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

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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.

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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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

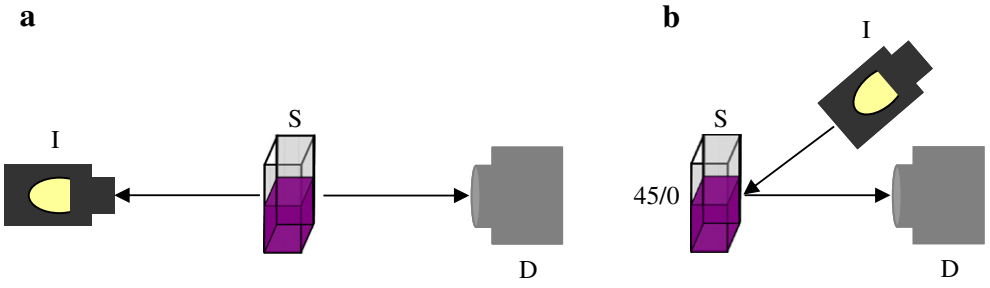

Fig. 1. Scheme (a) of a spectrophotometer used for the measurements of transmittance and (b) of a spectroradiometer for reflectance measurements.

Table 1
Mean concentration (mg/L. ±SD; n = 3) of the pigment families in the fractions obtained from the T. G. M and W wines.

Fraction	Polyphenols															
	Dp3G	Cy3G	Pt3G	Pn3G	Mv3G	TAnt3G	TAnt3dG	AcetAnt	CaffAnt	CoumAnt	TAcylAnt	TAnt	TPyran	DAcetF-A	DCF-A	TDer
	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Wine: Tempranillo (T)																
1	4.16 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.16 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.16 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	5.96 ± 0.02	3.97 ± 0.00	4.11 ± 0.01	1.32 ± 0.00	3.57 ± 0.01	18.93 ± 0.04	6.67 ± 0.00	2.49 ± 0.00	0.00 ± 0.00	4.35 ± 0.00	6.84 ± 0.00	32.44 ± 0.05	0.00 ± 0.00	2.47 ± 0.00	5.94 ± 0.00	8.41 ± 0.00
3	7.41 ± 0.03	0.00 ± 0.00	5.63 ± 0.02	1.40 ± 0.00	12.61 ± 0.10	32.04 ± 0.16	0.00 ± 0.00	6.83 ± 0.01	0.00 ± 0.00	27.27 ± 0.05	34.11 ± 0.06	66.14 ± 0.23	8.03 ± 0.01	3.08 ± 0.01	0.00 ± 0.00	11.11 ± 0.03
4	6.53 ± 0.03	3.80 ± 0.00	5.39 ± 0.02	2.06 ± 0.01	20.21 ± 0.18	38.00 ± 0.24	0.00 ± 0.00	6.78 ± 0.01	2.49 ± 0.00	24.18 ± 0.07	33.45 ± 0.08	71.45 ± 0.32	9.52 ± 0.02	2.79 ± 0.00	0.00 ± 0.00	12.31 ± 0.05
5	14.69 ± 0.11	5.91 ± 0.02	17.06 ± 0.14	7.19 ± 0.06	63.16 ± 0.61	110.84 ± 0.95	15.22 ± 0.05	9.09 ± 0.03	3.83 ± 0.01	33.67 ± 0.12	46.59 ± 0.16	172.65 ± 1.16	18.32 ± 0.07	3.45 ± 0.01	0.00 ± 0.00	21.77 ± 0.16
6	41.76 ± 0.38	5.59 ± 0.02	17.50 ± 0.14	3.92 ± 0.03	20.45 ± 0.18	95.93 ± 0.77	13.03 ± 0.02	15.17 ± 0.05	7.11 ± 0.05	37.76 ± 0.16	60.04 ± 0.26	169.01 ± 1.05	37.35 ± 0.19	3.83 ± 0.01	10.87 ± 0.01	52.73 ± 0.44
7	4.84 ± 0.01	3.66 ± 0.00	3.80 ± 0.00	1.25 ± 0.00	4.23 ± 0.02	17.79 ± 0.03	7.77 ± 0.00	2.53 ± 0.00	2.55 ± 0.00	12.94 ± 0.01	18.03 ± 0.01	43.59 ± 0.04	8.60 ± 0.00	2.55 ± 0.00	14.78 ± 0.01	25.93 ± 0.04
8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.79 ± 0.00	2.79 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.79 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Wine: Graciano (G)																
1	0.00 ± 0.00	3.62 ± 0.00	3.50 ± 0.00	0.00 ± 0.00	2.62 ± 0.00	9.74 ± 0.00	0.00 ± 0.00	2.46 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.46 ± 0.00	12.20 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	6.69 ± 0.03	5.65 ± 0.02	6.02 ± 0.03	1.63 ± 0.01	6.11 ± 0.04	26.11 ± 0.12	10.44 ± 0.01	6.05 ± 0.00	2.49 ± 0.00	15.78 ± 0.00	24.32 ± 0.01	60.87 ± 0.13	1.53 ± 0.00	2.56 ± 0.00	6.03 ± 0.00	10.12 ± 0.01
3	15.46 ± 0.12	3.99 ± 0.00	11.00 ± 0.08	5.07 ± 0.04	105.13 ± 1.04	140.65 ± 1.27	11.07 ± 0.01	7.21 ± 0.01	2.58 ± 0.00	31.50 ± 0.10	41.29 ± 0.11	193.02 ± 1.40	11.49 ± 0.01	3.09 ± 0.01	3.46 ± 0.00	18.05 ± 0.04
4	12.79 ± 0.09	5.04 ± 0.01	13.64 ± 0.10	15.59 ± 0.15	58.92 ± 0.57	105.98 ± 0.92	14.59 ± 0.05	3.72 ± 0.01	2.94 ± 0.01	36.20 ± 0.14	42.86 ± 0.16	163.43 ± 1.13	24.52 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	24.52 ± 0.17
5	20.41 ± 0.17	6.73 ± 0.03	25.38 ± 0.22	20.33 ± 0.19	2.96 ± 0.01	82.06 ± 0.63	15.74 ± 0.06	10.82 ± 0.04	3.03 ± 0.01	40.74 ± 0.19	54.58 ± 0.24	152.39 ± 0.93	31.26 ± 0.12	3.40 ± 0.01	2.53 ± 0.00	37.20 ± 0.26
6	25.65 ± 0.22	4.27 ± 0.01	7.00 ± 0.04	1.42 ± 0.00	4.89 ± 0.02	49.53 ± 0.30	12.36 ± 0.01	11.95 ± 0.02	3.93 ± 0.02	26.50 ± 0.05	42.38 ± 0.08	104.26 ± 0.39	21.98 ± 0.06	2.89 ± 0.00	7.16 ± 0.01	32.03 ± 0.16
7	4.72 ± 0.01	0.00 ± 0.00	3.74 ± 0.00	1.44 ± 0.00	4.60 ± 0.02	19.51 ± 0.04	4.14 ± 0.00	6.04 ± 0.00	2.52 ± 0.00	14.03 ± 0.01	22.59 ± 0.01	46.24 ± 0.05	2.57 ± 0.00	2.55 ± 0.00	15.02 ± 0.02	20.14 ± 0.04
8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Wine: Blend of grapes (M)																
1	4.23 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.23 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.23 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	5.43 ± 0.01	3.89 ± 0.00	4.41 ± 0.01	1.28 ± 0.00	3.33 ± 0.01	18.33 ± 0.04	4.10 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.12 ± 0.00	4.12 ± 0.00	26.56 ± 0.04	1.20 ± 0.00	0.00 ± 0.00	3.46 ± 0.00	4.65 ± 0.00
3	8.05 ± 0.04	3.69 ± 0.00	5.83 ± 0.02	1.37 ± 0.00	17.20 ± 0.15	36.15 ± 0.22	0.00 ± 0.00	3.74 ± 0.01	0.00 ± 0.00	22.39 ± 0.04	26.13 ± 0.05	62.28 ± 0.27	11.54 ± 0.01	2.81 ± 0.00	3.45 ± 0.00	17.81 ± 0.03
4	6.64 ± 0.03	3.80 ± 0.00	6.08 ± 0.03	2.38 ± 0.01	24.09 ± 0.22	43.01 ± 0.29	6.21 ± 0.00	6.82 ± 0.01	2.51 ± 0.00	26.05 ± 0.09	35.38 ± 0.10	84.60 ± 0.39	12.41 ± 0.01	2.60 ± 0.00	0.00 ± 0.00	15.02 ± 0.03
5	43.55 ± 0.40	7.75 ± 0.04	36.98 ± 0.34	5.72 ± 0.05	131.89 ± 1.31	233.42 ± 2.16	25.14 ± 0.11	13.86 ± 0.04	3.72 ± 0.01	48.10 ± 0.33	65.68 ± 0.38	324.24 ± 2.65	46.77 ± 0.30	3.84 ± 0.01	2.68 ± 0.00	53.29 ± 0.63
6	12.73 ± 0.09	4.18 ± 0.01	4.44 ± 0.01	1.20 ± 0.00	5.72 ± 0.03	28.27 ± 0.14	15.12 ± 0.01	6.10 ± 0.00	3.45 ± 0.01	14.95 ± 0.01	24.50 ± 0.02	67.89 ± 0.17	14.74 ± 0.01	2.49 ± 0.00	10.42 ± 0.01	27.63 ± 0.05
7	4.88 ± 0.01	0.00 ± 0.00	3.81 ± 0.00	1.29 ± 0.00	4.53 ± 0.02	14.51 ± 0.04	6.65 ± 0.00	0.00 ± 0.00	2.49 ± 0.00	13.63 ± 0.00	16.13 ± 0.00	37.29 ± 0.04	2.50 ± 0.00	2.48 ± 0.00	10.34 ± 0.01	15.32 ± 0.02
8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.65 ± 0.00	2.65 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.65 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.56 ± 0.00	2.56 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.56 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Wine: Blend of wines (W)																
1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	5.23 ± 0.01	3.89 ± 0.00	5.91 ± 0.03	1.55 ± 0.00	4.67 ± 0.02	21.24 ± 0.07	4.19 ± 0.00	6.01 ± 0.00	0.00 ± 0.00	11.85 ± 0.01	17.86 ± 0.01	43.29 ± 0.08	18.19 ± 0.02	2.51 ± 0.00	3.56 ± 0.00	24.26 ± 0.05
3	5.05 ± 0.01	3.72 ± 0.00	3.77 ± 0.00	1.68 ± 0.01	5.74 ± 0.03	19.97 ± 0.05	0.00 ± 0.00	2.53 ± 0.00	0.00 ± 0.00	20.82 ± 0.01	23.35 ± 0.01	43.32 ± 0.07	9.25 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.24 ± 0.01
4	11.45 ± 0.07	5.36 ± 0.02	10.94 ± 0.08	11.82 ± 0.11	79.38 ± 0.78	122.00 ± 1.06	12.25 ± 0.02	12.74 ± 0.03	2.58 ± 0.00	33.17 ± 0.14	48.48 ± 0.17	182.72 ± 1.25	31.89 ± 0.11	3.50 ± 0.01	3.50 ± 0.00	38.89 ± 0.25
5	51.94 ± 0.48	7.14 ± 0.04	34.41 ± 0.31	11.39 ± 0.10	75.31 ± 0.74	187.35 ± 1.70	19.08 ± 0.05	16.23 ± 0.10	3.03 ± 0.01	41.18 ± 0.19	60.44 ± 0.30	266.87 ± 2.05	33.89 ± 0.19	3.51 ± 0.01	8.74 ± 0.02	46.14 ± 0.45
6	6.50 ± 0.02	3.69 ± 0.00	3.89 ± 0.00	1.28 ± 0.00	3.36 ± 0.01	18.71 ± 0.04	0.00 ± 0.00	2.54 ± 0.00	2.54 ± 0.00	9.72 ± 0.00	14.80 ± 0.00	33.51 ± 0.05	10.13 ± 0.01	2.46 ± 0.00	3.85 ± 0.00	16.43 ± 0.03
7	4.62 ± 0.01	0.00 ± 0.00	3.75 ± 0.00	1.24 ± 0.00	3.58 ± 0.01	13.19 ± 0.02	4.14 ± 0.00	5.93 ± 0.00	0.00 ± 0.00	10.09 ± 0.00	16.02 ± 0.00	33.35 ± 0.03	2.48 ± 0.00	0.00 ± 0.00	6.54 ± 0.01	9.02 ± 0.01
8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.69 ± 0.00	2.69 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.69 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

