/TN and FAG values was also observed during the cold storage of the cheese test samples (from the 3rd to the 10th month) (Table 3). The data indicated that the trend towards more intensive proteolysis in the cheeses made from milk with high SCC observed in the ageing process persisted during their cold storage.

The more active proteolytic process established in this study during the ageing and cold storage of cheeses made from milks with SCC exceeding 500,000 cells/ml was a prerequisite for the appearance of taste defects, which was also confirmed by the organoleptic analysis of the samples investigated (data not shown). Some authors (Standarova et al. 2010; Le Maréchal et al. 2011; Murphy et al. 2016) explained this phenomenon by a greater hydrolysis of the αs1-CN in cheese made with high-SCC.

Taking into account the lower limits set for the working range of the HPLC method, the concentrations of biogenic amines found in the analyses to be below the quantitative evaluation limit were reported as lower than 10 mg/kg and results for the batches L and M were not presented.

Chromatograms of batch H cheese samples in the beginning, in the end of the ripening process and in the end of the cold storage are presented on Fig. 1a–c.

Table 3 Changes in the WSN/TN, NCN/TN, NPN/TN and FAG content in cheese samples during ripening period and cold storage

Sample	Concentration															
	WSN/TN, % (w/w)				NCN/TN, % (w/w)				NPN/TN, % (w/w)				FAG, mg/100 g			
	Ripening and cold storage period, months															
	1	3	6	10	1	3	6	10	1	3	6	10	1	3	6	10
L	10.1 ± 0.4^{a}	11.2 ± 0.6^{a}	15.1 ± 0.7^{a}	18.6 ± 0.8^{a}	8.2 ± 0.5^{a}	9.4 ± 0.6^{a}	13.1 ± 0.6^{a}	15.9 ± 0.7^{a}	4.6 ± 0.3^{a}	7.1 ± 0.4^{a}	10.4 ± 0.5^{a}	12.8 ± 0.6^{a}	20.4 ± 0.2^{a}	27.0 ± 0.3^{a}	35.1 ± 0.2^{a}	42.4 ± 0.2^{a}
M	10.8 ± 0.5^{a}	12.8 ± 0.5^{b}	18.2 ± 0.6^{b}	20.2 ± 0.9^{a}	8.7 ± 0.4^{a}	10.0 ± 0.6^{a}	15.2 ± 0.7^{b}	17.7 ± 0.7^{b}	4.8 ± 0.2^{a}	7.8 ± 0.5^{a}	12.5 ± 0.4^{b}	14.4 ± 0.7^{a}	21.5 ± 0.2^{b}	30.9 ± 0.4^{b}	40.2 ± 0.3^{b}	59.1 ± 0.4^{b}
H	12.7 ± 0.5^{b}	175. ± 0.6^{c}	22.1 ± 0.7^{c}	28.4 ± 0.8^{b}	10.5 ± 0.5^{b}	16.3 ± 0.5^{b}	20.1 ± 0.8^{c}	24.8 ± 0.9^{c}	5.9 ± 0.4^{b}	11.3 ± 0.6^{b}	17.4 ± 0.7^{c}	18.3 ± 0.9^{b}	27.3 ± 0.3^{c}	38.4 ± 0.3^{c}	57.6 ± 0.4^{c}	83.6 ± 0.3^{c}

^{a}, ^{b}, ^{c} Means with different letters within a column are significantly different ($p < 0,05$)

L low somatic cells count < 400,000 cells/ml, *M* medium somatic cells count between 500,000 and 600,000 cells/ml, *H* high somatic cells count > 1,000,000 cells/ml, *WSN/TN* water-soluble nitrogen in total nitrogen, *NCN/TN* noncasein nitrogen in total nitrogen, *NPN/TN* nonprotein nitrogen in total nitrogen, *FAG* free amino groups

Biogenic amine concentration in the cheese samples from the L and M batches remained below 10 mg/kg throughout the ripening and cold storage period, which was under the detection limit (Table 4). In contrast, the biogenic amines putrescine, cadaverine, and tyramine were found in low concentrations, in the cheeses made from milks with high SCC (batch H) in the end of the ripening process. This data were agreement with those reported by Linares et al. (2011).

The present study established an increase in the biogenic amine content at the end of the cold storage of the cheeses made from milks with high SCC (batch H) (Table 4). The main amines accumulated were tyramine, putrescine, and cadaverine. The putrescine concentration showed the most considerable increase, followed by cadaverine and tyramine. The higher content of biogenic amines in the cheeses made from milks with higher SCC could be explained by a more intensive proteolysis occurring in them. Traces of this biogenic amine histamine were only detected at the end of the cold storage of the batch H cheese samples, but its quantity remained below the 10 mg/kg quantitative evaluation limit of the method.

Conclusion

The results obtained in the present study showed that the SCC values of the raw cow milk did not have a significant effect on its physicochemical composition and those of the obtained cheeses. A relation between the high SCC and the TVC has been observed. The SCC values in the raw milk had no significant influence ($p > 0.05$) on the content of the main components, active acidity and lactic acid concentration in the produced cheeses. The high SCC of the raw milk had a significant effect on the proteolysis during ripening and storage of cheese ($p > 0.05$). Biogenic amine concentration in the cheese samples from the L and M batches remained below 10 mg/kg throughout the ripening and cold storage period. Significant biogenic amines accumulation in the end of the ripening period of cheese samples produced from milk with high SCC was established ($p < 0.05$). At the end of the storage (10th month), putrescine concentration showed the most considerable increase, followed by cadaverine and tyramine. Cheese samples of the present study could not be considered hazardous even for risk consumer groups. The more intensive proteolytic process during the ageing and cold storage of cheese made from milks with higher SCC was associated with the appearance of sensory quality defects and cheese shelf life reduction. Therefore, the appropriate control of SCC in raw cow milk is important both for the cheese quality and storability.

