Food Research International 50 (2013) 20–30

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Colorimetric study of the interactions between different families of red wine pigments using transmittance and reflectance measurements

Matilde García-Marino [a], M. Luisa Escudero-Gilete [b], M. Teresa Escribano-Bailón [a], M. Lourdes González-Miret [b], Julián C. Rivas-Gonzalo [a], Francisco J. Heredia [b,*]

[a] Grupo de Investigación en Polifenoles, Unidad de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, E37007 Salamanca, Spain
[b] Food Colour and Quality Laboratory, Dept. Nutrition and Food Science, Universidad de Sevilla, Facultad de Farmacia, E41012, Sevilla, Spain

ARTICLE INFO

Article history:
Received 6 July 2012
Accepted 20 September 2012

Keywords:
Red wine
Colour
Pigments
Colorimetric techniques
Tempranillo
Graciano

ABSTRACT

The aim of this work was to conduct a detailed colorimetric study, using transmittance and reflectance measurements, to evaluate the possible interactions occurring among the different families of pigments comprising to colour matter of red wines and their contribution to the colour in aged red wines.

To accomplish this, the phenolic material of monovarietal red wines obtained from Tempranillo and Graciano varieties, and their blends, were fractionated by gel permeation chromatography in order to separate the coloured fractions with different chemical compositions. The binary blends at different concentrations of the fractions having higher anthocyanin monoglucoside proportions with fractions having higher pyranoanthocyanin derivative contents and direct flavanol-anthocyanin condensation products were carried out in order to determine the effect of adding these derivatives on the colour of the anthocyanin monoglucosides, the major wine pigments. It was observed that the addition of derived pigments to the anthocyanin monoglucosides fraction resulted in colour differences perceptible by the human eye. These variations were mainly quantitative (changes in chroma and lightness), and were also qualitative (changes in hue) in monovarietal wines.

Studying the phenolic fractions of wines implies an approach to the chemical reality of the wines, more than the studies on model solutions, since they can lead to the knowledge of those components having more influence on the final colour of the wine. With these results the wineries could conduct the vinifications towards a higher extraction of the components or families of components more important for the intensity and stability of colour.

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1. Introduction

Wines, and especially red wines, have a complex phenolic composition coming from grapes as well as produced during the elaboration process. These phenolics are of great importance due to their contribution to the sensory characteristics of wines, such as colour, taste, astringency and bitterness (Brossaud, Cheynier, & Noble, 2001; Cheynier, Moutounet, & Sarni-Manchado, 2003; Haslam, 1980).

Many compounds are involved in the colour of the red wines. This is one of the main problems when studying this matter, together with the considerable differences existing in their concentrations and chromatic characteristics. Separating and identifying the anthocyanins and the derivative components of wines, which are responsible for the colour of red wine (Haslam, 1980) are generally the first steps to facilitate their study and characterization. With this purpose, different methods of fractionation have been developed (Alcalde-Eón, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2004; Asenstorfer, Hayasaka, & Jones, 2001; Guadalupe, Soldevilla, Sáenz-Navajas, & Ayestarán, 2006; He, Santos-Buelga, Mateus, & De Freitas, 2006; Mateus, De Pascual-Teresa, Rivas-Gonzalo, Santos-Buelga, & De Freitas, 2002; Mateus, Silva, Santos-Buelga, Rivas-Gonzalo, & De Freitas, 2002; Mateus, Silva, Vercauteren, & De Freitas, 2001; Oliveira, Santos-Buelga, Silva, De Freitas, & Mateus, 2006; Sarni-Manchado, Deleris, Avallone, Cheynier, & Moutounet, 1999; Shoji, Yanagida, & Kanda, 1999; Sun, Leandro, De Freitas, & Spranger, 2006; Vivar-Quintana, 2002). However, the anthocyanins isolated are very unstable and susceptible to degradation (Giusti & Wrolstad, 2003), their stability being influenced by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, enzymes, other phenols, proteins and metal ions (Brouillard, 1982; Giusti & Wrolstad, 2003; Kader, Rovel, Girardin, & Metche, 1997; Rein, 2005; Rivas-Gonzalo, 2003).

