Biogenic Amines and Polyamines in Milks and Cheeses by Ion-Pair High Performance Liquid Chromatography

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The proposed chromatographic method provides a complete resolution of twelve amines in a single run in milks and unripened cheeses, avoiding the losses of resolution linked to fluctuations in working temperature. We also propose an alternative chromatographic gradient, which can be useful for samples that have undergone long ripening periods, like ripened cheeses. According to the results of the reliability study, the method described was precise, accurate, and sensitive. The method was applied to several samples of milks and cheeses and the results showed that the biogenic amine profiles varied greatly, not only between different types of samples but also among the samples from the same kind of products. In unripened cheeses, milks, and yogurts, spermidine and spermine were the prevailing amines, but in ripened cheeses the major amine was tyramine, followed by putrescine and cadaverine.

Keywords: *Biogenic amines; polyamines; cheeses; milks; high performance liquid chromatography*

INTRODUCTION

The occurrence of biogenic amines (BA) in foods is mainly due to the decarboxylation of some amino acids by the action of microorganisms. In fermented milks and cheeses, the precursor amino acids are mainly produced as a result of the casein proteolysis (Joosten and Olieman, 1986). Small amounts of some BA can usually be found in some foods, because they play a natural role in microbial plant and animal metabolism. However, some amines, such as tyramine, histamine, and serotonin, can also have direct or indirect effects on the human vascular and nervous system. Thus, the consumption of large amounts of these BA can lead to undesirable effects as headache, nausea, hypo-or hypertension, cardiac palpitations, and anaphylactic shock syndrome (Stratton et al., 1991; Rodríguez et al., 1996). These effects can be especially adverse in patients treated with classical monoamine oxidase inhibitors (MAOIs) drugs (Gardner et al., 1996) or in individuals with genetic or acquired diaminoxidase deficiency.

The determination of the minimum toxic amount of BA in individuals is very difficult, because it depends on different factors, such as the individual efficiency of the detoxification systems, the potential simultaneous alcohol consumption, and the role of other amines as toxicity potentiators (Halász et al., 1994). No limits have been established for amines in milks or cheeses, although a strong hypertensive response (the "cheese reaction") can take place as a result of the interaction between classical MAOIs drugs and foods rich in tyramine. In fact, the only established amine maximum allowable content is for histamine in fish, and the limit is 50 ppm (Food and Drug Administration, 1995).

Cheese, like other fermented foods, is an ideal substrate for amine production, as its manufacturing process involves not only available free amino acids, but also the possible presence of decarboxylase-positive microorganisms and the environmental conditions that allow the growth of microorganisms, the decarboxylase enzymes activity (optimal pH, temperature, salt, and water availability), and the presence of suitable cofactors (pyridoxal phosphate) (Chang et al., 1985; Vale and Glória, 1997).

The first BA studies in cheeses only determined tyramine and histamine, but putrescine, cadaverine, tryptamine, and phenyethylamine have also been found in large amounts so far (Celano et al., 1992). The amine contents in cheeses varied according to samples, from non-determined (ND) to 4200 mg/kg (Evans et al., 1988; Martelli et al., 1993; Ordóñez et al., 1997; Schneller et al., 1997). This wide variability may result from the fact that the amine concentration depends on the type of cheese, the ripening time, and the conditions of the manufacturing process.

In addition, Halasz et al. (1994) reported that the amount and type of amine formed in foods depends on the nature of the commodity and, especially, on the microorganisms present. Some enterobacteriaceae, lactobacillus, pediococci, and enterococci actively produce BA. Moreover, some strains have proteolytic activity, which can favor the accumulation of BA in cheese (Halász et al., 1994).

The microorganisms involved in amine production may be the starter cultures used to control the raw milk flora, or may be introduced by contamination before, during, or after the cheese making and storage (Joosten and Stadhouders, 1987; Rodríguez et al., 1996; Ordóñez et al., 1997). Thus, the bacteriological quality of milk could be critical to controlling the amount of amines formed. Therefore, large amounts of BA in cheese could indicate a failure, from a hygienic point of view, in the milk used for cheese products or during the cheese making (Tarján and Jánossy, 1978; Antila et al., 1984).