M: Mean values (mg/L of wine) and standard deviation (±SD).
Dp3G: delphinidin-3-O-glucoside; Cy3G: cyanidin-3-Oglucoside; Pt3G: petunidin-3-O-glucoside; Pn3G: peonidin-3-O-glucoside; Mv3G: malvidin-3-O-glucoside; TAnt3G: total anthocyanidin-monoglucosides; TAnt3dG: total anthocyanidin-diglucosides; TAcylAnt: total acylated anthocyanins; AcetAnt: total acetyl anthocyanins; CaffAnt: total caffeoyl anthocyanins; CoumAnt: total p-coumaroyl anthocyanins; TAnt: total anthocyanins; TPyr: total pyranoanthocyanins; TAcetF-A: Acetaldehyde-mediated Flavanol-Anthocyanin condensation products; DCF-A: Direct Flavanol-Anthocyanin condensation products; TDer: Total derived pigments.

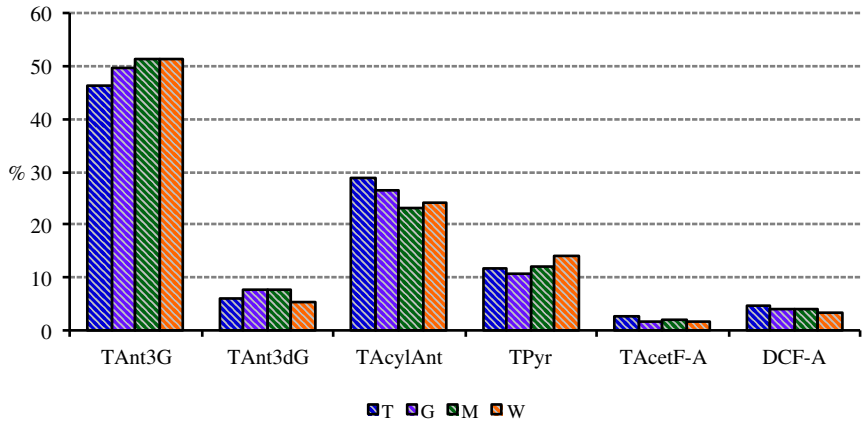

Fig. 2. Relative distribution (%) of the main pigment families (TAnt3G: total anthocyanidin-monoglucosides; TAnt3dG: total anthocyanidin-diglucosides; TAcylAnt: total acylated anthocyanins; TPyr: total pyranoanthocyanins; TAcetF-A: Acetaldehyde-mediated Flavanol-Anthocyanidin condensation products; DCF-A: Direct Flavanol-Anthocyanidin condensation products) in the total sum of the fractions obtained from fractionation of the Tempranillo (T), Graciano (G) wines, mixed grapes (M) and blended wines (W).

