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Determination of biogenic amines in beers and brewing-process samples by capillary electrophoresis coupled to laser-induced fluorescence detection

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Abstract

We have developed a sensitive capillary electrophoretic method for the simultaneous determination of 10 biogenic amine which usually appears in beer samples, using laser induced-fluorescence. Sample amines were first derivatized and filtered and then separated with an uncoated capillary tubing in the presence of 50 mM sodium borate and 20% acetone at pH 9.3 at 30 kV. It was possible to analyze biogenic amines in brewing-process samples and in beer samples in less than 30 min, obtaining detection limits between 0.3 $\mu g\,l^{-1}$ for ethylamine and 11.9 $\mu g\,l^{-1}$ for 1,6-hexanodiamine.

Keywords: Laser-induced fluorescence; Capillary zone electrophoresis; Beer analysis

1. Introduction

Beer has been commonly reported to be a health risk for some consumers due to the biogenic amines it contains (Kalac, Savel, Krizek, Pelikánová, & Prokopová, 2002). These substances are derived from the microbial decarboxylation of the corresponding amino acids or by the transamination of aldehydes by amino-acid transaminases (Moret & Conte, 1996). These are normal constituents of many foods and beverages and have been found in cheese, wine, beer, fishery products and meat, usually as a result of enzymatic degradation or fermentation processes (Busto, Miracle, Guasch, & Borrul, 1997; Subden, Brown, & Noble, 1978). Their presence in high amounts in foods is associated with food deterioration (Linares, Ayala, Afonso, & Diaz, 1998). Biogenic amines in low concentrations are essential for many physiological functions, whilst at high concentrations they may cause some damage effects. Several symptoms may occur following excessive oral intake of biogenic amines, such as headache, hypo- or hypertension, nausea, cardiac palpitations, renal intoxication and in more severe cases intra-cerebral haemorrhage and death (Kuhn & Lovenberg, 1982; Moret, Bortolomeazzi, & Lercker, 1992). Interest in the determination of amines in beverages is also because they may serve as precursors to the in vivo production of carcinogenic nitrosamines (Kataoka, Shindoh, & Makita, 1995; Fine, Ross, Rounbehler, Silvergleid, & Song, 1977).

Various methods, including gas chromatography (Baker & Coutts, 1982), high-performance liquid chromatography (Gennaro & Abrigo, 1991; Hornero-Méndez & Garrido-Fernández, 1994) and mass spectrometry (Shafi, Midgley, Watson, & Smail, 1989) have been developed to analyse biogenic amines in foodstuffs. Capillary electrophoresis (CE) has become a popular and powerful separation technique which possesses many advantageous features, such as high resolution, extremely high efficiency, rapid analysis and small consumption of samples and solvents and different applications of beer have appeared in recent years

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(Cortacero-Ramírez, Segura-Carretero, Cruces-Blanco, Hernáinz-Bermúdez de Castro, & Fernández-Gutiérrez, 2003a, 2003b, 2004). Thus, the development of a new and more sensitive analytical method using capillary zone electrophoresis to separate biogenic amines is desirable. An interesting alternative to enhance sensitivity, detection limits and linearity is the application of laser-induced fluorescence detection (LIF) to the capillary electrophoresis separation. The CE–LIF is considered as the highest reported sensitivity and a detection mode for separations (Albin, Grossman, & Moring, 1993) and numerous applications in different fields have been published (Armstrong & He, 2001; Arráez-Román, Segura-Carretero, Cruces-Blanco, & Fernández-Gutiérrez, 2004; Flaherty, Wark, Street, Farley, & Brumley, 2002; Pavski & Le, 2001).

Few papers relating to the presence and formation of biogenic amines during brewing have been published (Kalac, Hlavata, & Krizek, 1997). The presence of biogenic amines in foods and alcoholic beverages is important from both the toxicological and technological point of view. In brewing, the types and levels of biogenic amines are dependent on the raw materials present in the beverage, as well as on the brewing method, and any microbial contamination that may have occurred during the brewing process or during storage. Due to the high consumption of beer and the possible harmful effects of biogenic amines, it is important to determine their levels.

