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Simple, reliable determination of biogenic amines in Italian red wines. Direct analysis of underivatized biogenic amines by LC-ESI-MS

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ABSTRACT

Amines are ubiquitous compounds, and they are called “biogenic amines” (BAs) when they are synthetized by microbial decarboxylation of corresponding amino acids or “natural polyamines” when theyoriginate from endogenous metabolic pathway. BAs maybe both essential and harmful to human health. In wine, the composition of grape variety, the different types of fermentation and vinification processes and pH values are the most important contributors to BAs content. Several analytical methods have been reported for the BAs determination in wine (HPLC-UV/FLD, CE, LC-MS, etc.). As the most of them necessary require a long pre- or post-column chemical derivatization, LC coupled with MS spectrometry offers a reliable and faster determination of underivatized biogenic amines.The aim of the present work wasto investigate the content of nine BAs in 23 Italian red wines samplesusing LC-ESI-MS.

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Introduction

Aminesare ubiquitous bioactive compounds which may be both essential and harmful to human health[1]. They represent a class of organic, basic, and low-molecular weight compounds which can be natural/endogenous or exogenous in metabolism of plants, microorganisms and animals[2]. When originating from a natural metabolic pathway they are called “natural polyamines” and have physiological functions, especially for cellular metabolism as they are fundamental in membrane stabilization, protein synthesis and nucleic acid regulation[3]. Whereas, when they are synthetized through a microbial decarboxylation of the corresponding amino acids, they are defined Biogenic Amines (BAs), and are usually implicated into toxicological reactions[4]. BAs contamination in food involves symptoms that are similar to those of food poisoning: migraine headaches, nausea, gastric disorders, cardiac palpitations, respiratory suffering and psychoactive effects[5,6].Biogenic aminescan be synthesized both in fresh and perishable food(e.g. meat, fish, fruit, vegetables, etc.),which are directly exposed to decarboxylase-positive microorganisms[7];as well as fermented and/or processed food (e.g. wine, beer, cheese, coffee, chocolate, etc.) as a direct consequence of their transformation process (e.g. alcoholic and lactic fermentation)[8,9].BAs concentration mainly depends onthe protein composition of food matrix and the content of free amino acids, and it is also influenced by the presence of contaminating micro-organismsthat can be naturally present or added as starter cultureduring the transformation process[10]. However,BAs amount can be also linked tofood storage and contamination, caused by non-adequate hygienic conditions[11].

The occurrence of biogenic amines in wine depends on several factorsthat are mainly related to grape features, vinification or fermentation processes and pH values[12-14]. For the first case, different authorsreported that pedoclimatic conditions of the wine-growing area, nutritional status of vine, degree of grape ripeness and the composition of grape cluster in amino acids and natural polyamines are the main contributors to BAs content in wine[12-13,15].It has been reported that amino acids are the primary precursors of biogenic amines in wine. During alcoholic and malolactic fermentation in wine, altering yeasts and spoilage bacteria could have decarboxylating enzymes that metabolize amino acids and other substrates (e.g. aldehydes and ketones) into biogenic amines[16]. Moreover, the time of contact between the must, the grape marc and lactic acid bacteria (LAB), and the type of containers (stainless steel or oak barrels) used during vinification techniques could be significant for the synthesis of BAs in wine[17,18].pH is considered one of the most important factors influencing BAs content, as it controls the decarboxylating activity of microorganisms[10]. It is well known that high pH can positively affect the bacterial overgrowth and it consequentlypromotes the synthesis of biogenic amines in wine.Thus,explaining why higher pH values in red wines (pH: 3.4-3.5) are relevant for a higher BAs concentration compared to white wines (pH: 3.0-3.3)[18].

The main biogenic amines found and in wine are tyramine, histamine, putrescine and 2-phenylethylamine.Different authorshave shown that red wines contain more BAs than white and rosé wines, also quantifying tyramine and histamine as the most relevant BA reaching values above 8 mg/L[19-21].The European Union has not set BAs limits for the wine industry, but some countries have adopted their own regulations. Germany, Belgium and France have set a maximum histamine level of 2, 6 and 8 mg/L, respectively[21-22].

The biogenic amines determination is not simple because of their structure.