column, the solvent was changed to methanol/H_2O (80:20 v/v) until total elution of the pigments non-eluted with ethanol occurred. The different coloured bands formed during elution as well as the bleaching eluates were collected separately. In this way, nine fractions were obtained depending on the change of colour produced in the chromatographic column, each considered as different family of pigments according to the major compounds presents. All the fractions were acidified to pH = 1 in order to reverse the existing bisulphite-anthocyanin adducts, concentrated under vacuum, re-dissolved in water, and freeze-dried. Solutions of the freeze-dried fractions were prepared to have similar content as in the wines; thus, depending on the fraction, different amounts (mg) were dissolved in 5 mL of synthetic wine (pH 3.6, 0.2 mol/L).

2.3. HPLC-DAD-MS analysis

The solutions of fractions were acidified with 0.1 N HCl (Panreac® Barcelona, Spain) and injected into the chromatographic system after filtration through a 0.45-μm Millex® syringe-driven filter unit (Millipore Corporation, Temecula, CA, USA).

HPLC-DAD analysis was performed with a Hewlett–Packard 1100 series liquid chromatograph. The LC system was connected to the probe of the mass spectrometer via the UV cell outlet. The mass analyses were performed using a Finnigan™ LCQ ion trap detector (Thermoquest, San Jose, CA, USA) equipped with an API source, using an electrospray ionisation (ESI) interface. The HPLC-DAD-MS analysis of red pigments was carried out in accordance with García-Marino, Hernández-Hierro, Rivas-Gonzalo, and Escribano-Bailón (2010).

2.4. Quantification

For the quantitative analyses, calibration curves were obtained using standards of anthocyanin 3-*O*-glucosides (delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside and malvidin 3-*O*-glucoside). Anthocyanins were purchased from Polyphenols Labs., Sandnes, Norway.

Table 2
Proportion in pigments (%) of the fractions (1–9) obtained from the fractionation of the Tempranillo (T), Graciano (G) wines, grape mixture (M) and blended wines (W).

		% Polyphenols																
		Wine: Tempranillo (T)									Wine: Graciano (G)							
Pigment	Fraction	1[a]	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
	TAnt3G	100	46	41	45	57	43	26	100	–	80	37	67	56	43	36	29	–	–
	TAnt3dG	0	16	0	0	8	6	11	0	–	0	15	5	8	8	9	6	–	–
	TAcylAnt	0	17	44	40	24	27	26	0	–	20	34	20	23	29	31	34	–	–
TAnt		100	79	86	85	89	76	63	100	–	100	86	91	87	80	76	70	–	–
	Pir Totales	0	0	10	11	9	17	12	0	–	0	2	5	13	16	16	4	–	–
	CEt Flv-Ant	0	6	4	3	2	2	4	0	–	0	4	1	0	2	2	4	–	–
	CDr Flv-Ant	0	15	0	0	0	5	21	0	–	0	8	2	0	1	5	23	–	–
TDer		0	21	14	15	11	24	37	0	–	0	14	9	13	20	23	30	–	–
		Wine: Blend of grapes (M)									Wine: Blend of wines (W)								
Pigment	Fraction	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
	TAnt3G	100	59	45	43	62	30	28	100	100	–	31	38	55	60	37	31	100	–
	TAnt3dG	0	13	0	6	7	16	13	0	0	–	6	0	6	6	0	10	0	–
	TAcylAnt	0	13	33	36	17	26	31	0	0	–	26	44	22	19	30	38	0	–
TAnt		100	85	78	85	86	71	71	100	100	–	64	82	82	85	67	79	100	–
	Pir Totales	0	4	14	12	12	15	5	0	0	–	27	18	14	11	20	6	0	–
	CEt Flv-Ant	0	0	4	3	1	3	5	0	0	–	4	0	2	1	5	0	0	–
	CDr Flv-Ant	0	11	4	0	1	11	20	0	0	–	5	0	2	3	8	15	0	–
TDer		0	15	22	15	14	29	29	0	0	–	36	18	18	15	33	21	0	–

TAnt3G: total anthocyanidin-monoglucosides; TAnt3dG: total anthocyanidin-diglucosides; TAcylAnt: total acylated anthocyanins; TAnt: total anthocyanins; TPyr: total pyranoanthocyanins; TAcetF-A: Acetaldehide-mediated Flavanol-Anthocyanidin condensation products; DCF-A: Direct Flavanol-Anthocyanidin condensation products; TDer: Total derived pigments.

Owing to the considerable diversity of pigments identified, many of them did not have standards available. For this reason, the different pigments identified were quantified as the corresponding monoglucoside. All pigments were quantified from the areas of their chromatography peaks at 520 nm. The total content of the different groups of phenolic compounds studied was calculated as the sum of the individual concentrations obtained for each individual compound, expressed in mg/L of wine.

All experiments were performed in triplicate, and mean and standard deviation (±S.D.) were obtained.

2.5. Colorimetric measurements

The fractions were filtered through Millipore-AP20 filters (Millipore Corporation, Bedford, MA, USA) prior to the spectrophotometric analysis. Plastic cells (475×350×10 mm) were used for the measurements. The synthetic wine (pH 3.6; 0.2 mol/L) was measured previously as a blank.

The transmittance measurements (Fig. 1a) were made with a UV/Visible HP8452 (Hewlett–Packard, Palo Alto, CA, USA) diode-array spectrophotometer. The whole visible spectra were recorded (380–780 nm, Δλ = 2 nm). The CIE-1964 10° standard observer and CIE D65 standard illuminant (corresponding to day light) were considered as references to calculate the tristimulus values recommended by the "Comission Internationale de l'Éclairage" (CIE, 2004), applying the CromaLab® software (Heredia, Álvarez, Gonzalez-Miret, & Ramirez, 2004).