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Chromatographic methods used for the determination of BA are, among others, thin-layer chromatography (Evans et al., 1988; Shalaby, 1994), gas chromatography (Yamamoto et al., 1982), and high-performance liquid chromatography (HPLC) (van Boekel and Arentsen-Stasse, 1987; Joosten and Stadhouders, 1987; Martelli et al., 1993; Bockhardt et al., 1996; Vale and Glória, 1997). Several detection techniques, such as UV, electrochemical, and fluorescence detection, in combination with pre- and postcolumn derivatization with *o*-phthalaldehyde (OPT), fluorescamine, or dansyl chloride have been applied. Nevertheless, some of them present limitations, such as the detection of only a few amines and low specificity. The use of OPT as derivatization agent to obtain fluorescent compounds increases the selectivity and the sensitivity for primary amines (Izquierdo-Pulido et al., 1993).

The aim of the present work was to develop a reliable procedure for determining BA in milks and cheeses by liquid chromatography. The liquid chromatographic method proposed is based on methods previously developed in our laboratory for BA determination in other kinds of food, such as beers (Izquierdo-Pulido et al., 1993), fish and ripened-fish products (Veciana-Nogués et al., 1995), and cooked and fermented sausages (Hernández-Jover et al., 1996). Cheeses have a complex matrix because of their high proportion of fat and protein, which hinders the extraction of amines. Therefore, additional efforts in enhancing the analytical efficency were required.

Moreover, owing to the health implications of the intake of high BA amounts, a preliminary study on BA in cheeses, milk, and yogurts available in the Spanish market was carried out. The aim of this work was to determine whether any particular type of cheese presents high BA content, to recommend its withdrawal from the diet of patients taking MAOIs or with histamine or tyramine intolerance. Yogurts have also been included in this study because the ability of microorganisms to produce BA in these fermented products has scarcely been studied.

MATERIALS AND METHODS

HPLC Analysis. The HPLC system consisted of a Waters 600 E system controller pump, a Waters 717 plus autosampler, a Waters 501 postcolumn pump (Waters Chromatography, Milford, MA), and an SFM 25 spectrofluorometric detector (Kontron AG Instruments, Everett, MA). The Waters 501 was connected to a zero dead volume mixing T installed between the column outlet and the detector. A coil of stainless steel tubing, 200 cm × 0.01 in. i. d. was used to connect the T with the detector. Chromatographic data were collected and analyzed with a Millenium system (Waters). The separation was performed on a Nova Pack C_{18} column, 3.9 × 150 mm, 4 μm particle size (Waters), with a matching guard cartridge. The column was introduced into an oven (Waters model 600) to keep it at constant temperature.

An MR1812 centrifuge (Jouan, St. Nazaire, France) was used for the sample preparation. A Crison Micro-pH 2001 pH-meter (Crison, Capri, Italy) was used to adjust the pH of mobile phases. Filters for samples and standard solutions were HVLP 1300 membrane, 0.45 μm (Millipore, Bedford, MA). Filters for solvents were HVLP 4700 membrane, 0.45 μm (Millipore).

Reagents and Standards. Acetonitrile and methanol of HPLC grade were obtained from SDS (Peppin, France). Ultrapure water generated by the Milli-Q System (Millipore) was used. Other reagent-grade chemicals were: sodium acetate anhydrous, Brij 35 2-mercaptoethanol and *o*-phthalaldehyde (OPT), from Merck (Darmstadt, Germany); sodium octanesulfonate from Romil Chemicals (Cambridge, Great Britain) and boric acid, potassium hydroxide, hydrochloric acid (HCl), and perchloric acid 70% (PCA) from Panreac (Montplet & Esteban, Barcelona, Spain).

Table 1. Gradient Elution Program for Separation of Biogenic Amines in Milk, Yogurt, and Unripened Cheese

operation	time, min	mobile phase A%	mobile phase B%	curve	order
elution	0.01	80	20		
	30	50	50	exponential	5
	44	40	60	exponential	4
	46	20	80	logarithmic	3
return	50	80	20	linear	0
equilibration	60	80	20		0