The most common approach to analyzeBAs in wine includes high performance liquid chromatography (HPLC) coupled with ultraviolet detector (UV)[23,24] fluorescence detector (FLD)[18,25], mass spectrometry (MS)[26], and capillary electrophoresis (CE)[27]. As primary or secondary amines structure do not absorb in the visible and ultraviolet range nor do they show fluorescence, pre- or post-column chemical derivatization is a necessary analytical step required for the detection[25]. The derivatization step improves the sensitivity of the analytical method; nevertheless, it has some drawbacks such as analyte loss, side reaction amines compound and longer time for the analysis. This could result into a poor resolution of the chromatographic method. However, LC coupled with MS spectrometry represents a valid and rapid ifenate technique for the detection of raw amines, as it does not require the derivatization step. Table1 shows the chemical characteristics of the nine biogenic amines analyzed in Italian red wine samples.

The aim of the present work was to determine nine BAs (tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, serotonin, tyramine, spermidine and spermine) in 23 Italian red wines using LC-ESI-MS.

Material and methods

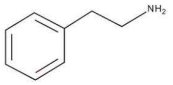
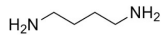
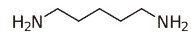
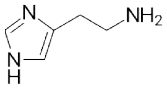
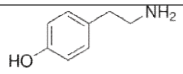
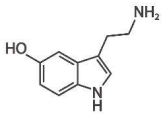
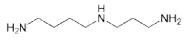
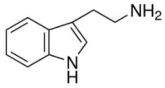
Sampling

The 23 red wine samples were purchased from local wine shop in different Italian regions: Piedmont, Tuscany, Veneto, Puglia and Sicily; for each region, red wines were respectively, for Piedmont: Barbera (Wine 1 and Wine 2), Dolcetto (Wine 3), Nebbiolo (Wine 4 and Wine 5); for Tuscany: Chianti (Wine 6 and Wine 7), Vernaccia (Wine 8), Montepulciano (Wine 9 and Wine 10); for Veneto: Pinot Nero (Wine 11), Merlot (Wine 12 and Wine 13), Cabernet (Wine 14 and Wine 15); for Apulia: Primitivo (Wine 16 and Wine 17), Negroamaro (Wine 18), Aglianico (Wine 19) and for Sicily: Nero d'Avola (Wine 20), Etna Rosso (Wine 21), Syrah (Wine 22 and Wine 23).

Chemicals

The nine biogenic amines studied were: Tryptamine (TRP), β-phenylethylamine (β-PEA), putrescine (PUT), cadaverine (CAD), histamine (HIS),serotonin (SER), tyramine (TYR), spermidine (SPD), spermine (SPM), all of which were supplied by Sigma Aldrich (St. Louis, USA), as well as heptafluorobutyric acid (HFBA) and 1,7-diaminoheptane (Internal Standard, IS).

Table 1. Chemical characteristics of the nine BA analyzed in this study

IUPAC nomenclature	*Molecular formula*	*Skeletal formula*	*Molar mass (g·mol^{-1})*
1-Phenyl-2-aminoethane (PHENYETHYLAMINE)	$C_8H_{11}N$		121.18
Butane-1,4-diamine (PUTRESCINE)	$C_4H_{12}N_2$		88.15
Pentane-1,5-diamine (CADAVERINE)	$C_5H_{14}N_2$		102.18
2-(1*H*-Imidazol-4-yl) ethanamine (HISTAMINE)	$C_5H_9N_3$		111.15
4-(2-Aminoethyl) phenol (TYRAMINE)	$C_8H_{11}NO$		137.18
3-(2-Aminoethyl)indol-5-ol (SEROTONIN)	$C_{10}H_{12}N_2O$		176.22
N'-(3-aminopropyl)butane-1,4-diamine (SPERMIDINE)	$C_7H_{19}N_3$		145.25
***N,N'*-bis(3-aminopropyl)butane-1,4-diamine (SPERMINE)**	$C_{10}H_{26}N_4$		202.35
2-(1*H*-Indol-3-yl)ethanamine (TRYPTAMINE)	$C_{10}H_{12}N_2$		160.22

Methanol of chromatographic grade was obtained from Carlo Erba (Milan, Italy) and distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

For the preparation of the amine standard solutions, an individual standard solution of 1.0mg $l^{-1}$of each amine were prepared in purified water and stored in darkness at 4±1 °C, while a standard solution containing all the amines (Mix 8) was obtained with 1 ml of each water solution diluted to 25 ml with purified water. Different aliquots of the standard solution were used to obtain the concentrations to construct calibration curves for BAs and to perform recovery experiments.

