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Establishment of influence the nitrogen content in musts and volatile profile of white wines associated to chemometric tools

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ABSTRACT

Many of the volatile compounds synthesized by yeast also change in response to the nitrogen concentration. It has been suggested that nitrogen management of wine fermentation can be an effective tool to modulate wine aroma composition and style. The aim of this study was to investigate the influence of clarification time on the must of the white grapes, and to establish a practical understanding of the effect of the nitrogen content (amino acids and ammonium) on the must in relation to volatile profile of the wine. The musts were clarified by applying two settling time: 12 h and 30 h, and submitted to the same microvinification procedure. The results showed that the must clarification time, influenced significantly the nitrogen compounds content, where the total amino acid content was higher in musts clarified for a shorter time. Moreover, the aromatic profile of these experimental wines was clearly influenced by the must clarification time. Principal components analysis showed a strong influence of the must clarification time on the separation of the wines. The results confirm that the management of the nitrogen content of the musts can be used as a tool to obtain wines with different aromas.

1. Introduction

Aroma is one of the most important factors determining the character and quality of a wine. It is known that wine aroma is strongly influenced by several parameters including grape variety, viticultural practices, climatic conditions, alcoholic fermentation and wine aging. The pre-fermentation steps have a strong influence on the quality and stability of a wine, particularly in the case of white wines, and they also greatly affect the must clarification process. Spontaneous settling at low temperature is the standard approach to removing the solid matter from white grape juice prior to fermentation. The favorable effect of clarification on wine quality is evidenced by the superior organoleptic characteristics of wines produced from clarified musts, particularly with regard to aroma. Furthermore, wines made from clarified musts have the advantage of being more stable and, importantly, more resistant to oxidation [1]. In contrast to the advantages afforded by clarification, problems associated with the excessive clarification of grape musts include slow or stuck fermentation [2], which results in wine with no varietal aroma, since this can be masked by the products of fermentation. On the other hand, excessive turbidity in the must leads to wine with an herbaceous aroma and has a tendency to reduce the varietal aromas [3].

The turbidity of the must obtained after the clarification step is manly related to the nitrogen content in the must. Nitrogen is present as both inorganic (ammonium) and organic (protein and, mainly, amino acid) forms, which are the two main sources of yeast assimilable nitrogen [4]. It has even been shown that the nature of the varietal aroma of certain cultivars can be partially explained by the amino acid composition of the grape must. During the early stages of fermentation, these compounds are rapidly accumulated by the yeast, thus fulfilling the biosynthetic requirements for the amino acids needed for protein synthesis and yeast growth [5]. The effect of must nitrogen content on the formation of yeast derived volatile compounds is complex and dependent on the type and concentration of nitrogen present as well as the class of volatile compounds. Moreover, the nitrogen composition of the grape may affect the kinetics of alcoholic fermentation [6,7].

Many of the volatile compounds synthesized by yeast also change in response to the nitrogen source and/or nitrogen concentration. The most important compounds include higher alcohols and esters, which are mostly associated with solvent/fusel odors and fruity/floral aromas, respectively. These compounds contribute to the fermentation *bouquet* of wine and are correlated with the concentration of amino acids in the must [8]. The catabolism of amino acids to yield the ketoacids and their corresponding alcohols and ester are an example of the important relation between nitrogen metabolism and these groups of volatile compounds.

Considering that nitrogen management of wine fermentation can be an effective tool to modulate wine aroma composition and style, the aim

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of this study was to investigate the influence of clarification time on the must of the white grapes, and to establish a practical understanding of the effect of the nitrogen content (amino acids and ammonium) on the must in relation to volatile profile of the wine, combined with chemometric analysis.

2. Material and methods

2.1. Chemicals and reagents

Standards of amino acids and biogenic amine were obtained from Sigma–Aldrich (St. Louis, MO, USA). Volatile compounds were obtained from Sigma–Aldrich (Saint-Quentin-Fallavier, France), with the exception of methyl butyrate, methyl hexanoate, isoamyl butyrate and ethyl trans-hexanoate (Alfa Aesar A Johnson Mattey Company, Bischheim, France), isoamyl acetate (VWR-Prolabo, Fontenay-sous-bois, France) and ethyl lactate (Fischer Scientific Labosi, Elancourt, France). All solvents were HPLC grade and purchased from Merck (Darmstadt, Germany). All solutions were filtered through membranes with 0.45 μm of diameter (Millipore). All reagents had purity greater than 95%.

2.2. Grape musts — experimental design

Experiments were carried out with musts obtained from white grapes of the *Vitis vinifera* varieties from the Southern Region of Brazil: Sauvignon Blanc, Vermentino and Viogner. The training system used for all plants was the V System, the rootstock was Paulsen 1103 (*V. berlandierli* Planch × *V. rupestris* Scheele), and the row and vine spacing were 2.7 × 1.3 m, respectively. All grape varieties at harvest showed good technical maturity with mean values of 20 °Brix, pH of 3.2 and total acidity of 7.5 g L^{-1} of tartaric acid. The grapes were destemmed and crushed to obtain the must. Original musts were taken as controls (must without treatment). In the experiment, must of each variety was distributed in to two fermentation tanks and the clarifying agents, bentonite (Majorbenton, AEB S/A) and silica sol (Baykisol 30, AEB S/A) were added to each tank. The dosage of the clarifying agents used was 7 mL L^{-1} for a bentonite solution 10% (v/v) and 2 mL L^{-1} for the sílica sol; these were recommended by the supplier for musts. The musts were clarified by applying two settling time: 12 h and 30 h, at 4 °C, according to winemaker's recommendation.

