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Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil

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ABSTRACT

Organic acids and sugars are related to the chemical balance of wines and grape juices, besides exerting a strong influence on the taste balance and sensorial acceptance by consumers. The aim of this study was to validate a method for the simultaneous determination of sugars and organic acids in wines and grape juices by high-performance liquid chromatography (HPLC) with refractive index detection (RID) and diode array detection (DAD) and to characterize commercial products from northeast Brazil. The method provided values for linearity ($R > 0.9982$), precision (CV% < 1.4), recovery (76–106%) and limits of detection (0.003–0.044 g L^{-1}) and quantification (0.008–0.199 g L^{-1}) which are considered acceptable for application in the characterization of these types of matrices. Principal components analysis (PCA) was used to verify the applicability of the method in the quality control of the products and resulted in the correct separation of the samples according to their type of processing. The results obtained in the characterization of the samples studied showed high levels of glucose and fructose in grape juice and the organic acids content was similar to those found in products originating from other regions around the world.

1. Introduction

Organic acids are primary metabolites present in grapes, and the profile and concentration of these compounds are important parameters in relation to the processing of grape juices and wines and the determination of their chemical composition (Ali et al., 2010). The content of acids, such as tartaric, malic and lactic, in these products directly influences the taste balance, chemical stability and pH (Silva et al., 2015). The presence of acetic acid, in particular, can be an indicator of microbiological changes or the use of low quality raw materials in the preparation of these products (Lima et al., 2014).

The analysis of organic acids in wines is necessary for quality control as well as to monitor the evolution of acidity during the different stages of winemaking, since important changes in wine can be detected by alterations in the acid content (Silva et al., 2015).

Regarding the bioactive capacity of organic acids, few studies have shown the beneficial effects of the consumption of foods rich in these compounds, with the exception of ascorbic acid, which has a high antioxidant power. However, citric and malic acids have significant protective effects on the myocardium and act on ischemic lesions, according to a study by Tang et al. (2013), where the importance of the addition of these compounds in the diet of patients under treatment for ischemic disease was highlighted.

Another group of primary metabolites present in grapes and related to the quality of juices and wines, are sugars. Glucose and fructose are the major sugars present in grapes and juices (Eyduran et al., 2015). In wines, due to the fermentation process, these compounds occur in low concentrations, and there is the presence of non-fermentable sugars such as xylose and arabinose. Grape derivatives may also contain sugars from the hydrolysis of polysaccharides through the action of pectinase enzymes, for instance, rhamnose (Apolinar-Valiente et al., 2015), and other sugars in small amounts, such as maltose (Morvai and Molnár-Perl, 1992).

The profile and concentration of sugars and organic acids in grapes

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and grape derivatives, like juices and wines, are mainly dependent on factors such as grape maturation stage, region of origin, climate, variety, cultural practices and process conditions (Soyer et al., 2003; Muñoz-robredo et al., 2011; Ribeiro et al., 2012; Lima et al., 2015). The sub-mid São Francisco Valley (SFV), in northeast Brazil, is a new world region for the production of tropical wines. It differs from other regions in terms of its climatic conditions, with an average temperature of 26 °C, high sunlight intensity (3000 h/year), and low annual rainfall (around 500 mm per year). The growth of grapes associated with crop irrigation management makes it possible for each vine to produce two harvests per year in this region (Padilha et al., 2016). Currently, the production of wines in the SFV is around 4 million liters per year, with 2.8 million liters (70%) of sparkling wines, 1.16 million liters of red wines (29%) and 40,000 Liters of white wines (1%) (Pereira et al., 2016). The main varieties used in wine production are Cabernet Sauvignon, Syrah, Tempranillo, Touriga Nacional, Moscato Canelli, Chenin Blanc, Verdejo (*Vitis vinifera* L.) (Padilha et al., 2016).

In recent years, in the SFV, there has also been investment in the production of grape juice from new Brazilian varieties developed for the preparation of high quality juices, such as Isabel Precoce (*Vitis labrusca*) and hybrids (*V. labrusca* x *V. vinifera*) BRS Violeta, BRS Cora and BRS Magna, and currently five companies produce around 1.5 million liters/year of commercial juices with these varieties (Lima et al., 2014; Padilha et al., 2017).

Given the importance of sugars and organic acids in relation to the quality of grapes and grape-derived beverages, various procedures have been described for the determination of organic acids in musts and wines, which is usually performed by high performance liquid chromatography (HPLC). In general, RP-C18 columns are used for the separation of these compounds and the detection is carried out by ultraviolet (UV) detection, photodiode array detection (DAD) or mass spectrometry (MS) (Scherer et al., 2012; Ehling and Cole, 2011; Lima et al., 2015). For the determination of sugars in musts and wines, the International Vine and Wine Organization (OIV) recommends the HPLC method coupled with refractive index detection (RID) (OIV, 2011).