For LC-ESI-MS analysis the amine standard solutions were acidified with HFBA to obtain a final acid concentration of 5 mM and ranged from 0.1 to 16 mg $l^{-1}$with 0.8 mg l^{-1} of IS.As for the wine sample preparation, wine samples were initially filtered through a 0.20 µm membrane Millipore filter. For LC-ESI-MS analysis 25 ml of the filtered wine samples were added with HFBA to obtain a final acid concentration of 10 mM. After a second filtration, a volume aliquot of 50 µl was injected in the chromatographic column.

All measurements in the LC-ESI-MS analysis were achieved using a Thermoquest (Manchester, UK) model P2000 with an Alltima (Alltech, IL, USA) C18 reverse-phase column (250 x 4.6 mm i.d., particle size 5 µm). Mass spectrometric analysis was carried out on a Finnigan AQA benchtop single-quadrupole mass spectrometer (Thermoquest). The ESI unit operated at 4.0 kV, the capillary was heated at 200°C and nitrogen was used as desolvation and nebulizer gas at a flow rate 300 and 50 L/hour, respectively. The ESI-MS system operated in the positive ionization mode (PI). Diagnostic fragment ions were obtained by in-source collisop-induced dissociation (CID) of the protonated molecule $[M+H]^+$ after optimization of the voltage of the skimmer cone. Selected ion monitoring (SIM) was applied for the time-scheduled recording of the analytes. Data acquisition parameters are reported in Table2. Instrument control, data acquisition and processing were carried out with Mass Lab (version 2.22) from ThermoquestFinnigan (Manchester, UK).

For the LC-ESI-MS analysis, themobile phase solvents A and B were methanol (10 mMheptafluorobutiric acid) and water (10 mMheptafluorobutiric acid)respectively, at a flow rate of 1 mL/min. The column was maintained at room temperature and analytes were eluted using aninitial linear gradient program from 10% of solvent A to 85% in15 min, then passing from 85% of solvent A to 100% in 1 min,followed by an isocratic elution of 100% of A for 3 min. An additional 10 min was added to reach the initial conditions.The injected volume was 50 µl.

Table 2. Data acquisition parameters used in LC-ESI-MS for the detection of biogenic amines (SIM conditions)[29].

Biogenic amines	MW	Channel, *m/z* (relative abundance)	Cone voltage (V)	Retention window (min)
Tyramine	137.2	121.2(30), 138.3 (100)	30	0-12.85
β-phenylethylamine	121.2	105.1 (10), 122.3 (100)	30	12.85-16.00
Putrescine	88.2	89.3 (100)	40	0-12.85
Cadaverine	102.2	86.2 (10), 103.3 (100)	30	0-12.85
Histamine	111.1	95.2 (30), 112.1 (100)	40	0-12.85
Serotonin	176.2	160.3 (10), 177.2 (100)	40	0-12.85
Tryptamine	160.2	144.3 (40), 161.2 (100)	30	12.85-16.00
Spermidine	145.2	112.3 (10), 129.2 (10), 146.3 (100)	40	12.85-16.00
Spermine	202.3	129.2 (20), 112.3 (10), 203.4 (100)	40	12.85-16.00