In this work, the determination of biogenic amines in beers and brewing process samples has been carried out after derivatization with fluoresceine isothiocianate (FITC) by capillary zone electrophoresis (CZE) using acetone as an organic modifier and laser-induced fluorescence detection (LIF).

2. Materials and methods

2.1. Instrumentation

The instrument used for the electropherograms was a Beckman 5500 capillary electrophoresis. The system comprises of a 0–30 kV high-voltage built in power supply, equipped with a laser-induced fluorescence detector, an argon ion laser was used as excitation source (488 nm) and the electropherograms were recorded by monitoring the emission intensity at 520 nm. All capillaries (fused silica) used were obtained from Beckman instruments Inc. (Fullerton, CA, USA) and had an inner diameter of 75 μm and an outer diameter of 375 μm, a total length of 57 cm and an effective separation length of 50 cm. The temperature was controlled using a fluorocarbon based cooling fluid. Data acquisition and processing were carried out with GOLD software installed in a personal computer.

2.2. Chemicals and samples

The analytes studied were agmatine, 1,6-hexanodiamine, cadaverine, putrescine, dibutylamine, dipropylamine, tryptamine, histamine, amylamine, 2-phenyletylamine, tyramine, butylamine, dimethylamine, ethylamine and methylamine, and all were obtained from Sigma (St. Louis, MO, USA). Standard stock solutions of different concentrations of mixtures of all analytes were prepared in doubly deionized water. FITC stock solution (10 mM) was prepared by dissolving fluorescein isothiocyanate isomer I in acetone. This solution was kept in a refrigerator at 4 °C. Fresh solutions were prepared daily. Reagent grade acetone, methanol and acetonitrile were obtained from Scharlau. A 0.2 M sodium carbonate/bicarbonate buffer (pH 9) was used for the derivatization procedure while a sodium borate solution (50 mM, pH 9.3) was selected for the separation. All solutions were filtered through 0.45 μm Millipore (Bedford, MA, USA) membrane filters before injection into the capillary. Doubly distilled water was obtained by Milli-Q water purification system.

2.3. Sample preparation

Since beers have a water-based sample matrix, very little sample preparation is required. Brewing-process samples of an extra beer were analysed: cold wort (Mc1), fermented wort (Mc2), matured and stored beer (Mc3), filtered beer (Mc4) and a bottled extra beer (see Fig. 1). Two other types of beers (special and non-alcoholic) have also been analysed.

Eight hundred microlitres of each sample previously degassed by ultrasonication was derivatized using 495 μl of 0.2 M carbonate buffer at pH 9, 1000 μl of FITC solution (10 mM), 1000 μl of acetone and doubly deionized water to a final volume of 5 ml in a test tube. This solution was put into a thermostatic bath for 2 h at 50 °C and 2 ml of the resulting solution was diluted to a final volume of 10 ml with doubly deionized water before the analysis by CE. In the case of extra, special and non-alcoholic beer 1500 μl was taken for the derivatization. After the derivatization procedure, the sample was passed through a 0.45 μm membrane filter prior to injection.

Peaks were identified by comparing their migration times with those of the standards spiked in the samples at several concentrations and quantified using standard additions.

2.4. Derivatization procedure

The derivatization procedure was optimised using our previous experience because we have optimised previously different parameters which affect the reaction of the chemical composition, concentration and pH of the buffer, the amount of FITC, the addition of organic solvents, reaction time and the temperature (Arráez-Román et al., 2004). The conditions chosen for the beer samples were: 495 μl of 0.2 M carbonate buffer (pH 9), 1000 μl of FITC solution (10 mM) and 1000 μl of acetone were mixed with 200 μl of a mixture of amines of different concentrations and doubly distilled water to a final volume of 5 ml in a test tube.

