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Dynamic of biogenic amines and precursor amino acids during cabernet sauvignon vinification

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ABSTRACT

The present study reports for the first time the dynamic of biogenic amines (BA) and precursor free amino acids (FAA) during Chilean Cabernet Sauvignon vinification applying a multi-matrix chromatographic method. BA (phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermine, spermidine) and their precursor FAA (phenylalanine, ornithine, lysine, histidine, and tyrosine) were dansylated and quantified using liquid chromatography with fluorescence detection. Contrarily to previous studies, from the five wineries analyzed, only one showed the highest BA formation during malolactic fermentation ($9.2 \pm 0.1\ mg\ L^{-1}$). The other wineries showed the highest formation during alcoholic fermentation ($n = 1$; $40.2 \pm 0.3\ mg\ L^{-1}$), in bottled wine ($n = 2$; 38.3 ± 0.0 and $168.6 \pm 0.2\ mg\ L^{-1}$), or without significant differences along vinification ($< 16\ mg\ L^{-1}$). In all wineries a tendency between FAA decrease and BA increase was observed. Different BA and FAA profiles were observed with putrescine and lysine as the most prevalent amines. Toxicologically important amines, histamine and tyramine, were found in low concentrations ($< 3.5\ mg\ L^{-1}$), except in one bottled wine that showed values of $8.4\ mg\ L^{-1}$ and $8.1\ mg\ L^{-1}$, respectively. It can be concluded that BA formation during Cabernet Sauvignon elaboration was moderately related with FAA content but without relation with FAA profile or a specific vinification stage.

1. Introduction

BA are organic nitrogenous compounds with essential physiological activities. These compounds are naturally produced in nature; in foods and feeds their presence is commonly associated with food deterioration and contamination as well as with food safety problems (EFSA, 2011). The intake of foods with high BA content could cause several adverse effects such as hypertension, migraines, kidney failure, anaphylactic shock and death (Anli & Bayram, 2009; Shalaby, 1996). Thus, BA has become a worldwide concern and several efforts have been made to reduce their prevalence in fermented food products (European Community, 2011). BA are widely present in foods (Bedia Erim, 2013; Pradenas, Galarce-Bustos, Henríquez-Aedo, Mundaca-Uribe, & Aranda, 2016; Spano et al., 2010). In wines the principal BA reported are putrescine, cadaverine, histamine, tyramine, spermine and spermidine. These BA come from two main sources, grapes (considered natural) and vinification process (associated with microbial activity). The main BA found in grapes are spermine, spermidine and putrescine (Bover-Cid, Iquierdo-Pulido, Marine-Font, & Vidal-Carou, 2006) and the ones associated with microbial activity are histamine, cadaverine, tyramine and phenylethylamine, which are mainly produced by decarboxylation of FAA during alcoholic (AF) and malolactic (MLF) fermentations. Even when BA are produced in both processes, the highest level is formed during MLF as a result of lactic acid bacteria enzymatic activity (Henríquez-Aedo, Durán, Garcia, Hengst, & Aranda, 2016; Lerm, Engelbrecht, & du Toit, 2010). The presence of BA has been extensively studied in diverse type of wines from different grape varieties and countries. The reported levels show a wide range from no detection up

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to 130 mg L^{-1} (Ancin-Azpilicueta, Gonzalez-Marco, & Jimenez-Moreno, 2008). Our research group evaluated the BA content in Chilean young and reserve varietal wines finding concentrations from 2.2 to 65.1 mg L^{-1}, where putrescine, histamine, tyramine and spermidine were the most abundant BA (Henríquez-Aedo, Vega, Prieto-Rodríguez, & Aranda, 2012; Pineda, Carrasco, Pena-Farfal, Henriquez-Aedo, & Aranda, 2012). These levels are relevant for a winemaking country like Chile, which in 2016 ranked within the ten principal worldwide wine producers and 4th of largest wine exporter (Organisation Internationale de la Vigne et du Vin (OIV), 2017). In order to decrease the BA levels found in Chilean wines, particularly in Chilean ensign wine, Cabernet Sauvignon, it is necessary to identify the principal sources or stages of BA formation/contribution during vinification. Establishing the BA dynamics, it will be possible to carry out corrective actions to reduce or prevent their presence, developing or modifying some enological and/or technological practices. To determine the contribution of grapes and each vinification step (AF, MLF and wine) to BA formation, it is necessary to evaluate BA content and precursors FAA during the entire vinification process. For that a multi-matrix chromatographic method was developed and validated. At the beginning of vinification FAA content shows a great variability because it depends on several factors such as grape variety and maturity, soil type, fertility, crop area, etc. (Ancin-Azpilicueta et al., 2008), then, along the process, FAA shows variable levels due to three main reasons: i) they are the main source of yeast assimilable nitrogen, ii) yeasts activity could increase FAA content through enzymatic protein degradation and iii) BA formation from FAA via enzymatic decarboxylation decreases its content (Ancin-Azpilicueta et al., 2008; R. M.; Callejon, Troncoso, & Morales, 2010). The simultaneously evaluation of both group of compounds has been reported in just a couple of articles (Martinez-Pinilla, Guadalupe, Hernandez, & Ayestaran, 2013; Wang, Ye, Zhu, Wu, & Duan, 2014). Conversely, many research groups have studied BA content in wines (Henríquez-Aedo et al., 2012; Izquierdo-Cañas, García Romero, Gómez Alonso, & Palop Herreros, 2008; Mazzucco et al., 2010; Pineda et al., 2012) but only a few have reported its presence in grapes and musts (Wang et al., 2014). To the best of our knowledge, the present work reports for the first time the dynamic of BA and precursor FAA during Chilean Cabernet Sauvignon vinification.