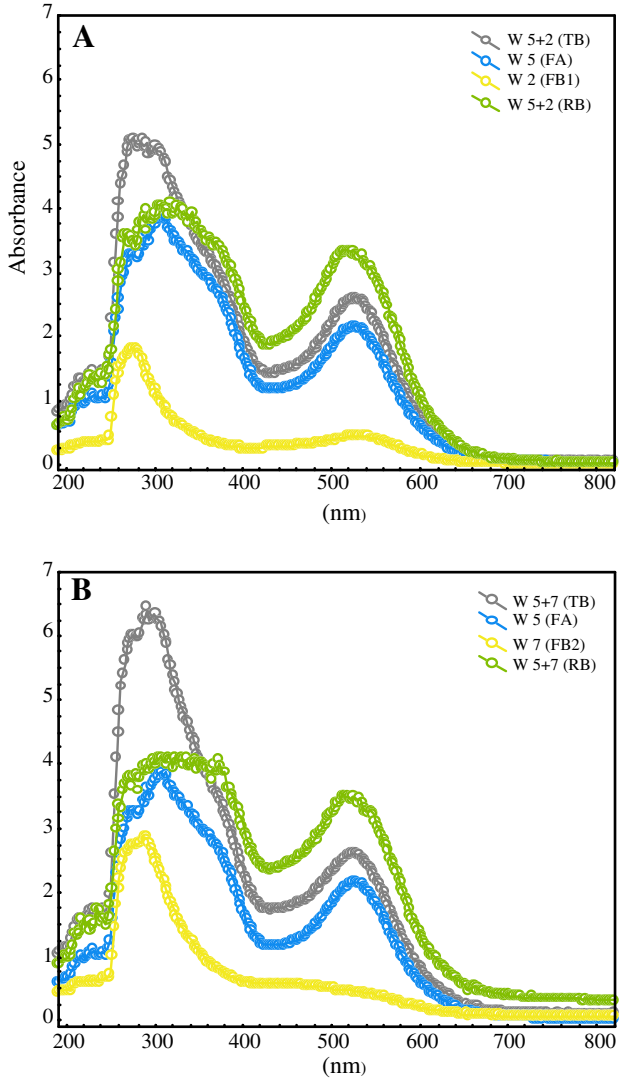

Fig. 3. Absorbance spectra of the fractions A (W5(FA)), B1 (W2(FB1)) and B2 (W7(FB2)), of the blends of the real fractions (RB) and of the theoretical blend (TB): (A) mixture of fraction A–fraction B1 (W5 + 2), (B) mixture of fraction A–fraction B2 (W5 + 7) from W wine.

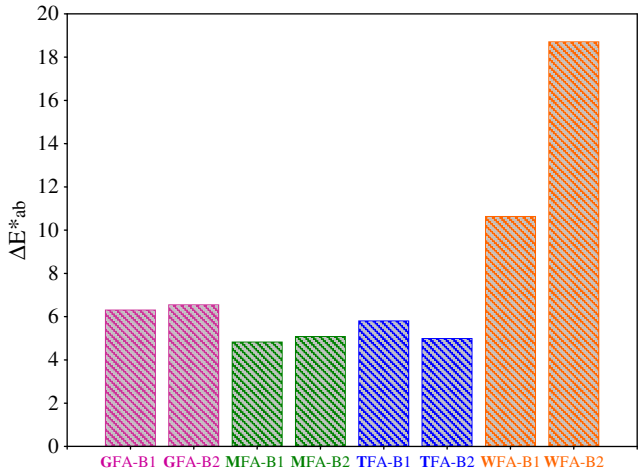

Fig. 4. Colour differences (ΔE^*_{ab}) between the mixture of real fractions and the theoretical blend of fractions of the T, G, M and W wines.

The reflectance measurements (Fig. 1b) –technique that best reproduces the evaluation of colour as is performed by the human eye– were performed by spectroradiometry, with the spectroradiometer connected to a TOP 100 telescopic optical probe (Instrument Systems, Munich, Germany) and a Tamron SP 23A zoom (Tamron USA, Inc., Commack, NY, USA), coupled to the CAS 140B (Instrument Systems, Munich, Germany). In this case, the CIELAB parameters were calculated by IS-Specwin v.1.8.1.6 software (Instrument Systems, Munich, Germany).

2.6. Assays of binary blends of fractions

Assays of binary blends of fractions with the highest anthocyanin monoglucoside proportions (fraction A: constant volume = 1 mL), with fractions of the highest contents in pyranoanthocyanin derivatives (fraction B1) and direct flavanol-anthocyanin condensation products (fraction B2) at different concentrations (increasing dilutions of fraction B with synthetic wine pH 3.6: 0, 33, 66 and 100%) were carried out in order to establish the influence in the colour of these derivatives on major wine pigments. Next, colour of blends was measured with the two techniques described previously.

3. Results and discussion

3.1. Chemical characterization of pigments

With a view to conduct a study on the composition of the different families of pigments, we performed the analysis by HPLC-DAD/ESI-ITMS on the different fractions obtained from the different wines. We identified and quantified a total of 37 pigments, including anthocyanins, monoglucoside, diglucoside, and acylated anthocyanins and other pigments derived from anthocyanins (anthocyanin-flavanol derivatives obtained by direct condensation and by ethyl bridges, and pyranoanthocyanins). All the pigments identified in the fractions analysed have been previously described in samples of wines and fractions (García-Marino, 2011; García-Marino et al., 2010).

Table 1 shows the mean concentration of the pigment families of the fractions from wines: T, G, M and W. Initially, it may be seen that the fractions were not obtained in the characteristic order in reverse phase chromatography but as a mixture of pigments. In all the fractions obtained the presence of monoglucoside anthocyanins

Table 3
Colorimetric parameters (L^*, a^*, b^*, C^*_{ab} y h_{ab}) of the original fractions (fraction A, fraction B1, fraction B2) and their blends (fraction A + fraction B1; fraction A + fraction B2) obtained from the T, G and M and W by spectrophotometry and spectroradiometry.