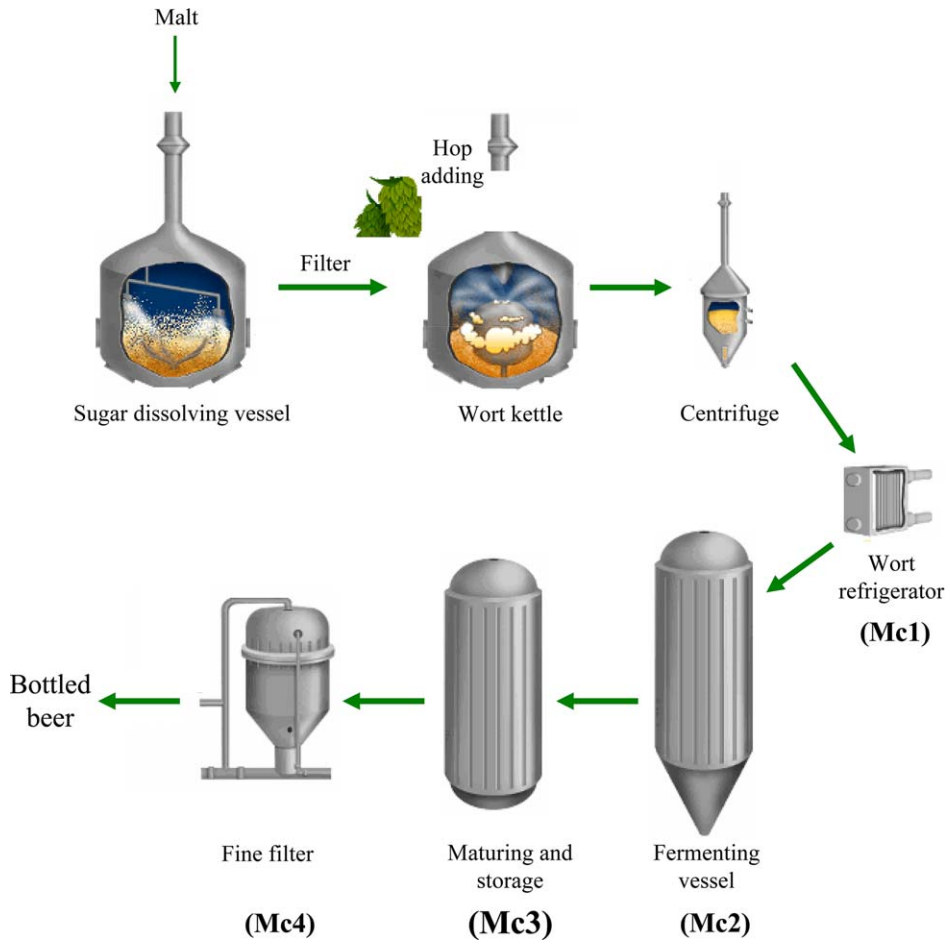

Fig. 1. Brewing process indicating the points of sample taking: Mc1, cold wort; Mc2, exit of fermented wort; Mc3, matured and stored beer; Mc4, filtered beer and bottler beer.

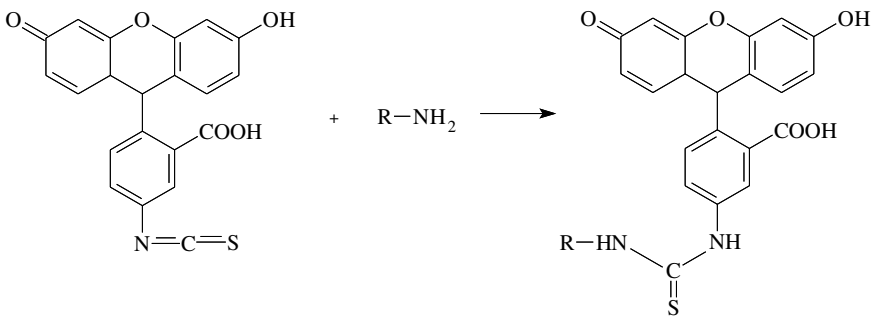

Fig. 2. Derivatization reaction between FITC and amine compounds.