Resultsand Discussion

Optimization of the LC-ESI-MS conditions

In order to investigate the separation of the nine BAs based on their mass/charge ratio, single amine standard solutions were injected without any column and analyzed in the full scan mode. These analytes have low relative molecular mass resulting in very small number of fragments. The MS conditions optimized to obtain maximum fragments are summarized in Tab. 3. For quantitative determination in select ion monitoring, the quasi molecular ion $[M+H]^+$ was selected for all compounds. Nevertheless, the detection of two or three confirming ions was carried out. In particular, the quasi molecular ion which has lost a NH_3 group and for spermidine and spermine the quasi molecular ion without two NH_3 molecules were selected. In the case of putrescine its low molecular weight allowed the monitoring of the quasi molecular ion only. Successively a reverse phase C18 column was installed to achieve amine separation. Biogenic amines are organic bases without any large hydrophobic side-chains; as a consequence, reverse phase chromatography is ineffective, eluting them with the dead volume. To overcome this problem, underivatized amines can be separated by ion-pair reversed phase liquid chromatography. The choice of the ion-pairing reagent has to fit two conditions: the first is to permit sufficient retention for good chromatographic separation and the second, most important, is that this reagent has to be volatile with minimum signal suppression. The additive has to allow at the same time optimum separation and recovery of amines and optimum detection by LC-ESI-MS. The addition of an acid to the mobile phase increases the retention times of the different analytes. This effect was due to the interactions between the negative charges on the inner column surface provided by the acid and the positive charges of the amines. Among the acid ion-pairing agents HFBA has demonstrated to work well in LC-ESI-MS. Moreover,a lower pH ($2<pH<3$) improves the analyte ionization in efficiency and analytical sensitivity due to the capacityof HFBA to facilitate nebulization and desolvation in the electrospray ionization source. Therefore, the use of HFBA allowed to obtain a longer total run time for best amine separation and the elution of other components present in the matrix that could co-elute with the analytes. The concentration of the ion-pairing reagent HFBA was studied as it is recommended to use a concentration as low as possible to avoid any signal suppression of the analytes. Some standard amine solutions were studied at four different HFBA concentrations (in the range from 1mM to 10 mM) of the mobile phase. Fig. 1 shows that increasing amounts of HFBA up to 5 mM result in an increase of the signal/noise ratios but at 10 mM the signal is strongly suppressed. For this reason, 5 mM was chosen as the optimal HFBA concentration for further experiments. The volume of sample injected in the column was also optimized. Fig. 2 shows the relative signal response and signal/noise ratio obtained for various injected volumes of a standard solution of tyramine in HFBA 5 mM. The best signal/noise ratio was obtained with a volume injected of 50 mL and this volume waschosen for further experiments.

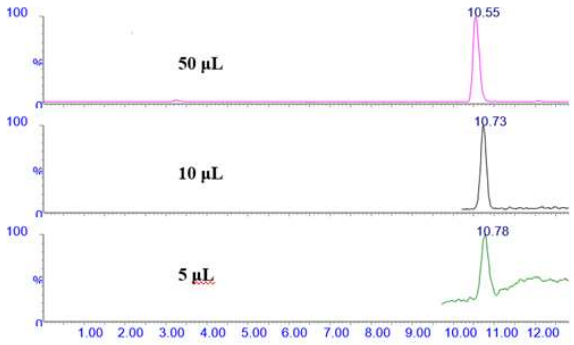


Figure 1. The increasing amounts of HFBA[29]

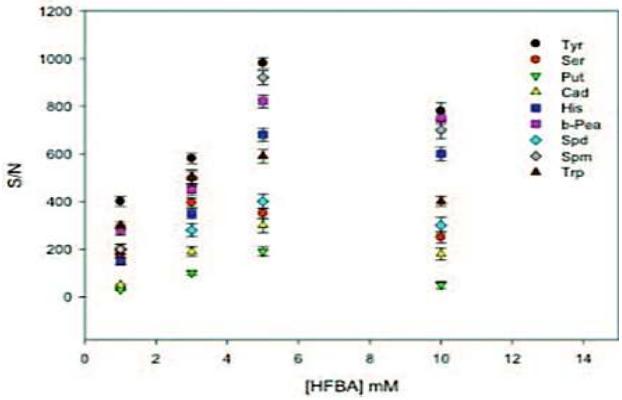


Figure 2. Signal response and signal/noise ratio obtained for various tyramine standard solution[29].

Performance characteristics of the LC-ESI-MS method

Linearity was tested using standard solutions of amines in acidified water (5 mM HFBA). Tab. 3 summarizes the results obtained. The response was linear in the range 0.1-16 µg L^{-1} and the correlation coefficients (R_2) were above 0.98, with the only exception of putrescine. The linearity "on line" (LIN) and the analytical sensitivity (AS) were calculated as reported above. The limits of detection were calculated according to the criterion of S/N=3, resulting in the range between 6.2 µg l^{-1} for tryptamine and 105.5 µg l^{-1} for putrescine.

Table 3. LC-MS method performances[29].