Colorimetric parameters	Spectrophotometry					Spectroradiometry				
	L^*	a^*	b^*	C^*_{ab}	h_{ab}	L^*	a^*	b^*	C^*_{ab}	h_{ab}
Fraction A										
T	94.02 ± 0.01	8.18 ± 0.01	−1.17 ± 0.00	8.26 ± 0.01	−8.11 ± 0.03	45.85 ± 0.01	47.19 ± 0.01	16.53 ± 0.01	50.00 ± 0.01	19.30 ± 0.01
G	93.35 ± 0.01	10.07 ± 0.02	−1.66 ± 0.01	10.21 ± 0.02	−9.34 ± 0.03	42.85 ± 0.07	46.39 ± 0.06	14.84 ± 0.01	48.70 ± 0.06	17.74 ± 0.01
M	85.64 ± 0.01	18.94 ± 0.01	−2.33 ± 0.01	19.08 ± 0.01	−7.01 ± 0.03	30.56 ± 0.05	28.02 ± 0.02	10.95 ± 0.00	30.09 ± 0.02	21.34 ± 0.01
W	88.25 ± 0.01	15.27 ± 0.01	−1.49 ± 0.00	15.34 ± 0.01	−5.56 ± 0.01	31.92 ± 0.06	30.37 ± 0.08	12.25 ± 0.05	32.75 ± 0.10	21.97 ± 0.03
Fraction B1										
T	84.92 ± 0.01	14.93 ± 0.03	2.15 ± 0.01	15.08 ± 0.02	8.21 ± 0.06	32.87 ± 0.09	20.59 ± 0.07	7.88 ± 0.05	22.04 ± 0.08	20.95 ± 0.07
G	92.24 ± 0.01	7.54 ± 0.00	1.72 ± 0.00	7.73 ± 0.00	12.84 ± 0.04	38.29 ± 0.05	35.72 ± 0.01	16.77 ± 0.03	39.46 ± 0.02	25.16 ± 0.03
M	94.94 ± 0.00	4.44 ± 0.00	2.13 ± 0.00	4.92 ± 0.00	25.61 ± 0.01	44.46 ± 0.04	38.46 ± 0.04	23.25 ± 0.02	44.94 ± 0.04	31.16 ± 0.00
W	97.22 ± 0.00	3.25 ± 0.00	−0.27 ± 0.00	3.26 ± 0.00	−4.78 ± 0.02	58.78 ± 0.26	37.86 ± 0.28	7.26 ± 0.02	38.55 ± 0.28	10.85 ± 0.05
Fraction B2										
T	94.30 ± 0.00	4.31 ± 0.00	1.99 ± 0.00	4.75 ± 0.00	24.73 ± 0.04	45.57 ± 0.10	38.86 ± 0.06	22.57 ± 0.04	44.94 ± 0.06	30.14 ± 0.04
G	93.29 ± 0.00	4.67 ± 0.00	3.14 ± 0.00	5.63 ± 0.00	33.91 ± 0.03	40.92 ± 0.09	38.54 ± 0.06	24.16 ± 0.02	45.48 ± 0.06	32.08 ± 0.01
M	93.44 ± 0.00	4.82 ± 0.00	2.75 ± 0.00	5.54 ± 0.00	29.72 ± 0.00	41.38 ± 0.05	36.90 ± 0.04	21.86 ± 0.03	42.89 ± 0.05	30.64 ± 0.03
W	96.81 ± 0.01	2.05 ± 0.00	2.91 ± 0.00	3.56 ± 0.00	54.81 ± 0.02	60.61 ± 0.05	33.96 ± 0.03	37.25 ± 0.05	50.41 ± 0.05	47.65 ± 0.04
Fraction A + fraction B1										
T	79.10 ± 0.08	20.34 ± 0.28	2.56 ± 0.12	20.50 ± 0.27	7.18 ± 0.44	29.71 ± 0.02	14.11 ± 0.05	5.83 ± 0.01	15.26 ± 0.05	22.45 ± 0.10
G	84.17 ± 0.00	18.29 ± 0.03	−0.62 ± 0.01	18.30 ± 0.03	−1.95 ± 0.02	25.96 ± 0.07	26.55 ± 0.02	10.13 ± 0.02	28.42 ± 0.02	20.89 ± 0.03
M	80.09 ± 0.03	21.96 ± 0.05	0.70 ± 0.03	21.98 ± 0.05	1.83 ± 0.09	24.70 ± 0.01	16.95 ± 0.04	6.34 ± 0.01	18.10 ± 0.04	20.49 ± 0.04
W	81.67 ± 0.04	22.32 ± 0.10	−1.46 ± 0.05	22.36 ± 0.11	−3.74 ± 0.12	25.73 ± 0.04	25.57 ± 0.07	10.07 ± 0.03	27.48 ± 0.07	21.49 ± 0.06
Fraction A + fraction B2										
T	89.05 ± 0.00	10.37 ± 0.01	1.31 ± 0.00	10.45 ± 0.01	7.19 ± 0.03	32.57 ± 0.05	38.98 ± 0.06	17.64 ± 0.05	42.78 ± 0.07	24.35 ± 0.04
G	85.80 ± 0.02	14.52 ± 0.03	0.98 ± 0.02	14.55 ± 0.03	3.87 ± 0.08	27.95 ± 0.07	30.02 ± 0.04	12.51 ± 0.04	32.53 ± 0.05	22.62 ± 0.05
M	79.81 ± 0.02	21.37 ± 0.04	1.57 ± 0.02	21.43 ± 0.04	4.21 ± 0.07	24.64 ± 0.02	15.68 ± 0.02	6.02 ± 0.01	16.79 ± 0.02	21.01 ± 0.03
W	79.96 ± 0.19	20.05 ± 0.09	0.98 ± 0.03	20.08 ± 0.09	2.80 ± 0.08	25.89 ± 0.08	21.02 ± 0.06	8.16 ± 0.02	22.54 ± 0.07	21.22 ± 0.04

Mean values and standard deviation (±SD; n = 3).

was generalized. It is also possible to note that fractions 1, 8, and 9 featured a low presence of pigments. Regarding the diglucoside anthocyanins, these were distributed between fractions 2 and 6, while the acylated anthocyanins were also present in fraction 7. The derived pigments condensed by an ethyl bridge and the vinyl-derived adducts were present in fractions 3, 4, 5 and 6 of some wines. In contrast, those derived from direct condensation were present almost exclusively in fractions 6 and 7 of some wines.

Fig. 2 shows the relative proportion of these pigment families (%) (glucoside, diglucoside, and acylated anthocyanins, pyranoanthocyanin derivatives, anthocyanin-flavanol derivatives condensed by an ethyl bridge and those derived by direct condensation) of all the fractions obtained from the T, G, M and W wines. It was possible to observe that the fractions obtained from the monovarietal T and G wines had a higher proportion of acylated anthocyanins and a lower proportion of glucosylated anthocyanins than the blended wines (M and W). Wine W contains the highest proportion of pyranoanthocyanins. These differences in the proportion of the different families of pigments could define the variations in the chromatic behaviour described below among the fractions obtained from the T, G, M and W wines.

According to Brouillard (1982), the contribution of derived pigments to the colour of wine is greater than that expected from their low concentration, mainly due to the fact that, unlike the anthocyanins, at the pH of wine the derived pigments are present in their coloured form, whereas scarcely 15% of the anthocyanins found in the flavyl cation form at these pH values. Accordingly, in order to check the effect of the colour of these pigments on the major pigments in wines (glucosylated anthocyanins), of all the data collected, we only show those appearing as fraction A (FA) –those with the greatest richness in glucosylated anthocyanins– and as fractions B1 (FB1) and B2 (FB2) those with the greatest richness in pyranoanthocyanins and derivatives by direct condensation respectively.

As shown in Table 2, the proportion of total anthocyanin pigments was high in all the fractions, being 100% in fractions 1, 8 and 9, so that these latter were excluded from the assays. The anthocyanidin glucosides were present in higher proportions in fraction 5 of the T (57%), M (62%) and W (60%) wines, and in fraction 3 of the G wine (67%). Pyranoanthocyanins and pigments derived by condensation were those present at the greatest concentration among all the derived pigments identified in these fractions. The pyranoanthocyanins were present at the highest proportions in fraction 6 of the T (17%), G (16%) and M (15%) wines and in fraction 2 of W wine (27%), while the derivatives by direct condensation were present at higher proportions in fraction 7 of the T (21%), G (23%), M (20%) and W (15%) wines.

3.2. Colorimetric parameters. Assays of binary blends

First, in order to check that the colour change of the binary blends of fractions depends on their chemical composition, we calculated the theoretical spectra of the blends of 100% of FA fraction and 100% of FB1 or FB2 fraction (1 mL FA + 1 mL FB1 and 1 mL FA + 1 mL FB2) by means of the theoretical sum of the real fractions. Fig. 3 shows the absorbance spectra of the whole fractions (FA, FB1 and FB2), the real blends (FA + FB1 or FA + FB2) and the theoretical blends, confirming that real colour of blends not satisfied the Beer–Lambert law, therefore the colour changes of the real blends could be due to chemical changes such as those involved in copigmentation reactions. Next, colour differences (ΔE^*_{ab}) between the real and theoretical blends of fractions were calculated to value the colorimetric effect due to the mixture (Fig. 4). To accomplish this, we took into account the CIELAB parameters –rectangular coordinates L^*, a^* and b^* and cylindrical coordinates C^*_{ab} and h_{ab}– of the fractions selected: FA, FB1, FB2 and blends thereof (Table 3), obtained by transmission spectrophotometry. We observed that the ΔE^*_{ab} between the real and theoretical blends were detectable by the human eye (>2.7 CIELAB units; (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001)), being