This solution was introduced into a thermostatic bath for 2 h at 50 °C and 2 ml of the resulting solution was diluted to a final volume of 10 ml with doubly distilled water. The final solution was introduced into the CE–LIF for analysis. Fig. 2 shows the derivatization reaction.

2.5. Electrophoretic method

Separation was performed in an uncoated fused silica capillary of 75 μm internal diameter (i.d.) and a total length of 57 cm (50 cm to the detector) using a running buffer formed by 50 mM $Na_2B_4O_7$ (pH 9.3) and 20% acetone; samples were injected hydrodynamically at 0.5 psi for 12 s, detection was carried out by on-column measurement using a LIF detector with a laser power of 3 mW at 488 nm, a beam diameter of 100 μm and equipped with a 488 nm notch filter and laser-mounted band pass filter and 520 nm for the detection. The voltage applied was 30 kV and the current achieved was 170 μA. The capillary was conditioned before each run for 1 min with sodium

hydroxide 0.1 M, then for 1 min with water, and finally for 3 min with the separation buffer.

3. Results and discussion

3.1. Selection of analytes and optimization of electrophoretic separation

Selection of the analytes was carried out by spiking a beer sample with standard amines which could be beer constituents in accordance with the bibliography and with information of the real samples analysed previously (Cortacero-Ramírez et al., 2003a, 2003b). The 15 selected analytes are: agmatine, 1,6-hexanodiamine, cadaverine, putrescine, dibutylamine, dipropylamine, tryptamine, histamine, 2-phenylethylamine, amylamine, tyramine, butylamine, dimethylamine, ethylamine and methylamine. Fig. 3 shows the chemical structures of these analytes.

The experimental and instrumental conditions such as pH, electrolyte composition, voltage, injection time were studied to obtain the best sensitivity and resolution for the chosen analytes. The optimum voltage value was 30 kV, because short analysis times were obtained. To study the hydrodynamic injection time, different times (between 5 and 15 s) were applied for introducing the analytes into the capillary, selecting 12 s as optimum. The effect of pH between 8 and 11 on the separation of analytes was studied using 50 mM sodium borate buffer solution, obtaining the best resolution between all peaks at 9.3.

In this work, the influence of ionic strength and organic modifiers on the separation of these biogenic amines were the most significant variables. The effect of ionic strength on resolution, for this mixture of 15 amines, was investigated by using 15% acetone and different concentrations of sodium borate (35–65 mM) varying the current between (130 and 210 μA) at pH 9.3, obtaining the best resolution with a concentration of 50 mM, since higher concentrations lengthen the analysis time.

Three organic modifiers were studied, acetone, methanol and acetonitrile, obtaining the best results with acetone. To establish the optimum value of acetone, its percentage was varied between 15% and 25% obtaining the best resolution using a percentage of 20%. The optimum electropherogram from a standard mixture of 15 amines is shown in Fig. 4(a). An electropherogram of a blank sample is shown in Fig. 4(b).

Ten amines were quantified without any interference. The remaining amines could not be quantified due to an overlap between them (dipropylamine and tryptamine; 2-phenylethylamine and amylamine) or due to the overlap with the labelling peaks such as methylamine.

