Food Chemistry 145 (2014) 395–403

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fingerprinting of anthocyanins from grapes produced in Brazil using HPLC–DAD–MS and exploratory analysis by principal component analysis

Karina Fraige [a], Edenir R. Pereira-Filho [b], Emanuel Carrilho [a,c,*]

[a] Instituto de Química de São Carlos, Universidade de São Paulo, 13560-970 São Carlos, SP, Brazil
[b] Departamento de Química, Universidade Federal de São Carlos, 13565-905 São Carlos, SP, Brazil
[c] Instituto Nacional de Ciência e Tecnologia de Bioanalítica, 13083-970 Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 31 July 2013
Accepted 16 August 2013
Available online 26 August 2013

Keywords:
Grapes
Anthocyanins
Chromatographic fingerprint
Principal component analysis

ABSTRACT

The anthocyanin profile was studied for 11 types of grapes of different varieties and geographical origins, including 10 wine grape varieties (*Vitis vinifera*) and one hybrid variety. Twenty anthocyanins were identified by means of the absorbance spectrum and fragmentation pattern by tandem mass spectrometry. The multivariate method of principal component analysis (PCA) was used to evaluate differences amongst the cultivars. The results show the hybrid grape separated from a cluster represented by vinifeiras grapes, mainly due to anthocyanin diglucosides. Within this group, it was possible to discriminate the different wine grapes. Clusters discriminating according to geographical origin were not observed.

1. Introduction

Grapevine (*Vitis* spp.) is one of the most cultivated fruit plants throughout the world. Grapes and products derived from them constitute an economically important factor worldwide. In Brazil, viticulture practice is recent when compared to traditional European grape producing countries, however, there has been observed a marked improvement in the quality of Brazilian wines due to the improvement of fine cultivars and winemaking techniques. A variety of species are cultivated throughout the world, but *Vitis vinifera* is known for the best quality wines. About 20% of the grapes cultured in Brazil come from *V. vinifera* species, and 80% from *Vitis labrusca*, *Vitis bourquiana*, and hybrid varieties (Mello, 2007). Amongst the *V. vinifera* species cultured in Brazil are Merlot, Cabernet Sauvignon, and Syrah. The first two are more adapted to the southern states whilst the latter is found in the northeastern and southeast states. The most widespread are non-*V. vinifera* species consumed as table grapes, for example Isabel, Moscato, Niagara, and Itália. The hybrid grape IAC 138-22, popularly known as Maximo, is a cross of Syrah (*V. vinifera*) and Seibel 11342 (hybrid), which was part of a genetic improvement program by Empresa Brasileira de Agropecuária (EMBRAPA) to produce a grape with fine traces and more resistance to pathogens. It is a well-adapted cultivar to São Paulo state and is used to make wines (Santos Neto, Pereira, Martins, & Leitão Filho, 1968).

Phenolic compounds have been extensively studied due to their potentially beneficial antioxidant, anti-inflammatory and anti-carcinogenic properties, which are spurring the interest of both industry and consumers for flavonoid-rich foods (Dugas et al., 2000; Moure et al., 2001; Ng, Liu, & Wang, 2000; Pérez-Gregorio, Regueiro, Simal-Gándara, Rodrigues, & Almeida, 2013). Many foods or products that present functional activities are rich in flavonoids content, such as anthocyanidins and flavonols, as quercetin, contributing to the overall intake of these components that present antioxidant activity (Pérez-Gregorio et al., 2013; Yang, Meyers, Van Der Heide, & Rui, 2004). The effect of the degradation of flavonols and anthocyanins on processing foods and beverages is largely studied. For example, it has been reported losses of flavonoids upon boiling of vegetables, as a result of its migration into the cooking water. However, quercetin conjugates showed to be remarkably resistant to degradation during normal processing operations, pointing out that it is an important source of flavonols even after cooking if the broth were consumed too (Rodrigues, Pérez-Gregorio, García-Falcón, & Simal-Gándara, 2009).