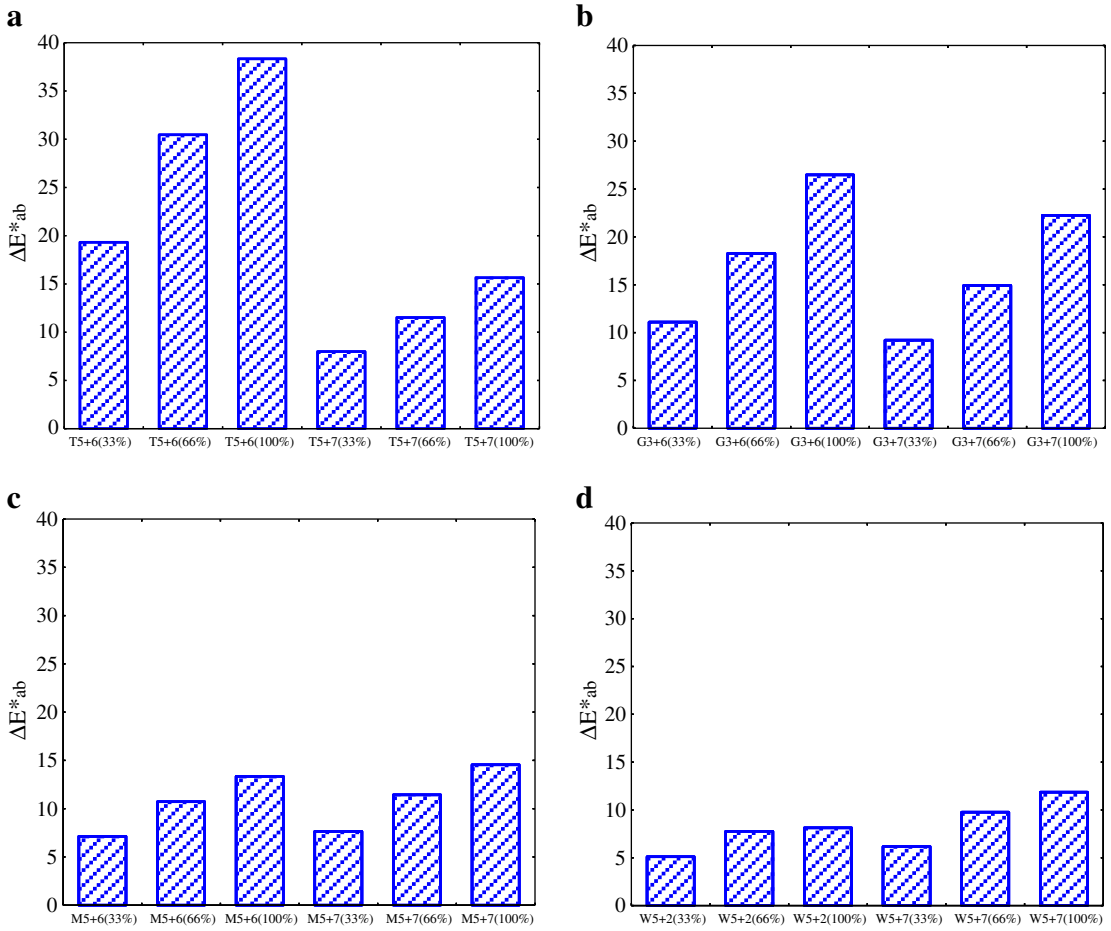

Fig. 5. Colour differences (ΔE^*_{ab}) of the blending of fraction A–fraction B1 and fraction A–fraction B2. (a) T wine: fraction A (T5)–fraction B1 (6) and fraction A (T5)–fraction B2 (7); (b) G wine: fraction A (G3)–fraction B1(6) and fraction A (G3)–fraction B2 (7); (c) M wine: fraction A (M5)–fraction B1 (6) and fraction A (M5)–fraction B2 (7); (d) W wine: fraction A (W5)–fraction B1 (2) and fraction A (W5)–fraction B2 (7).

considerably greater in blends from W wine fractions than those obtained from the other wines (T, G and M) (Fig. 4).

The detailed study of the phenolic composition fractions only showed differences in the Df/Mv ratio of the FA of wines, observing that the Df/Mv ratio of the FA obtained from the W wine (0.69) was greater than in T, G and M wines (0.23, 0.15 and 0.33, respectively), which might lead to thinking that the delphinidin anthocyanin showed an extraordinarily high reactivity compared to other anthocyanins conferring it greater susceptibility to change and explaining why the colour differences in the W wine were greater than those seen in the other wines. Likewise, Fig. 4 shows that, except in the blends obtained from T wine fractions, there seems to be a tendency for the blend of FA + FB2 to show a higher real–theoretical colour differences, ΔE^*_{ab}, than the blend FA + FB1, especially higher for W wine fractions. The different proportion of acylated pigments in FB1 and FB2 could explain it. It is well known that the acylated anthocyanins may undergo processes of intramolecular copigmentation (Dangles, Saito, & Brouillard, 1993; Eiro & Heinonen, 2002) that confer them greater stability. In this study, a detailed examination of the composition fractions just allowed to observe that the FB2 of G, M and W wines (34, 31 and 38%, respectively) had a greater proportion of acylated anthocyanins than the FB1 (31, 26 and 26%, respectively), whereas in the T wine the FB2 had a lower proportion of acylated anthocyanins (26%) than the FB1 (27%) (Table 2), which would lead to thinking that the acylated anthocyanins might get to play a significant role in the colour of wine fractions, hindering the possible interaction with FA and leading to lower values of ΔE^*_{ab}.

Then, with the colorimetric parameters measured by reflection by means of spectroradiometry (Table 3), ΔE^*_{ab} and its components, lightness ($\Delta^2 L^*$), hue ($\Delta^2 H$) and chroma ($\Delta^2 C^*_{ab}$), of the binary blends of fractions A and B (FB1 and FB2) of each of the four wines were calculated in order to observe which mixture (33, 66 and 100%) provided the best variation in colour (Figs. 5 and 6).

In Tempranillo, the binary blends of the fraction with the highest proportion of anthocyanins (FA, fraction 5) with the fraction with the greatest proportion of pyranoanthocyanins (FB1, fraction 6) and direct condensed derivatives (FB2, fraction 7) (Table 1) elicited colour differences (ΔE^*_{ab}) perceptible by the human eye (Fig. 5a), these being more marked in the case of fraction 6 (FB1) than with fraction 7 (FB2). This greater increase in colour due to the addition of fraction 6 than that due to the addition of fraction 7 to the blend could be related not only to the higher proportion of pyranoanthocyanins and direct condensed pigments present in them but also to the greater anthocyanin glucoside concentration in fraction 6 (169.01 mg/L of wine) as compared with fraction 7 (43.59 mg/L of wine) (Table 1). We also observed that as the concentration of fraction B (FB1 or FB2) in the blend increased the colour differences were also greater. Thus, with 33% of fractions B1 and B2 a ΔE^*_{ab} of 19.32 and 7.99 CIELAB units were obtained respectively. The colour differences between 33% and 66% (ΔE^*_{ab} [33%–66%]) of fractions B1 and B2 were also greater than 2.7 CIELAB units, being observed a ΔE^*_{ab} with B1 and B2 of 11.14 and 3.53 CIELAB units respectively. Regarding ΔE^*_{ab} [66%–100%] between 66% and 100% of fraction B1 decreased with respect to the previous case, being 7.87 CIELAB units, while of ΔE^*_{ab}