After this step, the must samples were collected and frozen until the analysis. The experiments were carried out in duplicate.

After the clarification process, the musts were submitted to the same microvinification procedure carried out by EPAGRI (Empresa de Pesquisa e Extensão Agropecuária de Santa Catarina), in Videira, SC, Brazil. The alcoholic fermentations were carried out with the addition of *Saccharomyces cerevisae* (20 g/100 kg) (Fermol Blanc, Pascal Biotech) at 17 °C. The experimental wines were cold-stabilized and the sulfite content was then adjusted to 30 mg L^{-1} of free SO_2 (Noxitan, AEB). After bottling the wines were stored at 13 °C.

2.3. General analysis of musts and wines

The following standard chemical parameters were determined: pH, total acidity (g L^{-1} of tartaric acid) and turbidity (nephelometric turbidity units; NTU) for the musts and pH, total acidity (g L^{-1} of tartaric acid), volatile acidity (g L^{-1} acetic acid) and ethanol (ebulliometry) carried out according to OIV [9] for the wines. The organic acid (tartaric, malic and succinic acids) contents were determined by HPLC-DAD, according to Burin et al. [10].

Protein nitrogen (PN) was determined using the Bradford method, and the results expressed in mg L^{-1} of BSA (bovine serum albumin), based on a calibration curves constructed from data obtained in white wine [11].

2.4. Nitrogen compounds

For all must samples, the quantitative determination of the nitrogen compounds, amino acids, ammonium ion and biogenic amines, was carried out, in triplicate, using a Shimadzu (Kyoto, Japan) liquid chromatograph (LC-20A) (HPLC-DAD). The column (4.6 × 250 mm, 5 μm) and pre-column (4.6 mm × 12.5 mm) were C18 reversed-phase columns (Shimadzu, Kyoto, Japan).

The analyses were carried out using the method described by Gómez-Alonso, Hermosín-Gutiérrez and García-Romero [12], with modifications. The mobile phases were acetate buffer (25 mM) pH = 5.8 (solvent A) and acetonitrile:methanol (80:20) (solvent B). The compounds were eluted applying a binary gradient: 90% of solvent B for 10.5 min, 90–83% of solvent B for 8.5 min, 83–80% of B for 11.5 min and maintained under this condition for 3 min, 80–60% of solvent B for 26.5 min, 60–18% of B for 5 min, 18–0% of B for 3 min and maintained under this condition for 4 min. The oven temperature was 35 °C during the whole chromatographic run and the flow was 0.9 mL min^{-1}. For the quantification an internal standard (2-aminoadipic acid, 1 g L^{-1}) was used, and the identification of the analytes was performed at 280 nm, with the exception of proline and the ammonium ion (NH_4^+) (292 and 268 nm, respectively). Briefly, the derivatization of these compounds was carried out through the reaction of 1.75 mL of borate buffer (pH = 9), 750 μL of methanol (Merck), and 1 mL of sample previously centrifuged (4000 rpm) for 20 min and filtered through a modified 0.45 mm PTFE membrane filter with 13 mm of diameter (Millipore, Bedford, MA), with the addition of 20 μL of internal standard and 30 μL of derivatization reagent diethyl ethoxymethylenemalonate (DEEMM). The derivatization reaction was carried out in a screw-cap test tube over 30 min in an ultrasound bath. The sample was then kept at 70 °C for 2 h to allow complete degradation of the excess DEEMM and reagent by products. The stability of the amino acid derivatives before the derivatization reaction was investigated, over 48 h, where the samples were placed in a dark room at 4 °C. All analytes showed stable concentrations (maintaining 100% of the initial concentration) 24 h after derivatization (data not shown).

The analysis was carried out in triplicate. The target compounds were identified according to the retention times and UV–vis spectral characteristics of the corresponding derivatized standards, expressed in mg L^{-1}. Quantification was performed using the calibration curves of the respective standards. The analytical performance of this method was investigated, indicating good linearity ($R^2 > 0.99$), repeatability (RSD < 5%) and accuracy (recovery > 80%).

2.5. Volatile compounds of the wines

The volatile profile of the wines (56 compounds) was obtained by gas chromatography (GC), using five different methods according to each class of compounds analyzed. All methods were developed and evaluated in the laboratory of *Institut des Sciences de la Vigne et du Vin* (ISVV), University of Bordeaux 2, France. The identification of the compounds was performed by comparing the retention times and/or mass spectra (NIST database mass spectra) with those of pure standards. The quantification for all compounds was performed using calibration curves based on data obtained in white wine.

2.5.1. Apolar esters (HS-SPME-GC/MS)

This method, developed and validated by Antalick, Perello and de Revel [13], allows 32 apolar esters to be quantified: fatty acid ethyl esters, higher alcohol acetates, branched acid ethyl esters, isoamyl esters, methyl esters, ethyl cinnamates and minor esters. In accordance with this method, 20 μL of internal standard solution was added to 25 mL of wine. A mixture of ethyl-d5 butyrate, ethyl-d5 hexanoate, ethyl-d5 octanoate, and ethyl-d5 cinnamate in 20 mg L^{-1} in ethanol was used as the internal standard. An aliquot of 10 mL of this wine was introduced into a 20 mL standard headspace vial previously filled with 3.5 g of