Multivariate statistical analysis techniques have proven to be powerful tools when investigating complex samples such as wine (Saurina, 2010). Principal component analysis (PCA) is one of the most commonly used techniques in multivariate analysis, aimed at reducing a dataset to its main components and visualizing similarities (Vanhatalo and Kulahci, 2015). PCA has been used in several studies, for instance, to interpret data related to chemical and sensorial profiles (Parpinello et al., 2015) and to distinguish the varietal and/or geographical origin (Ziółkowska et al., 2016) of grape pomaces and wines.

Few methods described in the literature involve the simultaneous determination of sugars and organic acids in grape juice and wine by HPLC. Some previously published methods for the simultaneous determination of these compounds in grapes, wine and alcoholic beverages fermented from fruits involve the separation of these metabolites with the use of ion exchange columns (300 × 7.78 mm) filled with sulfonated resin particles, crosslinked styrene-divinylbenzene copolymer in the hydrogen form, 7 to 11 μm in diameter. An isocratic phase comprised of acidified ultrapure water was used and the temperatures ranged from 25 to 75 °C. The detection of sugars is usually carried out by RID and for organic acids UV detection or DAD, at between 210 and 215 nm, is used (Lopez and Gomez, 1996; Chinnici et al., 2005; Kelebek et al., 2009; Eyéghé-Bickong et al., 2012). In this context, the aim of this study was to validate the methodology for the simultaneous determination of the main sugars and organic acids present in wines and grape juices by HPLC-DAD-RID and characterize samples from the sub-mid São Francisco Valley (SFV), northeast Brazil. Additionally, principal component analysis (PCA) was applied in the characterization of the data to demonstrate the applicability of the method in the quality control of these products.

2. Material and methods

2.1. Chemicals

Glucose and fructose were obtained from Sigma-Aldrich (St. Louis, MO, USA). Maltose and rhamnose were obtained from Chem Service (West Chester, PA, USA). Tartaric, malic, lactic, citric and acetic acids were obtained from Química Vetec (Rio de Janeiro, Brazil), all with a purity level of ≥98%. Ultrapure water was obtained from a Millipore Milli-Q system (Bedford, MA, USA). Sulfuric acid was obtained from Merck (Darmstadt, Germany).

2.2. Samples

To validate the methodology, commercial samples of grape juice and wine from the SFV (09° 16′ 20″ South latitude and 40° 52′ 8″ West longitude) were analyzed. The sample characterization was carried out using a previously validated method and 12 commercial products from local companies were collected. For each sample, 3 bottles of different batches were purchased, totalizing 36 samples. The commercial products, originated from five wineries, correspond to seven labels of red wines produced with the varieties 'Barbera', 'Touriga Nacional', 'Petit Verdot', 'Ruby Cabernet', 'Syrah', and 'Tempranillo' and a mixture of cultivars 'Cabernet Sauvignon', 'Syrah' and 'Alicante Bouschet', which was called Assemblage, two labels of sparkling Asti-type muscatel produced with the varieties 'Moscato Canelli' and 'Moscato Italia', a white wine label 'Chenin Blanc' and two grape juice samples (*Vitis labrusca* L.) made with new Brazilian grape varieties ('Isabel Precocce', 'BRS Cora' and 'BRS Violeta'). Classical analysis techniques were used to determine the pH (potentiometer/pH analyzer, Tecnal, Piracicaba, SP, Brazil), soluble solids (°Brix) (digital refractometer HI 96801, Hanna Instruments, Ann Arbor, MI, USA), titratable acidity and alcoholic strength of the grape juice and wine samples (OIV, 2011) (see Table 1).

2.3. Equipment and analytical procedure

The analysis were performed using an Agilent HPLC system, model 1260 Infinity LC (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary solvent pump (G1311C model), degasser, thermostatted column compartment (G1316A model) and autosampler (model G1329B) coupled to a diode array detector (DAD) (G1315D model) and refractive index detector (RID) (G1362A model). Data were processed using OpenLAB CDS ChemStation Edition™ software (Agilent Technologies).

The analytical procedure was performed applying the chromatographic conditions previously described by Ball (2011), optimized for wine and grape juice matrices. A 500 μL aliquot of wine or grape juice was diluted in 1.0 mL of ultrapure water, filtered through a 0.45 μm nylon membrane (Allcrom-Phenomenex, Torrance, CA, USA), and a volume of 10 μL was injected. The ion exchange column was an Agilent Hi-Plex H (300 × 7.7 mm) with internal particles of 8.0 μm protected by a PL Hi-Plex H (5 × 3 mm) guard column (Agilent Technologies, Santa Clara, CA, USA). The temperature of the column compartment was maintained at 70 °C and the RID flow cell was kept at 50 °C. The flowrate applied was 0.5 mL min^{-1} with a run time of 20 min. The phase was 4.0 mM L^{-1} H_2SO_4 in ultrapure water.

Standard solutions were injected to obtain the retention time for each compound. For the determination of tartaric, malic, lactic, citric and acetic acids, detection was conducted in the DAD at 210 nm. For the sugars maltose, glucose, fructose and rhamnose sugar, detection was carried out by RID.

The chromatograms obtained for the standard solutions of the organic acids and sugars can be seen in Fig. 1.