Grapes are a rich source of phenolic compounds, which play an important role in oenology due to their influence on some important sensory properties of wines, such as colour, stability, bitterness, and astringency. Anthocyanins constitute a large family of

* Corresponding author at: Instituto de Química de São Carlos, Universidade de São Paulo, 13560-970 São Carlos, SP, Brazil. Tel.: +55 16 3373 9441.
E-mail addresses: emanuel@iqsc.usp.br, ecarrilho@me.com (E. Carrilho).

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http://dx.doi.org/10.1016/j.foodchem.2013.08.066

polyphenols in plants and are widely distributed in nature. They are responsible for some fruit and flower colours, including red grapes, in which they are located in the skins and appear mainly during ripening (Esteban, Villanueva, & Lissarrague, 2001; Mazza & Miniati, 1993; Ryan & Revilla, 2003). They play a very important role in plant metabolism and are important in the food industry. The main anthocyanins found in grapes are derived from cyanidin, peonidin, delphinidin, petunidin, and malvidin, and they generally occur as glycosides and acylglycosides; malvidin-3-glucoside is the most abundant in almost all grape varieties (Pomar, Novo, & Masa, 2005; Ryan & Revilla, 2003; Vian, Tomao, Coulomb, Lacombe, & Dangles, 2006). Some studies suggest that anthocyanins present higher antioxidant activity than vitamins C or E, being reported a linear relationship between antioxidant capacity and anthocyanin content in some fruits (Castañeda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009; Heinonen, Meyer, & Frankel, 1998). The antioxidant activity of the *Vitis coignetiae Pulliat* anthocyanins was determined via 2,2-diphenyl-2-picrylhydrazyl radical scavenging and 2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation assays and the results showed that anthocyanins are primarily responsible for the antioxidant activity of this grape variety, as well as previously reported in other grapes varieties. Moreover, the antioxidant activity of *V. coignetiae Pulliat* anthocyanins was higher than that of ascorbic acid (Choi et al., 2010).

Quantitative analysis and identification of individual anthocyanins has been widely studied using high-performance liquid chromatography (HPLC), generally coupled to diode array detection as the main analytical tool (Hong & Wrolstad, 1990; Ryan & Revilla, 2003; Vian et al., 2006; Wang, Tong, Chen, & Gangemi, 2010). Vian et al. (2006) identified nine anthocyanins in the extracts of skin from Syrah grapes by HPLC with diode array detection, including 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, and the acetylglucosides and *p*-coumarylglucosides of malvidin and peonidin. These authors observed that the content of anthocyanins in conventionally grown grapes was significantly higher than in those from organic production. In the last few years, HPLC coupled to mass spectrometry (MS) had become a very efficient tool providing an increased sensitivity and structural information compared to HPLC–DAD (Castañeda-Ovando et al., 2009; de Rosso et al., 2012; de Villiers, Vanhoenacker, Majek, & Sandra, 2004; Kammerer, Claus, Carle, & Schieber, 2004; Mazzuca, Ferranti, Picariello, Chianese, & Addeo, 2005; Wang, Race, & Shirkande, 2003; Wu & Prior, 2005), making it possible to elucidate the structure and identify the anthocyanins by the aglycone moiety, type and number of sugars by tandem mass spectrometry. The MS instruments used can vary from simple quadrupole to advanced tandem and high-resolution systems, and when reference standards for anthocyanins are not available, compound identification is often tentative and is usually based on elution order, UV–Vis spectra and mass spectral information (molecular weight and MS/MS fragmentation) (Abad-García, Berrueta, Garmón-Lobato, Gallo, & Vicente, 2009). Mazzuca et al. (2005) used a mass spectrometry procedure by LC–ESI-MS/MS to differentiate *V. vinifera* from hybrid grape cultivars from Italy based on the anthocyanin profile. The results provided evidence of the incorrect classification of a grape cultivar previously believed to belong to the *V. vinifera* species and the correct classification of the Pallagrello cultivar as *V. vinifera*. Application of MALDI-TOF/MS for analyses of anthocyanins in food samples has already been done (Carpentieri, Marino, & Amoresano, 2007; Grant & Helleur, 2008; Ivanova et al., 2011). Recently, four different MALDI matrices were tested for anthocyanin analysis in wine and grape samples; it was found that 2,5-dihydroxybenzoic acid (2,5-DHB) provided the best results, allowing the identification of the dominant anthocyanins present in these samples (Ivanova et al., 2011). Alberts, Stander, and de Villiers (2012) reported a high-efficiency ultra high pressure liquid chromatography (UPLC) procedure with tandem mass spectrometry using a triple quadrupole instrument for screening of red wine anthocyanins and derived pigments. They used MS/MS in neutral loss scanning mode to observe the loss of dehydrated sugar moieties to tentatively identify the compounds based on their molar mass and elution order, and in a second experiment the characteristic fragmentation patterns were used to identify the anthocyanidin. Using this method they observed significant chemical alteration of the anthocyanins profile during wine ageing identifying 121 different compounds belonging to anthocyanins, pyranoanthocyanins, and flavanol–anthocyanin condensation products in Pinotage wines (Alberts et al., 2012).