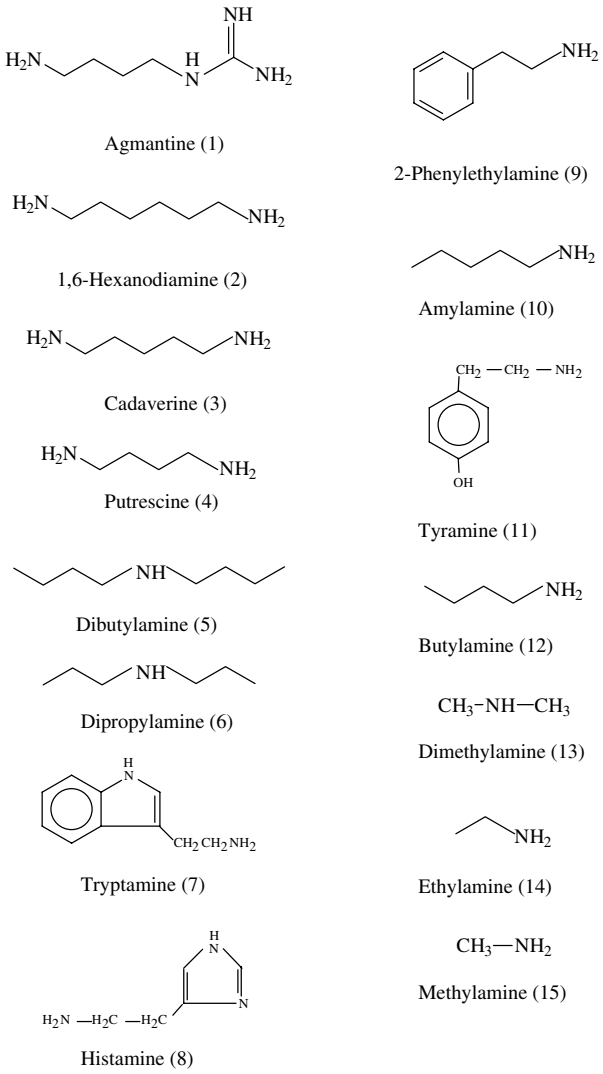

Fig. 3. Chemical structures of studied amines.

3.2. Quantification

The detection limits ($DL = 3S_o/b$) and quantification limits ($QL = 10S_0/b$) of the method were tested for the studied amines using the IUPAC method, where S_0 is the white standard deviation and b the slope of the calibration plots (Curie, 1995). The results obtained for the FITC-amines derivatives are summarized in Table 1. All calibration curves showed good linearity, agmatine was linear between QL and 950 μg l^{-1}, dibutylamine between QL and 4000 μg l^{-1}, 1,6-hexanodiamine and cadaverine between QL and 2400 μg l^{-1}, putrescine between QL and 5700 μg l^{-1}, histamine, butylamine, tyramine, dimethylamine and ethylamine between QL and 380 μg l^{-1}. Each point of the calibration plot was repeated three times in an independent solution prepared in the same way. The calibration plots indicate good correlation between peak areas and FITC-amine derivative concentrations; regression coefficients were 0.997 for all compounds quantified.

The precision of the method was evaluated by determining the repeatability of the peak areas and the migration time. The repeatability values obtained for three successive injections to peak areas of all analytes varied between 3.2% and 6.2% and the migration times varied between 2.0% and 4.1%.