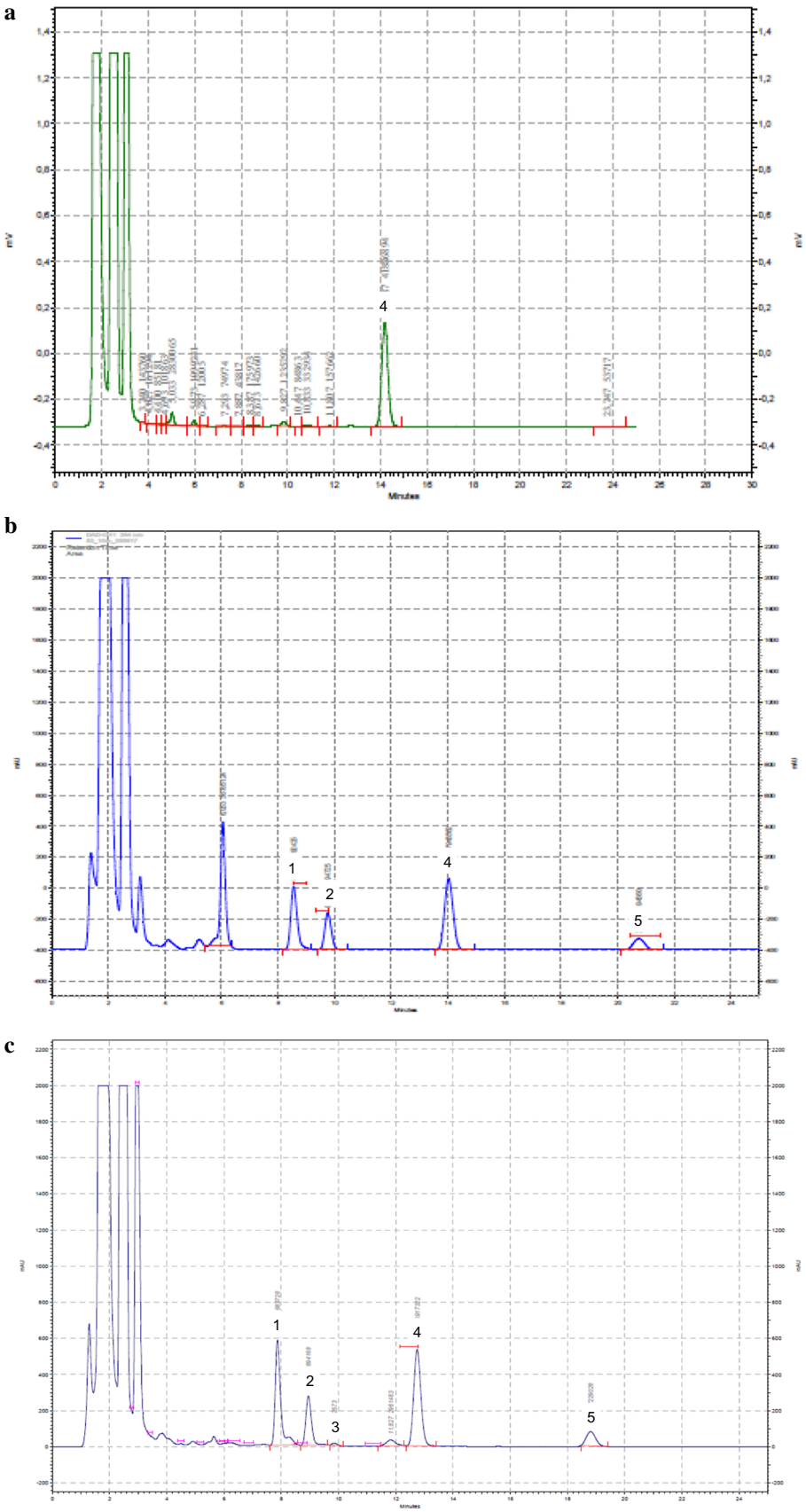

Fig. 1 HPLC chromatogram of biogenic amines in cheese samples produced from caw milk with high somatic cells count > 1,000,000 cells/ml (Batch H) at the 1st day of ripening (**a**), at the end of ripening (3rd month) (**b**) and in the end of cold storage (10th month) (**c**) (1-putrescine, 2-cadaverine, 3-histamine, 4-1,7-diaminoheptane, 5-tyramine)

Table 4 Changes in the biogenic amines content of cheese samples during ripening period and cold storage

Sample	Concentration of biogenic amines, mg/kg																	
	Putrescine				Cadaverine				Histamine				Tyramine					
	Ripening and cold storage period, months																	
	1	3	6	10	1	3	6	10	1	3	6	10	1	3	6	10		
L	0	< 10	< 10	< 10	0	< 10	< 10	< 10	0	0	0	0	0	< 10	< 10	< 10		
M	0	< 10	< 10	< 10	0	< 10	< 10	< 10	0	0	0	0	0	< 10	< 10	< 10		
H	0	15.4 ± 0.2[a]	25.8 ± 0.2[b]	31.7 ± 0.3[c]	0	13.2 ± 0.2[a]	16.3 ± 0.3[b]	20.5 ± 0.2[c]	0	0	0	< 10	0	10.4 ± 0.2[a]	11.8 ± 0.3[b]	14.6 ± 0.2[c]		

[a,b,c]Means with different letters within a row are significantly different ($p < 0.05$)

L low somatic cells count < 400 000 cells/ml, *M* medium somatic cells count between 500 000 and 600 000 cells/ml, *H* high somatic cells count > 1,000,000 cells/ml

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