Anthocyanins have been postulated as chemical markers to differentiate grape cultivars and the red wines made from, providing valuable information about the adulteration of juices and wines (Arozarena, Casp, Marin, & Navarro, 2000; Berente, De la Calle Garcia, Reichenbächer, & Danzer, 2000; García-Beneytez, Revilla, & Cabello, 2002). Statistical multivariate methods can be used to facilitate data interpretation when a large number of variables are analyzed. The multivariate method of principal component analysis (PCA) has been applied to reduce the number of dimensions of the original data system to visualize whether different groups of 23 Spanish wines could be related to specified anthocyanins or not. The authors found that the anthocyanin with the greatest discriminant power in the differentiation amongst varieties was malvidin-3-acetylglucoside, in addition to the relative contents of *p*-coumaric acid esters of malvidin 3-glucoside (Arozarena et al., 2000). A study on the composition of Tempranillo, Garnacha, and Cabernet Sauvignon grapes from high and low quality vineyards using different statistical multivariate methods showed that the anthocyanin profile was primarily determined by variety. The differences between vineyards, however, were very small (Arozarena et al., 2002). The anthocyanin and flavonol profiles of three varieties of grapes from Galicia, a Spanish wine-producing region, were investigated. The polyphenolic profile of Brancellao, Gran Negro and Mouratón red grapes, together with statistical analysis by cluster analysis and PCA allowed differentiation of the varieties. Gran Negro could be mainly characterized by peonidin and malvidin conjugates and syringetin-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside, Mouratón by their content on petunidin, delphidin and myricetin derivatives and Brancellao by the cyanidin content. Moreover the results showed that the biosynthesis of flavonols and anthocyanins are closely related (Figueiredo-González et al., 2012).

In the present study, the anthocyanin composition of Syrah, Cabernet Sauvignon, and Merlot grapes, all *V. vinifera* species from different locations of Brazil, and Maximo, a hybrid grape studied for the first time in relation to individual anthocyanin composition and used to produce wines in some regions of São Paulo state, was identified by HPLC coupled with a diode array detector and ion trap mass spectrometer (LC–DAD–MS). The relative amount of the identified anthocyanins was studied by PCA to visualize if the grape varieties or producer regions could be related to specific anthocyanins.

2. Materials and methods

2.1. Chemicals

HPLC grade acetonitrile, methanol, and formic acid were purchased from J.T. Baker (Phillipsburg, USA). Hydrochloric acid was purchased from Qhemis (São Paulo, Brazil), and sodium metabisulfite from Sigma (Saint Louis, USA). Water was acquired from a Milli-Q purification system (Millipore Corporation, Bedford, USA).