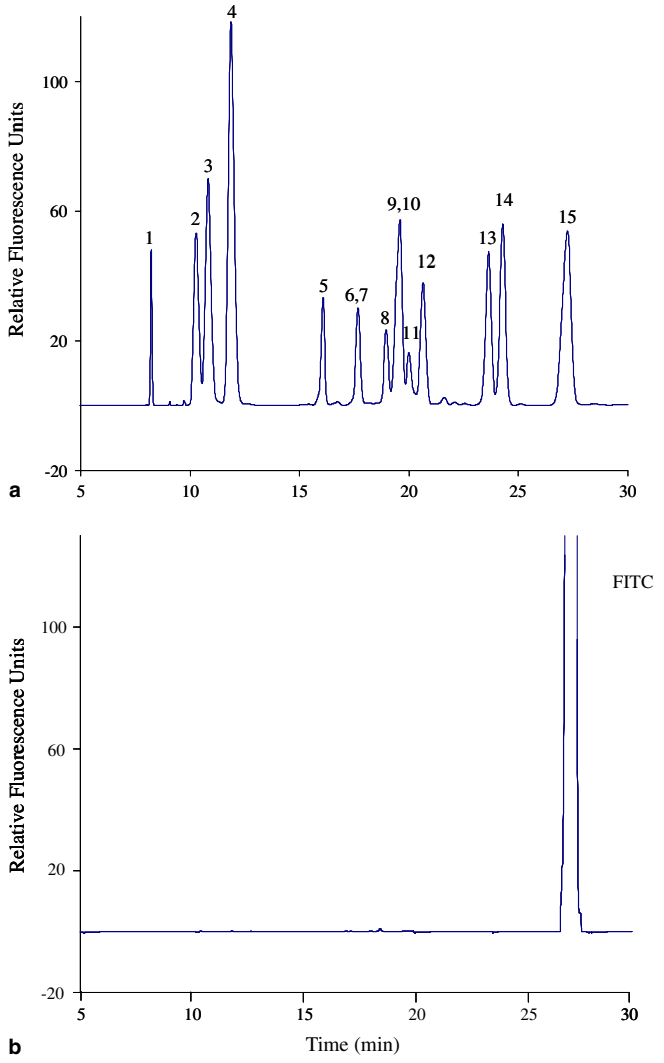

Fig. 4. (a) Electropherogram of a standard mixture of 15 biogenic amines. Experimental conditions: 50 mM sodium borate; pH 9.3; 20% acetone, fused silica capillary, 50 cm × 75 μm i.d.; voltage 30 kV; injection time 12 s. Peaks: 1, agmatine (400 μg l^{-1}); 2, 1,6-hexanodiamine (2400 μg l^{-1}); 3, cadaverine (2400 μg l^{-1}); 4, putrescine (2400 μg l^{-1}); 5, dibutylamine (400 μg l^{-1}); 6 and 7, dipropylamine and tryptamine (400 and 160 μg l^{-1}, respectively); 8, histamine (160 μg l^{-1}); 9 and 10, 2-phenylethylamine and amylamine (160 μg l^{-1}); 11, tyramine (160 μg l^{-1}); 12, butylamine (160 μg l^{-1}); 13, dimethylamine (160 μg l^{-1}); 14, ethylamine (160 μg l^{-1}); 15 methylamine (160 μg l^{-1}) with a peak corresponding to the labelling reagent. (b) Electropherogram of a blank.

Table 1
Characteristic parameters of studied analytes

Peak number	Analyte	Detection limit in beer (μg l^{-1})	Detection limit in injected solution (μg l^{-1})	Quantification limit in injected solution (μg l^{-1})
1	Agmatine	50.0	3.0	10.2
2	1,6-Hexanodiamine	198.3	11.9	39.7
3	Cadaverine	126.6	7.6	25.2
4	Putrescine	110.0	6.6	21.8
5	Dibutylamine	33.3	2.0	6.7
8	Histamine	16.7	1.0	3.4
11	Tyramine	23.3	1.4	4.8
12	Butylamine	53.3	3.2	10.6
13	Dimethylamine	6.7	0.4	1.3
14	Ethylamine	5.0	0.3	1.2