2. Materials and methods

2.1. Samples

Samples (grapes, AF, MLF and wines) from Cabernet Sauvignon vinification were collected in five wineries (A-E) located in three Chilean valleys: Itata (36°46′48.0″S and 72°13′0.05″W), Curicó (35°05′54.5″S and 71°18′37.0″W) and Limarí (30°34′29.1″S and 71°24.45′45.4″W). After harvest in March, each winery applied particular practices to carry out vinification process. The main and common activities can be described as follow: AF was carried out using freeze-dried yeasts in plastics bins (wineries A and B) or stainless-steel tanks (wineries C, D and E) at 22–25 °C for 7–21 days. Spontaneous MLF (without commercial starter) was performed immediately after AF in oak barrels (wineries A, B, D and E) or stainless-steel tanks (winery C) at 18–22 °C for 30–40 days. Samples of 1–3 kg of grapes were obtained before crushing. All liquid samples were randomly sampled in the cellar, 100 mL of different barrels (or tanks) were pooled to obtain 1 L of composed samples of each winery. Samples were stored at −20 °C until be processed.

2.2. Reagents and chemicals

L-phenylalanine (Phal ≥98%%), L-ornithine monohydrochloride (Orn ≥99%), L-lysine monohydrochloride (Lys, ≥98%), L-histidine monohydrochloride monohydrate (His ≥98%), L-tyrosine (Tyro ≥98%), putrescine dihydrochloride (Put ≥98%), cadaverine dihydrochloride (Cad ≥98%), 2-phenylethylamine hydrochloride (2-Phe ≥98%), spermidine trihydrocloride (Spmd ≥98%), histamine dihydrochloride (Him ≥99%), spermine tetrahydrochloride (Spm ≥98%), tyramine hydrochloride (Tyr ≥98%), 1,7- diaminoheptane (Dah ≥98%) used as internal standard (IS), polyvinylpolypyrrolidone (PVPP) and dansyl chloride (Dns-Cl ≥99%) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade), acetone, ammonia solution (250 mL L^{-1}), formic acid (98–100%), anhydrous sodium carbonate (> 99.5%) and sodium hydrogen bicarbonate (bicarbonate > 99%) were obtained from Merck (Darmstadt, Germany). Ultra-pure water (18 MΩ cm) was produced with Simplicity system from Millipore (Bedford, MA, USA). Filter paper N°4 and polyvinylidene difluoride (PVDF) 13 mm syringe filters (0.22 μm) were obtained from Whatman (Clifton, NJ, USA).

2.3. Standard solutions

Individual BA and FAA stock solutions were prepared in ultrapure water for a given concentration of 0.5 mg mL^{-1}. Pooled standard solutions were prepared mixing aliquots of each BA and FAA stock solutions in ultrapure water. All solutions stored at 4 °C were stable for at least 30 days. 1,7-Diaminoheptane (internal standard) stock solution of 0.4 g L^{-1} was also prepared in ultrapure water. Sodium carbonate-bicarbonate buffer, pH 10, was prepared weekly in ultrapure water. Dansyl chloride solution was prepared in acetone at a concentration of 10 mg mL^{-1} just prior to use.

2.4. Sample preparation

Sample preparation was carried out following the method proposed by (Pineda et al., 2012), with slight modifications. Briefly, 50 μL (20 μg) of internal standard and 0.5 g of PVPP were added to 10 mL of filtered (filter N°4) sample (grape juice, AF, MLF and wine). The mixture was shaken in a Boeco (Staufen, Germany) OS-20 orbital shaker for 15 min at 180 rpm and then filtered through filter paper N°4. One hundred microliter of filtrate were dansylated adding 300 μL of acetone, 400 μL of carbonate-bicarbonate buffer, pH 10, and 200 μL of Dns-Cl solution (10 mg mL^{-1}). The reaction mixture was vortex-mixed for 30 s and then incubated for 60 min at 47 °C. After this time, 100 μL ammonia (250 mL L^{-1}) were added to stop the reaction and after 30 min of storage in the dark, the sample was filtered through a 13 mm PVDF syringe filter (0.22 μm) and injected into HPLC system.

2.5. Chromatography

Simultaneous separation of AB and FAA (Fig. 1 & Fig. S1-S2 Supplementary material) was performed using Waters (Milford, MA, USA) HPLC equipment consisted of 600 controlled binary pump, 717 plus autosampler, 2475 multi-λ fluorescence detector, 5CH column oven and a VWR international (West Chester, Pennsylvania) L-7614 online degasser. Data were acquired and recorded by means of Empower 2 software from Waters. Chromatography was carried out on Merck Hibar Purospher C_{18} column (250 mm × 4.6 mm, 5 μm) connected to Waters C_{18} Symmetry (4.0 × 20 mm, 5 μm), both set at 45 °C using a binary mobile phase composed of acetonitrile (A) and 0.01 mol L^{-1} ammonium formate pH 7 (B). The following gradient was applied at a flow rate of 0.8 mL min^{-1}: 0–12 min 35–55% A, 12–16 min 55–70% A, 16–18 min 70-70% A (isocratic step), 18–25 min 70–80% A, 25–27 min 80–100% A, 27–35 min 100%–100% A (isocratic step), 35–37 min 100%–35% A, 37–45 min 35%A (column conditioning). Detection was performed by fluorescence using 330 nm and 520 nm as excitation and emission wavelengths, respectively.

2.6. Statistical analysis

Data were evaluated using descriptive statistics [mean, standard