Standards of tyramine (TY) free base and histamine (HI) dihydrochloride were from Merck. Octopamine (OC) free base, dopamine (DO) free base, putrescine (PU) hydrochloride, serotonin (SE) creatinine sulfate, cadaverine (CA) dihydrochloride, agmatine (AG) sulfate, β-phenylethylamine (PHE) hydrochloride, spermidine (SD) trihydrochloride, tryptamine (TR) hydrochloride, and spermine (SM) tetrahydrochloride were obtained from Sigma Chemical (St. Louis, MO). A stock solution (1000 mg/L), as the free base of each biogenic amine, was prepared in 0.1 N HCl. A 50 mg/L intermediate solution was prepared in 0.1 N HC1 from the stock solution. Standard solutions (ranging from 0.25 to 10 mg/L) were prepared in 0.1 N HCl from the intermediate solution. These solutions were filtered through a 0.45 μm filter, stored in a refrigerator, protected from light, and degassed before use.

Chromatographic Conditions. *Mobile Phase.* (1) Eluent A: Solution of 0.1 M sodium acetate and 10 mM sodium octanesulfonate, adjusted to pH 5.30 with acetic acid. (2) Eluent B: Solvent B: Acetonitrile (6.6:3.4). Solvent B consisted of 0.2 M sodium acetate and 10 mM sodium octanesulfonate solution, adjusted to pH 4.50 with acetic acid. (3) Postcolumn derivatization reagent: 31 g of boric acid and 26.2 g of potassium hydroxide were dissolved in 1000 mL of distilled water; 3 mL of 2-mercaptoethanol and a 3-mL aliquot of 30% Brij 35 solution were added. Then, 0.2 g of OPT, dissolved in 5 mL of methanol, were added, and the final solution was mixed. The reagent was prepared daily and protected from light.

The gradient elution program is shown in Table 1. The last step shown in the Table was for reequilibrating the column and returning it to the initial conditions, which is necessary to avoid changes in the retention time of amines in the subsequent run. The flow rate of the mobile phase was 1 mL/min and the flow rate of the derivatizating reagent was 0.4 mL/min. Mobile phases and derivatizating reagent were filtered and degassed before use. The temperature of the column was kept constant at 40 °C and the postcolumn reaction was set at room temperature. The eluate was monitored at 340 nm excitation and 445 nm emission wavelengths.

Sample Preparation. All samples were from Spanish retail stores and consisted of 5 milks, 5 yogurts, 10 unripened cheeses, and 10 ripened cheeses, representing different commercial brands.

Liquid or Fluid Samples (Milk and Yogurt). An aliquot of 10 g of sample was accurately weighed in a 50-mL centrifuge tube and thoroughly mixed with 6 mL of 0.6 N PCA in a magnetic stirring plate for 20 min. Thereafter, centrifugation at 30 000*g* at 4 °C for 10 min was carried out to separate the two phases. The centrifugation was repeated after re-suspending the resulting pellet in 4 mL of 0.6 N PCA, mixing it thoroughly for 20 min. The supernatants were combined, and the volume was adjusted to 20 mL.

Solid Samples (Unripened and Ripened Cheeses). Samples of the unripened cheese (10 g) and ripened cheese (5 g) were triturated and homogenized mechanically using a domestic mincer for about 1 minute, and the extraction with PCA was then carried out as described above. The extraction procedure was repeated three times and the final volume was 25 mL.