Anthocyanins, the main responsible of colour of red wines, may undergo alterations in their structure quite easily owing to the action of different agents, due to the electron-deficient flavylium nucleus. The many possibilities of substitution of the B ring and the hydroxyl functions afford anthocyanins specific properties; in particular, colour and stability, which are directly linked to structure (Brouillard, 1982).

The colour can be measured by both instrumental and visual analysis. In a previous study (García-Marino et al., 2012) the colour of

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[*] Corresponding author. Tel.: +34 954556495; fax: +34 954556110.
E-mail address: heredia@us.es (F.J. Heredia).

0963-9969/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2012.09.026

different phenolic fractions obtained from Tempranillo and Graciano wines was used to find the colorimetric technique (transmission spectrophotometry, diffuse reflectance spectrophotometry and spectroradiometry) which better correlate with the visual (sensory) appreciation of the colour, being the spectroradiometry the more adequate for this purpose.

During winemaking the anthocyanin proportion decreases and the derivative proportion increases. A series of mechanisms might be related to such changes, such as their adsorption by yeast, their degradation and oxidation, their precipitation with proteins, polysaccharides or condensed tannins, and the progressive and irreversible formation of more complex and stable anthocyanin derived pigments. Thus, the colour will be modified depending on the type of pigment formed. These derived pigments are mainly originated through two types of reaction; those of condensation between anthocyanins (A) and flavanols or tannins (T), either directly or mediated by aldehydes, and those of cycloaddition between anthocyanins and carbonyl compounds and vinylphenols (Bakker et al., 1997; Francia-Aricha, Guerra, Rivas-Gonzalo, & Santos-Buelga, 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Fulcrand, Cameira Dos Santos, Sarni-Manchado, Cheynier, & Favre-Bonvin, 1996; Mateus, De Pascual-Teresa, et al., 2002; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & De Freitas, 2003; Schwarz, Wabnitz, & Winterhalter, 2003; Somers, 1971; Timberlake & Bridle, 1977; Vivar-Quintana, Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1999). Pigments of the T-ethyl-A type are far more resistant to discolouration by SO_2 than free anthocyanins. These compounds are also more resistant than anthocyanins to variation in pH, probably as a result of a better protection against the nucleophilic attack by water (Escribano-Bailón, Álvarez-García, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001; Pissarra et al., 2004). Also, in comparison with the respective anthocyanin, they show a bathochromic shift of approximately 15 nm, with an absorption maximum at 540 nm that, according to the spectrum of the red wine, affords reddish-blue hues or violet hues at the pH of the wine (Atasanova, Fulcrand, Le Guernevé, Cheynier, & Moutounet, 2002; Escribano-Bailón et al., 2001; Francia-Aricha et al., 1997; Rivas-Gonzalo, Bravo-Haro, & Santos-Buelga, 1995; Salas et al., 2005; Timberlake & Bridle, 1976; Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002). The (A^+) anthocyanins could also react directly with flavanols or tannins (T), giving rise to polymeric A^+-T red and T-A^+ reddish-orange pigments (Salas et al., 2004).