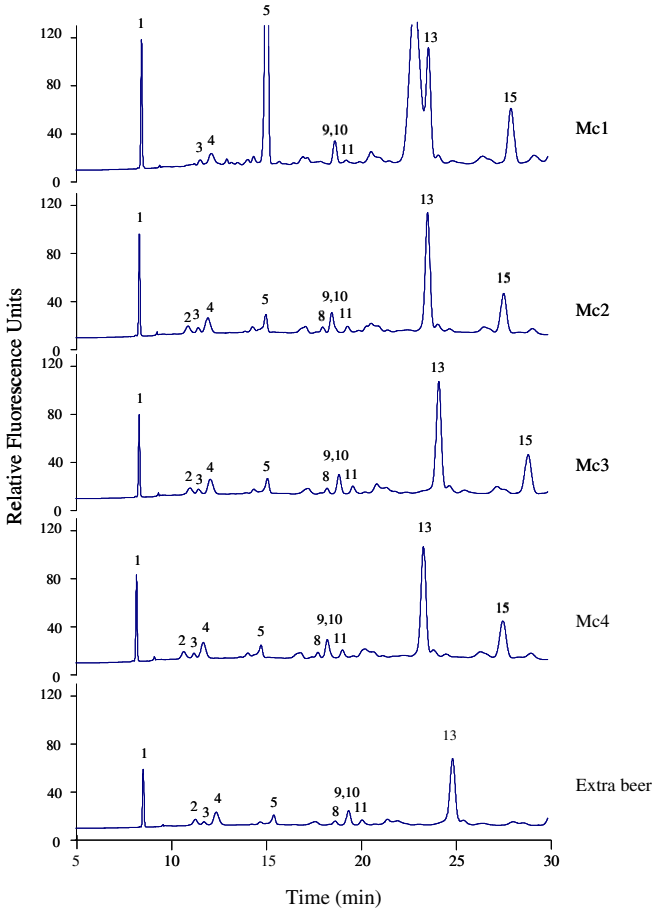

Fig. 5. Electropherograms of the samples during the brewing process of an extra beer. Capillary electrophoretic conditions as in Fig. 4.

3.3. Application to real samples

We have determined the concentration of biogenic amines in beers and brewing-process samples using an electrophoretic method based on derivatization by treatment with FITC as derivatizing agent for primary amines. Fig. 5 shows electropherograms of the samples analysed during the brewing process of an extra beer. The peaks were identified by standard additions at several concentrations to the matrix rather than by their migration time. Only small variations in migration time (lower 5%) were observed when real beer samples were analyzed.

As can be seen in this figure, agmatine, 1,6-hexanodiamine, cadaverine, putrescine, dibutylamine, histamine, 2-phenylethylamine, amylamine, tyramine, ethylamine and methylamine could be identified in all the analyzed samples, except in sample Mc1, where 1,6-hexanodiamine and histamine did not appear but their determination is possible in the rest of the samples.

Table 2
Concentration of biogenic amines during the brewing process and in beer samples (μg ml^{-1})

Peak numbers	Analytes	Mc1	Mc2	Mc3	Mc4	Extra beer	Special beer	Non-alcoholic beer
1	Agmatine	21.7 (10.3)[a]	20.3 (4.0)[a]	18.6 (10.1)[a]	17.7 (0.6)[a]	5.7 (5.6)[a]	3.1 (5.6)[a]	0.2 (5.7)[a]
2	1,6-Hexanodiamine	nd	51.7 (4.3)[a]	40.2 (8.0)[a]	28.2 (6.8)[a]	21.5 (8.7)[a]	13.8 (1.5)[a]	nd
3	Cadaverine	28.1 (2.0)[a]	31.9 (2.8)[a]	28.6 (7.4)[a]	29.8 (3.6)[a]	12.8 (7.0)[a]	13.6 (1.9)[a]	nd
4	Putrescine	38.6 (3.3)[a]	36.4 (1.1)[a]	40.6 (5.5)[a]	42.1 (7.2)[a]	22.4 (10.9)[a]	17.1 (3.9)[a]	8.3 (5.5)[a]
5	Dibutylamine	92.6 (8.1)[a]	6.4 (7.5)[a]	7.7 (7.4)[a]	6.1 (9.6)[a]	2.0 (10.8)[a]	2.7 (9.5)[a]	1.4 (4.5)[a]
8	Histamine	nd	1.3 (3.3)[a]	1.1 (5.9)[a]	1.4 (1.4)[a]	0.3 (8.7)[a]	0.6 (4.2)[a]	0.1 (1.5)
11	Tyramine	1.6 (4.3)[a]	1.8 (6.4)[a]	1.9 (0.8)[a]	1.9 (7.5)[a]	0.2 (7.2)[a]	0.6 (0.4)[a]	nd
12	Butylamine	nd	nd	nd	nd	nd	nd	nd
13	Dimethylamine	6.4 (8.3)[a]	6.6 (5.2)[a]	6.4 (1.9)[a]	6.9 (3.4)[a]	1.3 (1.5)[a]	1.6 (4.3)[a]	0.09 (11.6)[a]
14	Ethylamine	nd	nd	nd	nd	nd	nd	nd