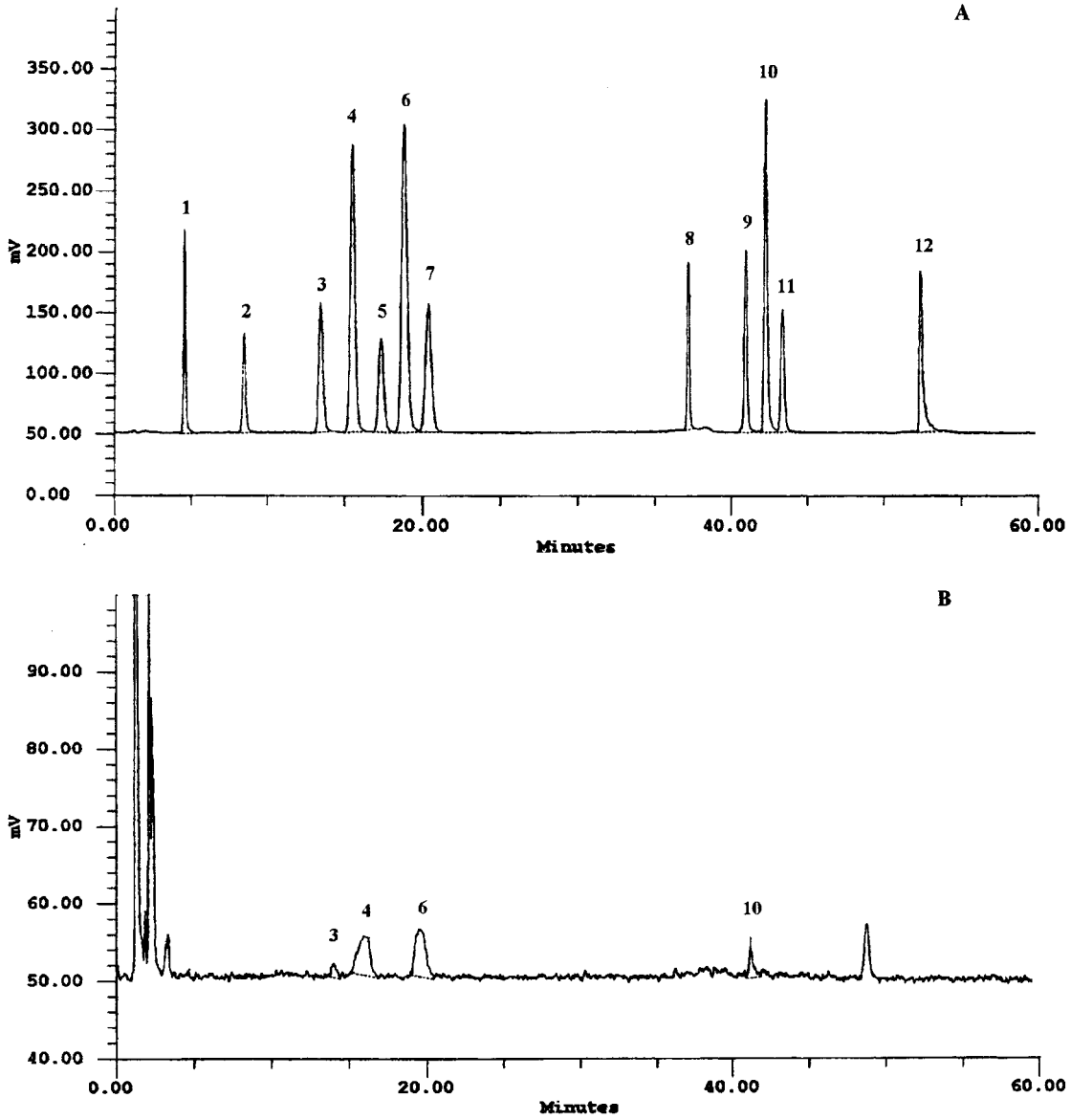

Figure 1. Liquid chromatograms of biogenic amines. (A) Standard solution; (B) unripened-cheese sample. Peak identities: OC (1), DO (2), TY (3), PU (4), SE (5), CA (6), HI (7), AG (8), PHE (9), SD (10), TR (11), and SM (12).

In all cases, if the amine content was higher than 50 mg/kg, the sample weight to PCA-extraction volume ratio had to be decreased. Perchloric extracts were passed through a 0.45-μm filter before HPLC analysis.

Statistical Analysis. The BA contents in cheese did not follow a normal distribution. Thus, the data were evaluated using the Mann Whitney test for nonparametric data. All statistical tests were performed by means of the Statistical Software Package for Windows 6.0.1. (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Previous procedures described for the determination of BA in beers, fish, and meat products (Izquierdo-Pulido et al., 1993; Veciana-Nogués et al., 1995; Hernández-Jover et al., 1996) showed lack of resolution in some amines when they were applied to some cheeses and, especially, when the working temperature was higher than 20 °C or when it fluctuated during the day. This could be avoided by modifying the final pH of the mobile phase A. However, in order to avoid changes in the daily preparation of mobile phases and so to automate the method, the working temperature was increased to 40 °C, which was determined to allow the best resolution. The increase in working temperature required a modification of the gradient elution program used for other kinds of samples. The new program involves the increase of eluent B following a combination of linear, logarithmic, and exponential curves to improve the resolution between amines (Table 1).