Regarding pyranoanthocyanins, their concentration in wines is much lower than that of other pigments (Bakker & Timberlake, 1997; Romero & Bakker, 2000) and differs from anthocyanins in many analytical aspects, especially the colour. In comparison to the genuine anthocyanins, hydroxyphenyl-pyranoanthocyanins, vitisins and vinylflavanol-pyranoanthocyanins possess ranges of maximum absorption between 495 and 520 nm (hypsochromic effect) (Schwarz, Quast, Von Baer, & Winterhalter, 2003). They also show an absorption maximum at 420 nm (Bakker et al., 1997; Fulcrand et al., 1998), which would explain why these molecules are related to the change in hue from reddish-violet to reddish-orange hue. Due to the protective effect of the new pyran ring against the nucleophilic attack of water which hinders the carbinol base, the colour of pyranoanthocyanins is not very sensitive to the pH (Francia-Aricha et al., 1997), SO_2 (Bakker et al., 1997; Vivar-Quintana et al., 1999) and even to temperature (Sarni-Manchado, Fulcrand, Souquet, Cheynier, & Moutounet, 1996) almost all these adducts participate in the colour of the wine (Zamora, 2003). As well as being structurally more stable than anthocyanins, pyranoanthocyanins are not strongly absorbed by the cell walls of yeast because they are formed in the mid/end of the alcoholic fermentation, when the walls are saturated by anthocyanins. However, most pyranoanthocyanins possess yellow to orange colour and contribute to the tawny colour shift associated with red wine ageing, except for the new pigments identified in Port red wines, such as flavanyl/phenyl-vinylpyranoanthocyanins (portosins) and pyranoanthocyanin. They possess a bathochromically shifted maximum of absorption resulting in bluish and turquoise colours, respectively (Mateus et al., 2003; Oliveira et al., 2006, 2010).

These different attributes of anthocyanin-derived pigments lead to many suspicions about their possible contribution to the colour of aged red wines, as important factor; and might offer the opportunity to use these molecules as a measure to determine the age of a red wine. For this reason, the aim of this work was to evaluate the influence of adding derivative pigments on the colour of the fractions having anthocyanin monoglucosides using transmittance and reflectance measurements.

2. Material and methods

2.1. Winemaking and samples

Three wines were elaborated separately from *Vitis vinifera* L. red grapes in Bodegas Roda S.A. (La Rioja, Spain): T from the Tempranillo variety, G from the Graciano variety, and M from an 80:20 blend of Tempranillo and Graciano grapes. A fourth wine W was elaborated by blending T and G wines (80:20 v/v) after finishing malolactic fermentation in each wine.

2.2. Sample fractionation

After three months of ageing in barrels, 180 mL of each wine sample (T, G, M and W wines) was collected and fractioned with a Toyopearl HW-40(s) gel column (Tosoh, Japan) (Alcalde-Eón et al., 2004). Previously, the wine samples were acidified, in order to convert all the anthocyanins present in the sample into their respective cationic and coloured forms and to favour the reactions between them and the sodium bisulfite (Acros Organics, New Jersey, U.S.A.) in excess that subsequently is added to the sample. The addition of sodium bisulfite to the acidified wine samples was made in order to induce a selective modification of the structure and/or chromatographic properties of specific pigments groups, facilitating their separation by compound groups according to their more or less resistance to attack by bisulfite.

The elution solvent was ethanol/H_2O (80:20 v/v). With this solvent the majority of the pigments retained in the column were eluted. When practically no more coloured compounds were eluted from the

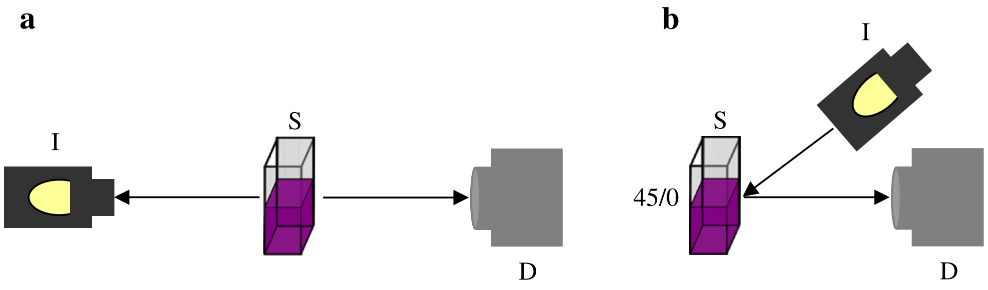

Fig. 1. Scheme (a) of a spectrophotometer used for the measurements of transmittance and (b) of a spectroradiometer for reflectance measurements.

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