Biogenic Amine	R_t (min)	Conc. Range (mg l^{-1})	R^2	LIN %	AS (µg l^{-1})	LOD (µg l^{-1})
Tryptamine	15.6	0.1 - 16	0.999	98.99	23.1	42.8
β-Phenyethylamine	16.1	0.1 - 16	0.998	99.20	37.3	64.2
Putrescine	17.6	0.1 - 16	1.000	99.70	3.3	8.0
Cadaverine	18.8	0.1 - 16	0.999	99.25	8.2	17.1
Histamine	19.2	0.1 - 16	0.999	99.85	27.1	50.4
Serotonin	22.0	0.1 - 16	0.990	98.75	37.7	66.9
Tryramine	23.9	0.1 - 16	0.999	99.10	30.3	61.2
Spermidine	24.9	0.1 - 16	1.000	99.97	11.1	20.4
Sperimine	29.2	0.1 - 16	0.999	98.96	14.7	27.0

R^2: square of regression coefficient; LIN: linearity on-line; AS: analytical sensitivity; LOD: detection limit.

Determination of biogenic amines in wine samples

23 wine samples were analyzed using LC-ESI-MS method under the selected experimental conditions. Four replicates for each determination were performed. Fig. 3 shows the MS spectra for each of the nine amines studied. The complete results obtained with LC-ESI-MS for all wine samples studied are reported in Tab. 4. Each column refers to a specific biogenic amine. In the last column is reported the total amine amount, calculated for each wine sample.

By comparing the values reported in the last columns of Tab. 4 it can be also easily noted that the total amine concentrations are much higher in red wine samples 4, 8, 9, 12, 18, 22. The significant differences observed in the values reported for the wine samples can be explained bythe fact that the biogenic amine amount in wines is strongly dependent on different variables such as pH, wine aging and wine-growing area.Moreover, pH is the most important factor determining not only the biological activity of bacterial cluster in wine but also their variety and cultivar, as reported above. As for wine aging and production area, the literature reports that old wines contain significantly higher amounts of biogenic amines than young wines[12] and that in some producing areas biogenic amines are found in higher levels than in others[18]. However, as it is known in literature, red wines are generally less acidic than white ones andtherefore, biogenic amines are produced in high amounts[10]. The higher the pH, the more complex the bacterial clusters. An easier total growth and a greater bacterial diversity is observed in red wines which, therefore, show a composition of grape variety rich in amino acids and polyamines.

This is related in part to the type of winemaking and whether it involves the type of fermentation orvinification processes[17]. The most abundant amines determined with the LC-ESI-MS method resulted to be putrescine, histamine and tyramine (Tab. 5). In particular putrescine was found to be the highest value in wine samples (7.59 mg l^{-1}), followed by tyramine (6.75 mg l^{-1}) and histamine (6.01 mg l^{-1}). The correlation between putrescine, histamine and tyramine has already been noted by Martuscelliet al.[21], especially in red wines where these amines are present in greater quantities. This fact could be a consequence of malolactic fermentation which is required after alcoholic fermentation for nearly all red wines. The concentration of these amines is low after alcoholic fermentation and increases in most wines during malolactic fermentation to a very variable extent[17]. Spermine and spermidine were found in the lowest amount in red wine samples, 1.43 mg l^{-1} and 0.65 mg $l^{-1,}$ which is in accordance with the study of Liu et al.[28]. The accuracy of LC-ESI-MS methods was calculated by means of a spiking and recovery study onall red wines samples. The recovery was calculated as mean spiked concentration minus the mean original sample concentration divided by the spiked concentration. The spiked levels were 0.2mg l^{-1} and 1.0 mg l^{-1}. The LC-ESI-MS method resulted to be almost accurate with the following recovery values at 0.2 mg l^{-1}: 95.6% for tyramine; 104.2% for β-phenylethylamine; 99.9% for cadaverine; 103.3% for histamine; 96.0% for serotonin; 99.3% for tyramine; 101.0% for spermidine and 98.0% for spermine.