Figure 1 shows typical chromatograms of amines in standard solution and in an unripened-cheese sample. Amines were identified on the basis of retention time by comparison with standard solutions. The relative standard deviations (RSDs) of retention times were satisfactory, and they ranged from 0.45 to 2.58%.

The reliability of the method was studied in terms of linearity, precision, recovery, and sensitivity. The lack of interferences from amino acids, which eluted in the first 4 min of chromatogram, was verified. The linearity of detector response to each amine, between 0.25 and 10 mg/L, was tested by analysis of variance of the linear regression. Least-squares analysis revealed a correlation coefficient $r \geq 0.9990$ ($p < 0.001$) for TY, PU, and AG, and $r \geq 0.9972$ ($p < 0.001$) for the other amines. The coefficient of determination (r^2) was higher than 99.45%

Table 2. Precision and Recoveries of the HPLC Method for the Determination of Biogenic Amines in Cheese

	precision		recovery	
biogenic amine	RSD (%)[a]	RSDH (%)[b]	Cochran's test[c] C_{exp}	recovery % mean (SD)
OC	1.42	5.73	0.814	89.68 (3.33)
DO	1.62	5.72	0.704	91.40 (2.66)
TY	1.80	5.71	0.537	92.65 (1.88)
PU	1.31	5.67	0.337	97.35 (1.06)
SE	1.46	5.70	0.562	94.61 (1.26)
CA	1.02	5.68	0.735	97.59 (1.71)
HI	1.12	5.67	0.631	97.33 (1.71)
AG	1.24	5.68	0.725	95.73 (2.84)
PHE	0.97	5.72	0.689	91.45 (1.53)
SD	2.13	5.67	0.798	96.70 (2.36)
TR	2.04	5.72	0.763	91.53 (2.34)
SM	1.63	5.67	0.812	96.56 (3.18)

[a] RSD, relative standard deviation. [b] RSDH, Acceptable range for relative standard deviation according to Horwitz's formula for intra-laboratory studies. [c] Cochran test: $C_{tab\ (7,\ 2,\ 0.05)} = 0.8332$.

for all standard curves. These results indicate a definitive linear relationship between amine concentration and detector response. To study the precision of the method, eight determinations of the same sample were performed using the same reagents and apparatus.

When an amine was not naturally present, samples were spiked with a known quantity of the corresponding amine standard. The RSDs obtained for each amine were always satisfactory (<2.5%), and they were acceptable according to Horwitz's formula for intra-laboratory studies (Horwitz, 1982). The recovery was determined according to the standard addition method by using two addition levels for each amine. Eight determinations were carried out for each addition level. Recovery was satisfactory, because it was, in general, higher than 90%. In addition, the Cochran's test showed that the method accuracy did not depend on the biogenic amine content in the samples. The results of precision and recovery are shown in Table 2. To determine the sensitivity of the method in the working conditions proposed, the detection limit (DL) and the determination of method (DtL) were studied. The criterion was the repeated analysis of a blank according to the formula of Long and Winefordner (1983). The blank used was 0.6 N PCA, because it was not possible to obtain a sample of cheese without BA. The DLs were lower than 0.10 mg/L for all the amines, while the DtLs were lower than 0.15 mg/L for all the amines except SM, which was slightly higher (0.20 mg/L).

The method described was accurate and precise, and was useful for the determination in one run of twelve BA in milk and some dairy products, such as unripened cheeses and yogurt. However, the procedure did not allow the correct identification and quantification of BA in cheeses whose manufacture involved a long ripening process, such as ripened cheese. Figure 2 (A) shows a chromatogram of a ripened cheese obtained with the method above described, in which some interferences hinder the amine resolution. To improve the results, some strategies to increase the cleanup efficiency of samples before injection were assayed. Trichloroacetic acid and HCl were used instead of PCA, as both had previously been reported for the sample preparation of dairy products (Martelli et al., 1993; Bockhardt et al., 1996). Moreover, phosphotungstic acid was also used, because it had been reported for nitrogenous fraction analysis (Beuvier et al., 1997). However, the chromatographic resolution obtained by using all these procedures was very similar to that obtained after PCA extraction.

