Analytical Methodologies for the Determination of Biogenic Amines in Wines: An Overview of the Recent Trends

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Abstract
Biogenic amines are naturally present in grapes or can occur during the vinification and aging processes, essentially due to the microorganism’s activity. When present in wines in high amount, biogenic amines may cause not only organoleptic defects but also adverse effects in sensitive human individuals, namely due to the toxicity of histamine, tyramine and putrescine. Even though there are no legal limits for the concentration of biogenic amines in wines, some European countries only recommend maximum limits for histamine. In this sense, biogenic amines in wines have been widely studied. The determination of amines in wines is commonly achieved by liquid chromatography, using derivatization reagents in order to promote its separation and detection. In alternative, other promising methodologies have been developed using capillary electrophoresis or biosensors, revealing lower costs and faster results, without needing a derivatization step. Nowadays, it is still a challenge to develop faster and inexpensive techniques or methodologies to apply in the wine industry. Thus, this review will be focused on the studies published in the last decade that involves the determination of biogenic amines in wines, highlighting the novelty, improvement and optimization of the analytical methods. The sample preparation procedures (such as derivatization reagents), the analytical methodologies and the new trends being followed by the wine industry are also described and discussed.

Introduction
Biogenic Amines (BAs) are nitrogenous low molecular weight compounds, naturally synthesized in plants, animals and microorganisms, with an important role on physiological functions, such as the regulation of body temperature and the secretion of gastric acid[1,2]. These compounds are essentially formed by decarboxylation of free amino acids by the activity of yeasts or bacteria, through substrate-specific enzymes, in a wide variety of protein-rich foods[1,3]. Also, other enzymatic reactions such as transamination, reductive amination and degradation of some precursors of amino compounds can also produce BAs. The knowledge about BAs in food and beverage’s gaining interest in the last years, not only to ensure the quality of the products, since that they have been used as an indicator of spoilage, but mostly to guarantee the consumer protection, due to the potential toxic effect of BAs to humans[2].

Wine is one of the most consumed fermented beverages in the world. Due to quality control and the growing consumers protection demands, the presence of BAs in wines has been largely studied[4]. Among the 25 different amines described in wines, the main ones are histamine, putrescine, tyramine and cadaverine[5] (Figure 1). Others are phenyl ethylamine, spermine, spermidine, agmatine and tryptamine. The presence of BAs in wines is frequent because these compounds can be naturally present in grapes and, therefore, its concentration depends on the grape variety, grape-growing conditions (climate, soil type, soil composition and fertilization, degree of maturation, etc.) and enological practices (skin maceration, SO₂ concentration, vinification conditions, amino acid levels, clarification treatments, microorganism strains and aging processes)[6-11]. However, some BAs, such as putrescine and cadaverine, are considered indicators of poor sanitary conditions of grapes or lack of hygiene during winemaking[6,12].
in susceptible individuals due to the presence of ethanol, which inhibit or reduce the activity of the enzymes responsible for the metabolism of BAs (monoamine and diamine oxidase)\[27\]. Thus, even though legal limits for BAs in wines are not yet established, some European countries have recommended maximum limits for histamine in wines, namely Germany (2 mg/L), France (8 mg/L) and Belgium (6 mg/L)\[19\]. Switzerland was the only country that had established an official upper limit of 10 mg/L, but in 2011 it was removed for imported wines\[19\].

The consumer safety, the wine quality control and the applied legislation are the main priorities for the wine industry. In order to facilitate the international trade, the International Organization of Vine and Wine (OIV) standardized the analytical methods and published two chromatographic methods for the determination of BAs\[20\]. However, even today, it is still a challenge the development of a simple, fast, low cost and reliable method for the determination of BAs in wines. In this sense, this review provides an overview of the recent advances observed in the analytical methodologies used for the determination of BAs in wines in the last decade, focusing the improvements, the optimization and the novelities in this field.

### Analytical methodologies

The determination of BAs in wines has been a challenge for the scientific community due to their low concentration, complex matrices, the lack of chromophores, strong polarity or even the presence of structurally similar compounds\[21\]. Typically, the determination of BAs relies on liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE). The detection is usually performed by diode array detection in the ultraviolet–visible region (DAD), fluorescence (FLD) and mass spectrometry (MS)\[22-30\]. Additionally, Sensors, Enzymatic and Immunoassays procedures as well as commercial test kits are already available, for the rapid detection of histamine and tyramine in wines\[31\].