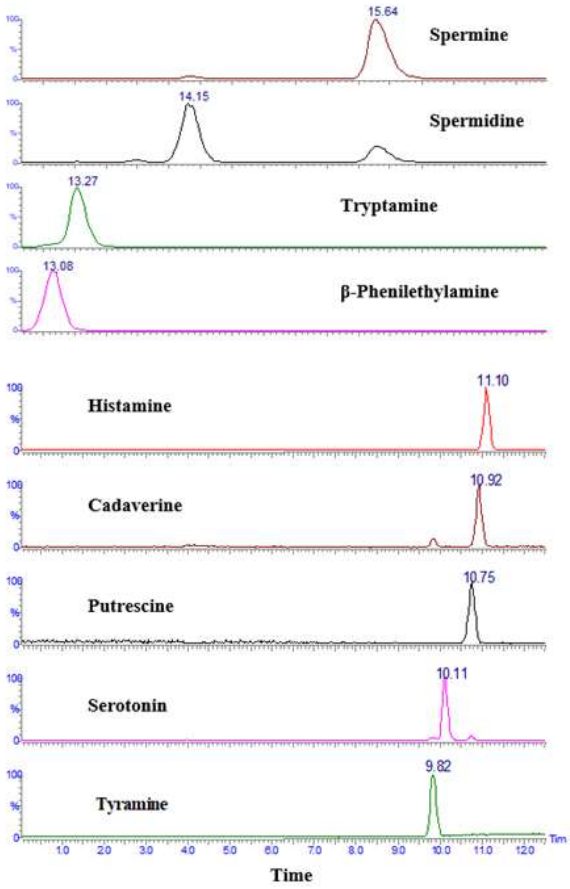


Figure 3. MS spectra of the nine biogenic amines

Table 5. Concentration range (mg l^{-1}) of the detected Biogenic Amines in wine samples, by LC-ESI-MS[29]

Biogenic Amine	Concentration Range (mg l^{-1})
Tryptamine	0.05 - 1.95
β-Phenylethylamine	0.15 - 4.05
Putrescine	0.26 – 7.59
Cadaverine	0.15 - 4.40
Histamine	0.44 - 6.01
Serotonin	0.25 - 3.55
Tyramine	0.15 - 6.75
Spermidine	0.18 - 0.65
Spermine	0.14 - 1.43

Whereas, the recovery values at 1.0 mg l^{-1} were:103.7% for tryptamine; 98.6% for β-phenylethylamine; 103.3% for cadaverine; 96.8% for histamine; 99.9 for serotonin; 97.6% for tyramine; 98.4% for spermidine and 103.1% for spermine, with theexception of putrescine whichshows lower recovery values rangingbetween 73.4%and 79.1%, respectively[29].

Table 4. LC-ESI-MS concentration (mg l^{-1}) and relative deviation standard (±RSD) of the nine biogenic amines in 23 Italian Red wines (Nd: not detectable)