Small peptides were thought to be involved in the lack of resolution, because the interferences were observed only in ripened cheeses with a higher degree of proteolysis. Thus, the usefulness of Ultrafilters (Ultrafree-15, MW cutoff: 5000D, Millipore) to avoid the interfering peaks was assayed. The results obtained were not satisfactory, because the interferences were not completely eliminated. To avoid the potential interference of peptides, Carboxipeptidase A and Pronase E (Merck) were also used in sample preparation, as they hydrolyze peptides. Using both enzymes, the presence of interferent peaks in the chromatogram was less evident, but the results were not entirely satisfactory because of the irregular losses of BA, which ranged from 3.5 to 18.5%.

The best solution for increasing the resolution among amines and their interferents in ripened cheeses was the use of an alternative gradient elution program. Mobile phases were the same as described above, and the new gradient elution program designed is shown in Table 3. The latter elution program is slower than that previously described, because it uses only logarithmic curves. The resolution obtained between peaks is better in these conditions, as Figure 2(B) shows. The sample preparation was also as described above.

Results showed that the proposed procedure is reliable and allows the BA determination in milk, yogurts, and unripened cheeses. Moreover, by using the same general procedure and only modifying the gradient elution program, it is possible to determine BA in dairy products with a more complex matrix such as those found in ripened cheeses.

Biogenic Amine Contents. The range of concentrations, the median value, and the quartile deviation for the BA contents in milk, yogurt, and cheese are shown in Table 4 for diamines and polyamines, and in Table 5 for biogenic monoamines. SE was the only nondetected BA in any of the samples analyzed, in agreement with Petridis and Steinhart (1995) who did not find SE in ripened cheeses. However, Vale and Glória (1997) found SE in Parmesan cheese at low levels. No data were found on SE in milk and yogurt. OC and DO were only detected in 40% and 30% of ripened cheeses, respectively, but always at levels lower than 5 mg/kg ww. No previous data were found on the presence of these minor amines in milk or other dairy products.

In milks and yogurts, only the diamine CA and some polyamines were detected, and always at low levels. In milk, SD was the prevailing polyamine and AG was found in 50% of samples. In yogurt, the levels of the polyamines AG and SD were slightly higher than in milk. In addition, the polyamine SM and the diamine CA, which were not found in milks, were also found in yogurts. That could imply that the microorganisms involved in the yogurt fermentation process produce these BA. However, according to our results, the differences in amine profile were not so relevant, indicating that further studies are needed to support this hypothesis. Other authors also found low levels of amines in milks and yogurts (ten Brink et al., 1990; Petridis and Steinhart, 1996; Bardócz et al., 1993). In addition, Bardócz, (1995) has reported that PU, CA, AG, SD, and SM can be naturally found in foods, and so do not necessarily result from bacterial metabolism. This ac-