### Table 1: Chromatographic methodologies for the determination of biogenic amines in wine published in the last decade.

<table>
<thead>
<tr>
<th>Chromatographic technique</th>
<th>Sample pretreatment</th>
<th>Derivatization reagent</th>
<th>Matrix</th>
<th>Amines</th>
<th>Detection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP-HPLC</td>
<td>PVPP</td>
<td>DNS-CI</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad, Phe, Spm, Spmd</td>
<td>FLD</td>
<td>[17]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>PVPP</td>
<td>DNS-CI</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad, Phe, Spm, Spmd</td>
<td>UV-Vis</td>
<td>[18]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>PVPP</td>
<td>RTIL - DNS-CI</td>
<td>Wine</td>
<td>Tyr, Put, Cad, Try, Phe, Spm</td>
<td>FLD</td>
<td>[19]</td>
</tr>
<tr>
<td>TLC</td>
<td>PVPP</td>
<td>DNS-CI</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad</td>
<td>Densitometry</td>
<td>[30]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>-</td>
<td>OPA</td>
<td>Wine and honey</td>
<td>His, Tyr, Cad, Try, Phe, Isop</td>
<td>FLD</td>
<td>[21]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>-</td>
<td>OPA</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad, Met, Try, Eth, Ety</td>
<td>FLD</td>
<td>[22]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>N2 liquid and TCA (10%)</td>
<td>BzCl</td>
<td>Wine, salami, cheese, tuna, chocolate and yogurt</td>
<td>His, Tyr, Put, Cad, Try, Spm, Spmd, Phe, Eth, Mpa, 3-Mba, 2-Mba, 3-Mpa</td>
<td>MS/MS</td>
<td>[23]</td>
</tr>
<tr>
<td>RP-HPLC (core-shellcolumn)</td>
<td>-</td>
<td>AQC</td>
<td>Wine</td>
<td>His, tyr, Put, Cad</td>
<td>FLD</td>
<td>[24]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>-</td>
<td>DEEMM</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad, Phe, Spm, Spmd, Agm, Eth, Ser</td>
<td>UV-Vis</td>
<td>[25]</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>-</td>
<td>FNB-T</td>
<td>Wine</td>
<td>His, Tyr, Try, Phe</td>
<td>UV-Vis</td>
<td>[26]</td>
</tr>
<tr>
<td>IEC</td>
<td>PVPP</td>
<td>-</td>
<td>Wine, salami, cheese, tuna, anchovies and olives</td>
<td>His, Tyr, Put, Cad, Agm, Spm, Spmd, Tea, Tma</td>
<td>CD</td>
<td>[27]</td>
</tr>
<tr>
<td>GC</td>
<td>Centrifugation</td>
<td>IBCF</td>
<td>Wine and grape juice</td>
<td>22 BAs</td>
<td>MS</td>
<td>[28]</td>
</tr>
</tbody>
</table>

**Technique:** RP-HPLC: Reversed-Phase High Performance Liquid Chromatography; TLC: Thin-Layer Chromatography; IEC: Ion-Exchange Chromatography; GC: Gas Chromatography.

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**Figure 1:** Chemical structure of the main biogenic amines found in wines.

The BAs found in grapes and musts are usually present in low concentrations, since they are produced and then degraded during winemaking. The fermentative stages (alcoholic and mostly malolactic) and the aging in barrels are the main winemaking processes that mostly contribute for their occurrence in wines. There are only some bacteria or yeast strains, carrying a specific metabolic pathway, that succeed to convert amino acid precursors into BAs and for that reason wines may contain different amounts of amines\[13\]. The BAs levels usually depend on the type of wine. For instance, red wines present higher levels of BAs than white wines, reaching levels up to 130 mg/L in Spanish red wines\[14\]. This fact is mainly due to the maceration process with grape skins, usually performed in red wine vinification, which promotes the increase of amino acids and elevates the fermentation temperature. On the contrary, generally, the malolactic fermentation is not applied or has a short duration in the white wine production\[15,16\].