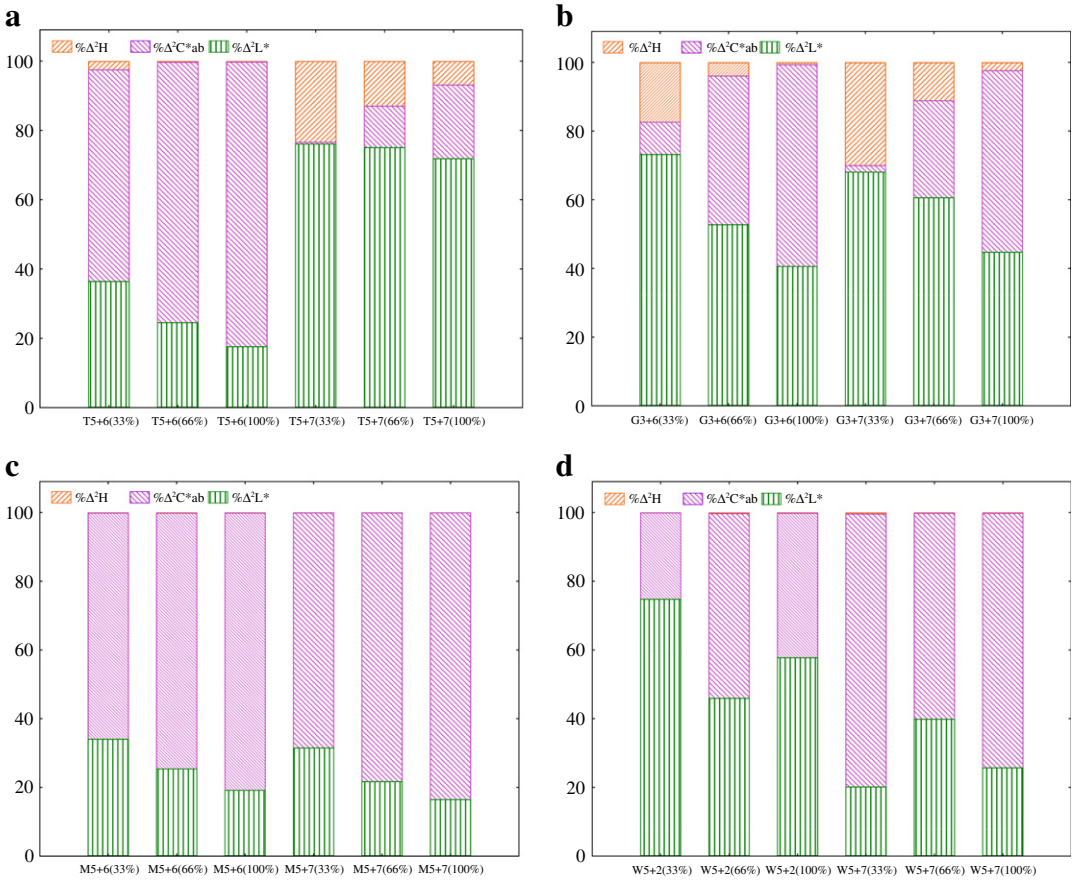

Fig. 6. Hue, chroma and lightness differences proportions (%Δ^2H, %$\Delta^2C^*_{ab}$, %Δ^2L^*) of the blending of fraction A–fraction B1 and fraction A–fraction B2. (a) T wine: fraction A (T5)–fraction B1 (6) and fraction A (T5)–fraction B2 (7); (b) G wine: fraction A (G3)–fraction B1(6) and fraction A (G3)–fraction B2 (7); (c) M wine: fraction A (M5)–fraction B1 (6) and fraction A (M5)–fraction B2 (7); (d) W wine: fraction A (W5)–fraction B1 (2) and fraction A (W5)–fraction B2 (7).

[66%–100%] between 66% and 100% of fraction B2 increased with respect to the previous case with 4.12 CIELAB units (Fig. 5a). This suggests the existence of a trend towards colour stabilization of the anthocyanin fraction (FA) with the increase in the proportion of FB1 (pyranoanthocyanins); but not with FB2 (direct condensed derivatives) that continues to increase.

In Graciano, as with the T fractions, the anthocyanin–pyroanthocyanin (FA + FB1) and anthocyanin-direct condensed derivatives binary blends (FA + FB2) brought about changes in colour (ΔE^*_{ab}) that were perceptible by the human eye; these were also greater with fraction 6 (FB1) than with fraction 7 (FB2) (Fig. 5b). As in T, in G the concentration of glucosylated anthocyanins of fraction 6 (104.26 mg/L of wine) was higher than that of fraction 7 (46.24 mg/L of wine) (Table 1). Likewise, we observed that as the concentration of fractions B1 and B2 increased in the blend the colour difference was more marked (Fig. 5b). However, unlike what was observed in T, the increase in the proportion of B1 and B2 fractions involved a gradual increase in both ΔE^*_{ab} [33%–66%] (B1 and B2, 7.18 and 5.69 CIELAB units, respectively) and in ΔE^*_{ab} [66%–100%] (B1 and B2, 8.21 and 7.33 CIELAB units, respectively). In this case, the trend towards stabilization did not seem to be present.

Regarding ΔE^*_{ab}, the blendings obtained in M (Fig. 5c) and W (Fig. 5d) showed a very similar behaviour. Fig. 5c shows that in M, the blending of fraction A with the B1 and B2 fractions produced perceptible colour differences for the human eye, with the exception of anthocyanin–pyranoanthocyanin blending (FA + FB1) at 100% where ΔE^*_{ab} [66%–100%] were of 2.64 CIELAB units. Unlike what was observed in T and G, the B fractions (B1 and B2) obtained from the M wine showed a similar anthocyanin glucoside concentration, being 26.56 and 37.29 of mg/L of wine in B1 and B2 respectively (Table 1).

In W, the addition of 33% of B1 and B2 fractions elicited colour differences appreciable by the human eye of 5.10 and 6.16 CIELAB units, respectively. However, the increase in the proportion of fractions B1 and B2 did not produce an important increase of ΔE^*_{ab}. Thus, the colour differences between 33% and 66% (ΔE^*_{ab} [33%–66%]) of fractions B1 and B2 were 2.66 and 3.6 CIELAB units respectively, while ΔE^*_{ab} [66%–100%] between 66% and 100% fractions B1 and B2 decreased from the previous; being with B1 of 0.37 CIELAB units and with B2 of 2.06 CIELAB units (Fig. 5d).

In W (Fig. 5d), the addition of fractions B1 and B2 led to smaller colour changes in fraction A than in blendings of T (Fig. 5a) and G (Fig. 5b), and very similar to M (Fig. 5c). This could be because the proportion of anthocyanins of the A fractions of the T (89%) and G (91%) wines was higher than in M (86%) and W (85%); this supposed a higher proportion of derived pigments (M, 14% and W, 15%) (Table 1). What might confer greater stability to the blend could explain the smaller differences in colour observed.