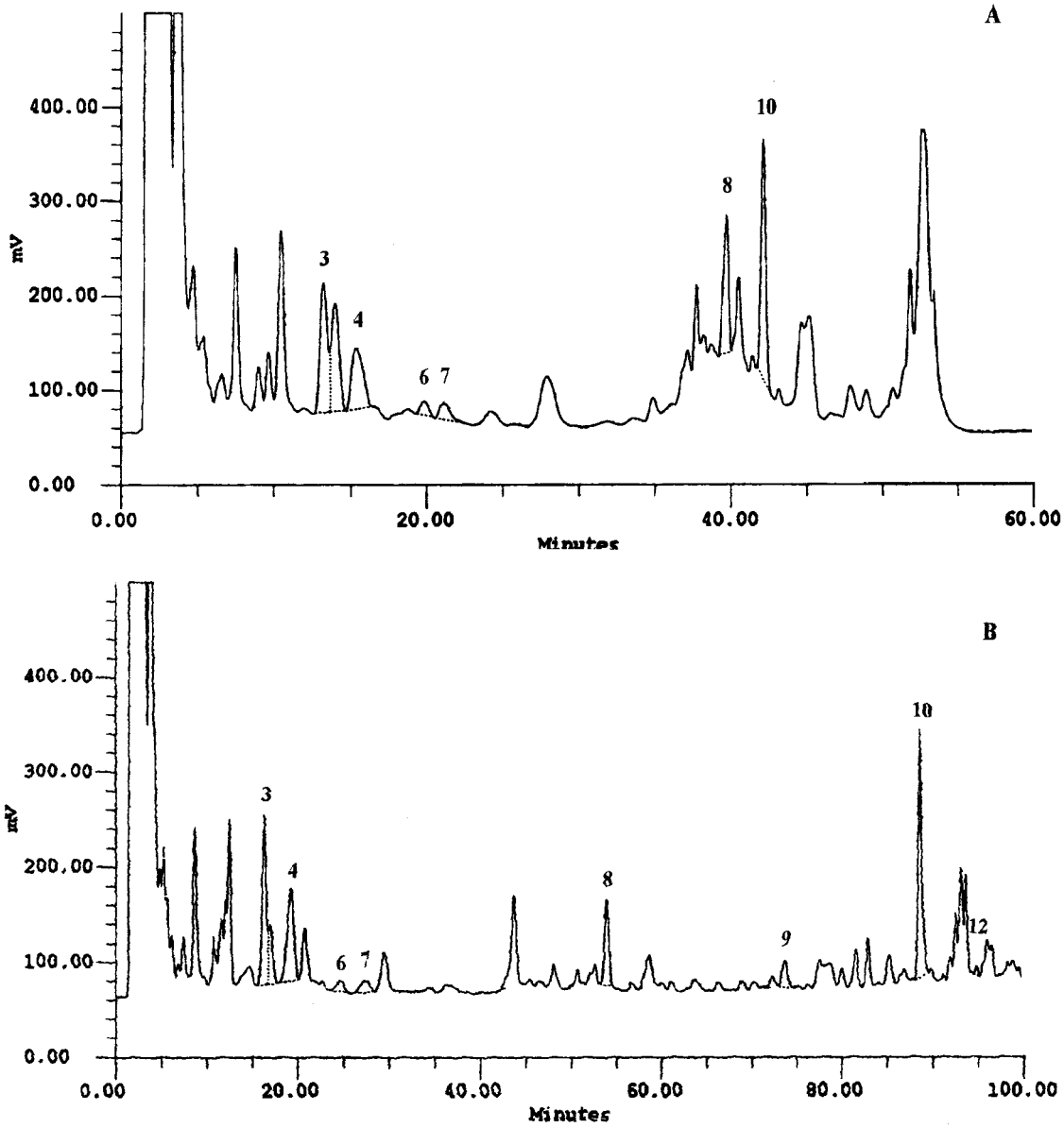

Figure 2. Liquid chromatograms of biogenic amines. (A) Ripened cheese obtained with method described for unripened cheeses, milks, and yogurt; (B) ripened cheese obtained with method described for ripened cheese. Peak identities: TY (3), PU (4), CA (6), HI (7), AG (8), PHE (9), SD (10), and SM (12).

Table 3. Gradient Elution Program for Separation of Biogenic Amines in Ripened Cheese

operation	time, min	mobile phase A%	mobile phase B%	curve	order
elution	0.01	82	18		
	35	70	30	linear	0
	85	46	54	exponential	3
	88	35	65	exponential	3
return	96	82	18	linear	0
equilibration	100	82	18		0

counts for the absence of amines or their low levels found.

The BA contents varied markedly between the two types of cheese: unripened and ripened (Tables 4 and 5). Thus, the amounts of BA in ripened cheese were much higher and showed much more variability than those in unripened cheese. Statistical differences ($p < 0.05$) in the content of all amines between the two kinds of cheese were found by a Mann Whitney test. These remarkable differences could be attributed to specific conditions of their manufacture, such as prolonged ripening time, which seem to affect the production of amines. Likewise, it is well-known that the ripening process due to microflora increases proteolysis, which results in a progressive increase of the BA precursor amino acids (Giraffa et al., 1995). Several research groups have reported production of amines throughout the ripening period (Reuvers et al., 1986; Evans et al., 1988; Stratton et al., 1991; Diaz-Cinco et al., 1992; Martelli et al., 1993). Thus, high BA levels in ripened cheeses could be explained by their long ripening period.

Regarding diamines, although the PU and CA contents were relatively similar in unripened cheeses, their levels fluctuated greatly in ripened cheeses, from ND to 611.68 mg/kg ww, and from 4.23 to 215.28 mg/kg ww, respectively. The contents reported by Halász et al. (1994) for PU and CA in cheeses were even much higher than the maximum found in our samples. However, lower levels of these amines have also been found by others (ten Brik et al., 1990; Sieber et al., 1994; Ordóñez et al., 1997).