High amounts of BAs in wine may cause organoleptic and toxicological effects. Higher levels of putrescine and cadaverine can promote a negative flavor in wines, associated with specific metabolic pathways. The fermentative stages (alcoholic and mostly malolactic) and the aging in barrels are the main wine-making processes that mostly contribute for their occurrence in wines. There are only some bacteria or yeast strains, carrying a specific metabolic pathway, that succeed to convert amino acid precursors into BAs and for that reason wines may contain different amounts of amines\[13\]. The BAs levels usually depend on the type of wine. For instance, red wines present higher levels of BAs than white wines, reaching levels up to 130 mg/L in Spanish red wines\[14\]. This fact is mainly due to the maceration process with grape skins, usually performed in red wine vinification, which promotes the increase of amino acids and elevates the fermentation temperature. On the contrary, generally, the malolactic fermentation is not applied or has a short duration in the white wine production\[10,13\].

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Determination of Biogenic Amines in Wines


**Detection:** UV-Vis: Ultraviolet–Visible Spectroscopy; FLD: Fluorescence Detector; MS: Mass Spectrometry; CD: Conductimetric Detector.

**Amines:** His: Histamine; Tyr: Tyramine; Put: Putrescine; Cad: Cadaverine; Met: Methionine; Agm: Agmatine; Phe: Phenylethylamine; Spm: Spermine; Spmd: Spermidine; Try: Tryptamine; Ety: Ethylamine; Ser: Serotonin; Eth: Ethanolamine; Met: Methylamine; Agm: Agmatine; Phe: Phenylethylamine; Arb: Arbutamine; Spm: Spermine; Spmd: Spermidine; Try: Tryptamine; Ety: Ethylamine; Tma: Trimethylamine.

**Chromatographic methods**

In wines, the most commonly employed technology for the determination of BAs is the LC with Reversed-Phase (RP) separation, using C18 columns. Table 1 summarizes the chromatographic methods used to quantify BAs in wine, in the last decade. Typically the wine samples are analyzed directly or after a simple treatment with Polyvinylpolypryrolidone (PVPP) to remove some phenolic compounds[17-32]. Nowadays, most methodologies usually involve sample preparation prior to analysis, not only to remove some compounds that may interfere with the analysis but also to concentrate the analytes. Also, pre-column or post-column derivatization is often needed for appropriate detection, since BAs do not have enough absorption in the UV-Vis or FLD wavelength ranges. The derivatization step is also used to improve the separation in the RP columns, reducing the polarity of the original compounds[17,26,28,29,32,36]. Several derivatization reagents have been reported[37] however, dansyl chloride (DNS-Cl) has been commonly used in the last years, since that its derivatives can be detected using DAD, FLD and MS[39].

The determination of dansylated amines in wines has been the target of several studies[37,38]. The derivatization and chromatographic conditions have been optimized using the central composite designs, with BAs being detected by fluorescence[32] or UV-Vis[17]. FLD revealed better sensitivity to detect dansylated amines[17,32]. DNS-Cl has been largely used as pre-column derivatizing agent in the determination of BAs in wines and it produces stable derivatized compounds. However, the dansylation reaction is a time consuming process that requires the application of external temperature: 10 to 60 min at 40 to 70 °C[38,39]. Jiang, et al.[35] introduced a new method to perform the dansylation at room temperature, during 20 min, using ionic liquids as media for the derivatization of BAs in wines. A low-cost method based on thin layer chromatography using densitometry to quantify the dansylated BAs was also developed[40] and it can be used for routine analysis of histamine, tyramine, putrescine and cadaverine in wine.

**Ortho-Phthalaldehyde (OPA)** is another recognized reagent for the derivatization of biogenic amines. OPA is one of the most used derivatization reagent and for that reason several studies report its use for the determination of BAs in wines. OPA derivatives are less stable but the reaction can occur at room temperature in a short time[41-43]. Pereira, et al.[29] proposed a methodology using a pre-column derivatization with OPA, performed in 3 min into the sample injection loop of the HPLC-FLD system, for the simultaneous determination of BAs and amino acids in wine, without using any preliminary separation or clean-up. The OIV also propose OPA as derivatization reagent for the BAs evaluation in musts and wines using HPLC-FLD[20]. In alternative, they also propose a methodology that uses Diethyl ethoxymethylenelemalonate (DEEMM) to derivatize the BAs present in wine when it is used an HPLC-DAD system[20]. Also, Wang, et al.[44] reported the development of a pre-column derivatization method with DEEMM for the quantification of 10 BAs and 23 amino acids, using RP-HPLC-DAD, shortening the analysis time for 30 min. This pre-column derivatizing agent enables the quantitative determination of secondary and primary amines, with no side-reaction products and produces stable derivatives at room temperature for several days, which can be detected by an UV detector, usually available in most chromatography laboratories[45]. Recently, a new derivatization reagent, namely 1-fluoro-2-nitro-4-(trifluoromethyl)benzene (FNBT), was also used for the determination of histamine, tyramine, tryptamine and phenyl ethylamine in wines using RP-HPLC-DAD analysis, showing simple and less-time consuming derivatization when compared to other methods[21].