nd, non-detected.
[a] Mean concentration of 7 replicates (relative standard deviation).

The effect of maturing and storage (Mc3) and fine filter (Mc4) are minimal in the content of biogenic amines analysed except in the case of 1,6-hexanodiamine whose concentration decreases considerably.

A large fall in dibutylamine (peak 5) between Mc1 and other samples was observed.

Table 2 shows the content of biogenic amines during the brewing process of an extra beer and two other beers (special beer and non-alcoholic beer). As can be observed in this table the differences between the Mc4 data and the concentrations in the bottled end product "extra beer" are due to a final stage in the brewing process, the bottled beer is diluted with water. The electropherogram corresponding to Mc1 shows an unknown large peak migrating just before dimethylamine (peak 13), for this reason, the concentration determined of this compound could be considered as an approximation.

Only agmatine, putrescine, dibutylamine, histamine and dimethylamine were detected in the bottled non-alcoholic beer. In bottled extra and special beer putrescine, cadaverine and 1,6-hexanodiamine were detected in major concentrations while histamine and tyramine are present in small concentrations.

Quantification was also studied using standard additions method, adding increasing quantities of the analytes to a constant volume of sample to corroborate that the quantity of each analyte found was that obtained by the direct method of analysis and to check that no matrix effect exists (Cuadros-Rodríguez, García-Campaña, Alés-Barrero, Jiménez-Linares, & Román Ceba, 1995; García Campaña, Cuadros-Rodríguez, Aybar-Muñoz, & Alés Barrero, 1997). The slopes of the calibration graph and the addition method were according to which the statistical t of the slopes should be smaller than the t of Student's *t-test* tabulated for a α (probability) of 0.01 and $n_S + n_A - 4$ grades of freedom (n_S = number of data used to establish the calibration plot with patterns; n_A = number of data to establish the calibration plot of pattern addition) (t tabulated value 2.9). The concentrations of biogenic amines during the brewing process of an extra beer found by the standard additions method can be seen in Table 3. In this table we can observe that there does not exist significant differences with the found concentration using the calibration plots (Table 2).

Table 3
Concentration of biogenic amines during the brewing process of an extra beer using standard addition method (μg ml^{-1})

Peak numbers	Analytes	Mc1	Mc2	Mc3	Mc4	Extra beer
1	Agmatine	21.9	20.0	18.5	18.2	5.3
2	1,6-Hexanodiamine	nd	52.0	40.3	28.5	21.1
3	Cadaverine	28.0	31.0	29.3	29.4	13.1
4	Putrescine	38.9	37.0	39.9	41.8	22.0
5	Dibutylamine	91.9	6.8	7.1	6.4	1.8
8	Histamine	nd	1.2	1.3	1.2	0.4
11	Tyramine	1.5	1.7	1.8	2.1	0.2
12	Butylamine	nd	nd	nd	nd	nd
13	Dimethylamine	6.5	6.4	6.5	6.8	1.2
14	Ethylamine	nd	nd	nd	nd	nd

nd, non-detected.

4. Conclusion

We have developed a new method for the determination of amines in beer at μg l^{-1} levels and brewing-process samples by capillary electrophoresis coupled to laser-induced fluorescence detection. The results of this work demonstrate that this is a useful, simple and rapid method for the separation, identification and determination of amines in many types of these samples.

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