Table 4. Diamine and Polyamine Contents (mg/kg wet weight) in Milks, Yogurts, and Cheeses

sample	PU	CA	AG	SD	SM
milk ($n = 5$)	nd[a]	nd	nd−0.18 0.08 (0.09)	0.16−0.18 0.17 (0.09)	nd
yogurt ($n = 5$)	nd	nd−0.27 0.11 (0.13)	nd−0.39 0.16 (0.18)	nd−0.43 0.21 (0.21)	nd−0.34 0.15 (0.16)
unripened cheeses ($n = 10$)	nd−1.43[b] 0.00 (0.27)[c]	nd−1.49 0.00 (0.29)	nd	0.39−0.82 0.50 (0.06)	nd−1.12 0.37 (0.34)
ripened cheeses ($n = 10$)	nd−611.68 14.16 (50.98)	4.23−215.28 11.08 (14.69)	nd−22.01 4.02 (5.85)	nd−43.01 11.17 (7.48)	nd−18.69 2.17 (5.92)

[a] nd, not detected. [b] Range (minimum − maximum). [c] Median (quartile deviation).

Table 5. Biogenic Monoamine Contents (mg/kg wet weight) in Milks, Yogurts, and Cheeses

sample	TY	HI	PHE	TR
milk ($n = 5$)	nd[a]	nd	nd	nd
yogurt ($n = 5$)	nd	nd	nd	nd
unripened cheeses ($n = 10$)	nd−0.51[b] 0.00 (0.26)[c]	nd	nd	nd
ripened cheeses ($n = 10$)	nd−241.92 44.29 (93.07)	2.21−163.56 5.62 (5.65)	nd−29.03 6.45 (3.56)	nd−45.05 0.00 (3.73)

[a] nd = not detected. [b] Range (minimum − maximum). [c] Median (quartile deviation).

Among the polyamines, only AG was not found in any sample of unripened cheese and was only detected in 6 samples of ripened cheeses, with a median of 4.02 mg/kg ww. In contrast, SD and SM were found in most samples of both type of cheeses. In unripened cheeses, contents of SD were similar to those of SM, whereas in ripened cheeses, SD was the prevailing polyamine. Few data on the contents of AG, SD, and SM in cheeses are available. For AG, Petridis and Steinhart (1995) and Simon-Sakardi and Hodosi (1995) have reported lower levels, and Sieber et al. (1994) and Simon-Sakardi and Hodosi (1995) have found a wide range of SD and SM contents, from nondetectable to 112.86 mg/kg.

The levels of polyamines in unripened cheeses were higher than those in milks. That difference could only reflect the effect of the milk concentration. Higher differences in AG, SD, and SM concentrations have been found (Table 4) between ripened cheeses and milks. The higher contents observed in ripened cheeses could hardly be explained by a concentration effect only. Thus, the production of polyamines throughout the ripening process seems to take place.

The contents of biogenic monoamines were higher and showed wider variability in ripened than in unripened cheeses. In unripened cheeses, only TY, at very low levels (from ND to 0.51 mg/kg ww), was found, while in ripened cheese, TY and the other biogenic monoamines were detected in many samples. TY and PHE were detected in 80% of the samples, TR in 40%, and HI in all of the samples tested. In ripened cheese, as well as in unripened cheese, TY was the major amine. The levels of biogenic monoamines reported elsewhere are also characterized by a wide range of variability. The TY and HI contents found here were lower, in general, than those previously reported for ripened cheese (Evans et al., 1988; Martelli et al., 1993; Petridis and Steinhart, 1995; Schneller et al., 1997). The low levels of PHE and TR found here agree with those reported by other authors (Petridis and Steinhart, 1995; Ordóñez et al., 1997).

Owing to the significant presence of BA in ripened cheeses, and the well-known unhealthy effects of the intake of large amounts of some BA, the consumption of ripened cheeses should be avoided, particularly in sensitive individuals. However, the wide variability of BA contents in ripened cheeses, and the very low levels of BA found in some of these cheeses, suggest continued study of the critical control points in order to reduce the BA accumulation in this type of cheese.

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