On the other hand, the combination of a core-shell C18 column with 6-Aminoquinolinyl-N-Hydroxysuccinimidyl Carba-mate (AQC) derivatization reagent, using FLD detection, was studied by Berbegal, et al.[33]. They reported that this combination enables the analysis of BAs in wine reducing the run time and the use of organic solvents. Stable isotope dilution assays, with 10 isotopically labeled BAs as internal standards, have also been used for the quantification of BAs in several food matrices, including wine, with benzyl chloride (BzCl) as derivatization agent using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS)[45]. Ion-exchange chromatography combined with a conductometric detector (IEC-CD) was successfully applied to determine BAs in wines. The LODs was not lower than those obtained by RP separation, however this method has the advantage that BAs do not need derivatization to be separated and detected[46].

Finally, a gas chromatography–mass spectrometry (GC-MS) method was also used for the quantification of volatile and nonvolatile biogenic amines in Port wines, using Isobutyl Chloroformate (IBCF) as derivatization agent. Even though the method implies a complex procedure, it provides an accurate identification and enables the quantification of a higher number of BAs compared to the typical LC methods[24].

**Electrophoretic methods**

Capillary electrophoresis (CE) is also used as a separation technique for the determination of BAs in wine (Table 2), with the advantage of being rapid and effective, with low reagent consumption. Automated on-line combination of capillary isotachophoresis–capillary zone electrophoresis (cITP–CZE) with UV detection was successfully used for determination of selected BAs in wines, without a derivatization step[47]. The authors concluded that this method can be more sensitive for the determination of BAs in wines when comparing to other chromatographic and electrophoretic methods, due to the online pre-concentration of selected analytes in the cITP step, the high separation efficiency of the CZE and the fact that BAs can be detected by selective photometric detection, without needing derivatization. Another Isotachophoresis (cITP) method was
proposed by Jastrzebska, et al.[49]. After a simple sample preparation, without needing derivatization, they determined a group of important BAs in wines, in a 14-min run. The CITP method revealed to be an attractive choice comparatively to an HPLC method based on a pre-column derivatization with DNS-Cl.

### Table 2: Electrophoretic methodologies for the determination of biogenic amines in wines published in the last decade.

<table>
<thead>
<tr>
<th>Electrophoretic technique</th>
<th>Sample pretreatment</th>
<th>Derivatization reagent</th>
<th>Matrix</th>
<th>Amines</th>
<th>Detection</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CITP</td>
<td>-</td>
<td>-</td>
<td>Wine and Beer</td>
<td>His, Tyr, Put, Cad, Phe, Try, Spm, Spmd</td>
<td>CD</td>
<td>[48]</td>
</tr>
<tr>
<td>CITP/CZE</td>
<td>-</td>
<td>-</td>
<td>Wine</td>
<td>His, Tyr, Phe</td>
<td>UV-Vis</td>
<td>[47]</td>
</tr>
<tr>
<td>CE</td>
<td>PVPP</td>
<td>FITC</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad, Spm, Spmd, Try, Phe, Uro</td>
<td>MS/MS</td>
<td>[25]</td>
</tr>
<tr>
<td>MECK</td>
<td>-</td>
<td>FITC</td>
<td>Wine</td>
<td>His, Tyr, Put, Cad, Spmd, Try, Phe</td>
<td>LIF</td>
<td>[57]</td>
</tr>
</tbody>
</table>

**Technique:** CE: Capillary Electrophoresis; CITP: Capillary Isotachophoresis; CZE: Capillary Zone Electrophoresis; MECK: Nonionic Micellar Electrokineic chromatography.

**Chemicals:** PVPP: Polyvinylpyrrolidone.

**Derivatization agent:** FITC: Fluorescein IsothioCyanate.

**Detection:** CD: Conductimetric Detector; UV-Vis: Ultraviolet–Visible Spectroscopy; MS/MS: Tandem Mass Spectrometry; LIF: Laser-Induced Fluorescence.

**Amines:** Hist: Histamine; Tyr: Tyramine; Put: Putrescine; Cad: Cadaverine; Phe: Phenylethylamine; Spm: Spermine; Spmd: Spermidine; Try: Tryptamine; Uro: Urocanic acid.