Sample	*TRP*	*B-PEA*	*PUT*	*CAD*	*HIS*	*SER*	*TYR*	*SPD*	*SPM*	*Total Amines*
	Mean (±RSD)	*Mean(±RSD)*	*Mean(±RSD)*	*Mean(±RSD)*	*Mean (±RSD)*	*Mean(±RSD)*	*Mean(±RSD)*	*Mean(±RSD)*	*Mean(±RSD)*	
Wine 1	1.29±2.5	1.41±1.8	0.74±1.9	0.16±2.7	1.88±2.4	2.53±1.9	1.44±1.2	0.04±4.0	0.64±3.4	10,13
Wine 2	ND	0.31±1.8	ND	3.19±3.0	ND	0.96±3.0	2.01±2.2	0.38±2.4	1.53±1.8	8.22
Wine 3	1.31±2.4	3.05±1.8	ND	1.75±1.8	1.10±3.1	2.02±2.7	3.60±1.5	0.22±4.0	0.22±4.2	13,27
Wine 4	0.59±1.5	3.22±1.8	1.01±2.0	ND	6.21±1.4	1.02±1.8	6.85±1.4	0.75±1.9	0.33±2.9	19.28
Wine 5	0.99±2.0	0.23±2.6	0.38±3.0	3.88±1.5	3.55±2.6	0.26±1.4	0.95±2.1	ND	0.20±4.0	10,44
Wine 6	ND	ND	0.36±3.1	2.11±2.0	0.41±2.8	1.55±2.7	0.51±2.0	0.18±3.9	ND	5,12
Wine 7	0.07±2.5	0.23±2.3	ND	1.72±1.8	2.32±2.1	2.42±1.4	0.18±2.6	0.03±3.9	ND	6,97
Wine 8	2.05± 2.5	ND	0.29±3.5	2.23±1.9	1.82±3.0	2.49±2.5	1.33±1.3	0.70±2.3	0.28±1.5	11.17
Wine 9	2.04± 2.2	2.55±2.0	1.00±2.8	2.00±1.7	1.22±1.5	0.98±2.5	1.22±1.8	ND	0.33±3.1	11,34
Wine 10	ND	ND	0.33±3.2	2.15±1.9	0.45±2.7	1.71±2.5	0.42±2.1	0.25±1.6	ND	5.11
Wine 11	0.09± 3.0	1.68±1.9	1.61±2.0	ND	ND	1.89±1.9	2.1±1.5	0.50±2.7	ND	7,87
Wine 12	0.78± 2.7	2.80±2.3	0.57±2.0	4.41±3.7	2.94±2.8	ND	4.05±1.4	0.21±4.2	0.15±5.3	15.85
Wine 13	ND	ND	0.66±3.4	0.88±1.9	1.20±2.9	ND	1.98±1.8	ND	0.16±4.2	4,88
Wine 14	0.60±2.5	ND	0.77±2.9	3.22±2.4	0.66±3.1	1.56±1.7	0.99±2.0	0.33±2.5	0.16±3.8	8,29
Wine 15	0.06±2.5	0.22±2.9	ND	1.45±1.9	1.18±3.3	2.42±1.5	0.19±2.6	ND	0.22±2.9	5.22
Wine 16	0.55±2.7	2.00±1.8	1.45±1.9	1.65±2.0	1.00±2.4	1.68±1.9	0.99±2.3	0.33±2.3	0.44±2.6	10,09
Wine 17	ND	ND	0.66±2.3	1.66±2.1	ND	2.57±1.7	0.39±2.9	0.09±3.9	0.12±3.8	5,49
Wine 18	1.15±1.8	0.24±2.5	0.48±3.1	1.67±1.6	3.97±2.5	0.27±1.3	0.93±2.2	ND	ND	19.63
Wine 19	2.40±2.4	3.99±1.5	1.85±2.1	1.68±1.8	0.55±2.8	3.74±1.2	2.00±2.0	0.41±3.0	0.36±3.3	16,98
Wine 20	0.28±4.2	0.18±2.4	1.30±2.4	1.69±1.5	ND	3.25±3.7	2.93±3.3	0.31±2.2	0.36±2.3	10.97
Wine 21	0.34±3.0	ND	1.05±2.8	1.70±1.4	0.75±3.1	ND	2.05±2.1	ND	0.57±2.8	6,46
Wine 22	1.25±3.0	4.05±1.9	2.57±2.5	1.71±2.2	2.95±1.9	ND	1.88±2.4	0.05±3.1	ND	15.60
Wine 23	ND	2.73±2.0	0.87±2.9	1.72±2.1	3.66±1.6	1.53±1.5	3.84±1.5	0.27±3.0	0.68±2.9	15,3

Conclusion

The concentrations of nine biogenic amines found in red wines from different regions of Italywas were investigated using LC-ESI-MS. The method enables qualitative and quantitative detection phases to be carried out simultaneously with good performances. This approach offers a reliable and faster determination of underivatized biogenic amines,when compared with other chromatographic techniques (e.g. HPLC-UV/FID), that require a long pre- or post- column chemical derivatization.All the 23 red wine samples originated from different regions of Italy show the presence of biogenic amines, ranged between 4.88 and 19.63 mg l^{-1} and, in accordance with the concentration range (mg l^{-1}) of the detected Biogenic Amines in wine samples by LC-ESI-MS,putrescine, histamine and tyraminewere the most abundant BAs in red wine samples. This could be probably related to multiple factors such as non-adequate hygienic conditions during winemaking practices or storage, fermentation processes, wine ageing, and the oenological procedure. In addition, the presence of ethyl alcohol in wine could have a negative synergistic effect on the increasing amount of biogenic amines. Since there is no international legislation establishing maximum tolerability limits for the biogenic amines in wine, it is important to notice that a high content of BAs could be harmful for consumers' health. Therefore, a fast determination with reliable analytical methods, such as LC-ESI-MS, could be a useful tool to monitor food quality and safety parameters in wine.

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Conflicts of interest

The authors declare no conflict of interest.

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