Capillary electrophoresis–tandem mass spectrometry (CE–MS/MS) was also applied for the eco-friendly, simple and fast determination of BAs in wines[23]. The electrophoretic separation took just 10 min for the migration of 9 BAs and the wine samples were just submitted to a PVPP clean-up and filtrated prior to analysis.

Finally, nonionic micellar electro kinetic chromatography with laser-induced fluorescence detection (MEK-C/LIF) was used for the fast quantification (less than 9 min) of 7 BAs in wine samples[49]. The MEK–LIF method used Fluorescein Isothiocynate (FITC) as derivatization reagent.

### Other methodologies

In alternative to chromatography and electrophoresis, other techniques have been used for the BAs determination in wines, namely sensors and flow-injection analysis.

The development of biosensors has recently gained much interest among the scientific community because these low-cost devices can give results in a few minutes, without needing any kind of sample pre-treatment and the possibility to be used outside the laboratory. BAs biosensors, resulting from the combination of different enzymes for the bio-recognition of the BAs, are then a good option for a rapid determination of these compounds in wine[50,51]. The most used signal transducers of these biosensors are electrochemical sensors, which are generally based on the fluorescence response between BAs and sensor molecules interactions. Henao-Escobar, et al.[52] produced a dual enzymatic sensor for the simultaneous determination of histamine and putrescine by measuring the oxidation current and Di Fusco, et al.[51] characterized and evaluated the use of a diamine oxidase (DAO) from Lathyrus Sativus, as a biocatalytic component of an electrochemical biosensor, for the determination of the total amount of BAs in wines. On the other hand, Basozabal, et al.[53] developed a sensor based on molecular imprinted nanoparticles with high affinity for histamine. Table 3 summarizes the use of biosensors for the determination of BAs in wines.

### Table 3: Recent studies using biosensors for the determination of biogenic amines in wines.

<table>
<thead>
<tr>
<th>Type of biosensor</th>
<th>Amines</th>
<th>Sample pre-treatment</th>
<th>Recognition element</th>
<th>LOD</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potentiometric sensor</td>
<td>Hist</td>
<td>pH 5 (NaOH) and then diluted (1:5 or 1:10) in Ac buffer 10 mM (pH 5).</td>
<td>Molecularly imprinted nanoparticles</td>
<td>1.12 µM</td>
<td>[53]</td>
</tr>
<tr>
<td>Electrochemical sensor (amperometry)</td>
<td>Hist, Put</td>
<td>Activated carbon</td>
<td>Histamine dehydrogenase and putrescine oxidase enzymes</td>
<td>8.1 for Hist and 10 µM for Put.</td>
<td>[52]</td>
</tr>
<tr>
<td>Electrochemical sensor (amperometry)</td>
<td>Total BAs</td>
<td>0.1 M phosphate and adjusted the pH for 7.4 with NaOH</td>
<td>Diamine oxidase from L. sativus</td>
<td>200 µg/L</td>
<td>[51]</td>
</tr>
</tbody>
</table>

Hist: Histamine; Put: Putrescine; BAs: Biogenic amines

Although the enzyme-linked immnosorbent assay (ELISA) presents high sensitivity and precision, in the last decade, no studies were found in literature reporting this procedure for determining BAs in wines. Indeed, this methodology is time-consuming and may be expensive for a small number of samples[54]. The last report was made by Marcobal, et al.[55], in 2005, which consisted in the development of a direct ELISA immunoassay method, specific for the determination of histamine in wines.

Finally, a flow injection method for the rapid determination of histamine in wine was reported by Hernández-Cassou, et al.[56]. This method was based on the reaction of histamine with 1,2-naphthoquinone-4-sulfonate to form a derivative that can be detected by a UV-Vis spectrophotometer[57]. The procedure is simple and can be automatized and miniaturized.

**Conclusion**

In the last decade, several methods for the determination of BAs in wines have been developed, in order to improve the precision and sensitivity. Most procedures use liquid chromatography and DNS-Cl as derivatizing reagent. However, oth-
er reagents have been studied and are showing very promising results, regarding the time-consuming for sample preparation, analysis and also lower solvent consumption. The new trend for the determination of BAs in wines will go through portable, faster and cheaper methodologies to meet the demands of the wine industry. Thus, the development of biosensors has been gaining interest. Similarly, CE also demonstrates lower costs and faster results, without using the derivatization step.

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References
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