The $\Delta^2E^*_{ab}$ components, lightness (Δ^2L^*), hue (Δ^2H) and chroma ($\Delta^2C^*_{ab}$), of the binary blends of fractions A and B (FB1 and FB2) for each wine are shown in Fig. 6. In the blend with pyranoanthocyanins (FA + FB1) from T, the variations in colour were mainly due to variations in $\Delta^2C^*_{ab}$, and to a lesser extent in Δ^2L^*. Thus, with the increase in the proportion of FB1 the variations in colour due to $\Delta^2C^*_{ab}$ increased, therefore it appears that the pyranoanthocyanin compounds of fraction FB1 influence mainly on the quantitative component of colour ($\Delta^2C^*_{ab}$), providing a greater amount of colour, that is, more C^*_{ab}. The differences in colour between monoglucosides and direct condensed derivatives (FA + FB2) were mainly due to changes in lightness (Δ^2L^*), although the involvement of $\Delta^2C^*_{ab}$ in the colour

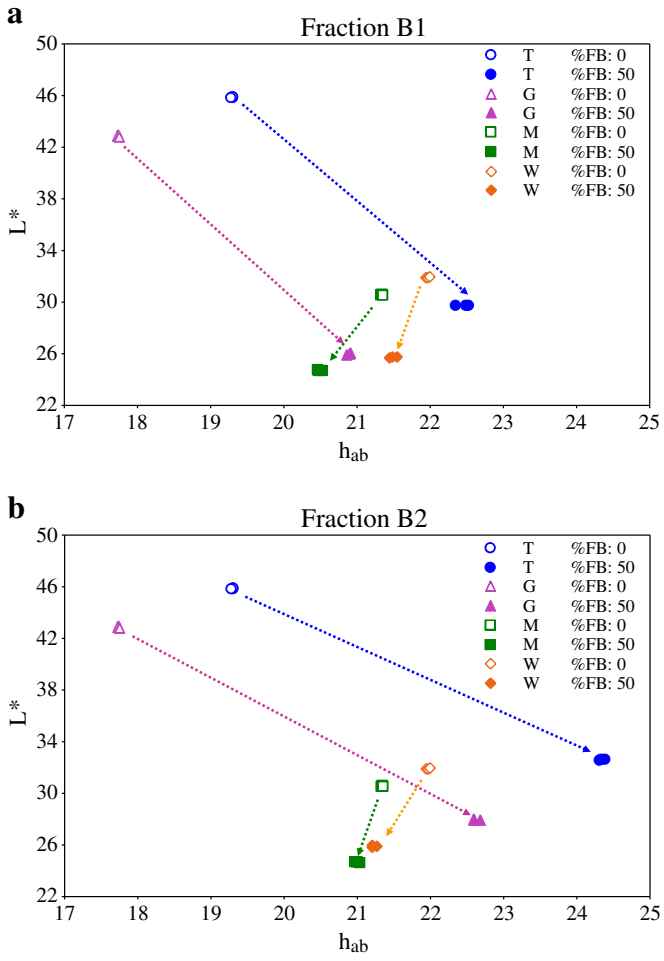

Fig. 7. Localisation area of the blending of fraction A–fraction B1 (a) and fraction A–fraction B2 (b) of the T, G, M and W wines on the (L^*, h_{ab}) diagram measured by spectroradiometry; %FB: 0, 100% of fraction A, and %FB: 50, fraction A and B (50:50).

differences increased with the increase in the proportion of fraction B2 and the involvement of Δ^2H decreased (Fig. 6a).

In general, the blends with fractions B1 and B2 in T wine increased the contribution of $\Delta^2C^*_{ab}$ in the colour differences and the involvement of Δ^2H decreased. Nevertheless, the blend with fraction B2 seemed to involve a difference in hue of the resulting solution, while with the B1 fraction this change did not occur. Accordingly, fraction B1 (pyranoanthocyanins) produces an essentially quantitative change on fraction A, confirming that observed in Table 3, showing that the C^*_{ab} values of FA decrease drastically in the blend with FB1 (FA + FB1). However, the B2 fraction (direct condensation derivatives) produces a qualitative change in colour on fraction A, related to an increase of h_{ab} values in the blend with FB2 (FA + FB2) compared with FA (see Table 3).

Furthermore, in G, unlike what happened in T, the behaviour of the colour components in the blending with pyranoanthocyanins (FB1) and direct flavanol-anthocyanin condensation products (FB2) was very similar. As shown in Fig. 6b colour variations with 33% of fraction B were mainly due to changes Δ^2L^* and to a lesser extent Δ^2H and $\Delta^2C^*_{ab}$. Nevertheless, with the increase in the proportion of fractions B1 and B2 the colour variations due to $\Delta^2C^*_{ab}$ increased and decreased the Δ^2H and Δ^2L^*.

In M, the colour differences observed in the mixture of both fractions were due primarily to changes in $\Delta^2C^*_{ab}$ (Fig. 6c) and to a lesser extent in Δ^2L^*. There were no variations in hue with blending; that is, both B fractions (B1 and B2) exerted a quantitative change in the colour of fraction A, as happened with the B1 fraction of the T wine. As in M, in W wine these colour differences between blendings were due primarily to variations in chroma and lightness (Fig. 6d).

Furthermore, in order to test the influence of the fractions with the highest proportion of anthocyanins derived in the blend, the location of these on the lightness versus hue diagram (L^*,h_{ab}) was used (Fig. 7).

In general, the fractions and their blendings were placed on the 20° hue (h_{ab}), which could be termed the zone of red-orange (Fig. 7). Although slight differences were seen, the blend of fractions % FB: 50 (1 mL FA + 1 mL FB) of T and G were located in areas further to the right than their A fractions, while the blends generated in M and W were placed more to the left than the corresponding A fractions. That is, the blends of fractions produced red-orange hues in T and G, and especially in the fractions of the T wine than with mixture of B2, located on the 25° hue (Fig. 7b), and in M and W more red-bluish hues. Regarding L^*, the A fractions showed higher L^* values than their blending, implying a decrease in the lightness of the blend.

Finally, Fig. 8a shows the location on the diagram (a^*,b^*) of the samples measured in the spectroradiometer, showing a clear separation between the monoglucoside fractions and blends. However, contrary to what was expected, the monoglucoside fractions were located farther from the axis of the coordinates than the corresponding blend, indicating a higher C^*_{ab} of the former. This could be because the colour of the blend became so intense than the spectroradiometer was interpreted as black, and decreased its value. This contrasts with the results obtained for L^* reported above, in which the L^* values in the mixtures were lower (darker) than the L^* values for the monoglucoside anthocyanins.

To clarify this aspect one resorted to the projection on the diagram (a^*,b^*) of these samples measured by the spectrophotometer (Fig. 8b). The fractions that only had monoglucosides (%FB: 0) were located in the zone between −10° and 0° of hue on the diagram (a^*,b^*), which indicates lower hues (bluish-red) of the blends while these would be located in the area between −5° and 10° (red) of the diagram (a^*,b^*). Furthermore, in contrast to what was observed with the spectroradiometer, and as expected, the mixtures were placed farther from the axis of the coordinates. Thus, the colour of blend fractions showed a high colour intensity and red-orange hues, being consistent with that observed previously on the plot (L^*,h_{ab}) for these same samples measured by spectroradiometry (Fig. 7). This study shows that use of a spectroradiometer affords the possibility of better appreciating colour differences between samples, although the spectrophotometer is more appropriate for determining, from an analytical point of view, the colorimetric behaviour of each mixture.

4. Conclusions

The colorimetric study of binary blends of phenolic fractions from Tempranillo and Graciano wines allowed us to observe that the addition of fraction B (pyranoanthocyanin derivatives –fraction B1– and direct flavanol-anthocyanin condensation products –fraction B2–) to the anthocyanin monoglucoside fraction (fraction A) resulted in colour differences perceptible by the human eye.

Through this study, which was performed by spectrophotometric and spectroradiometric techniques, it was found that the colour changes were mainly due to quantitative changes (changes in quantitative components of colour; lightness Δ^2L^* and chroma $\Delta^2C^*_{ab}$) and, to a lesser extent, qualitative changes (changes in hue Δ^2H) in the case of fractions obtained from the T and G monovarietal wines.

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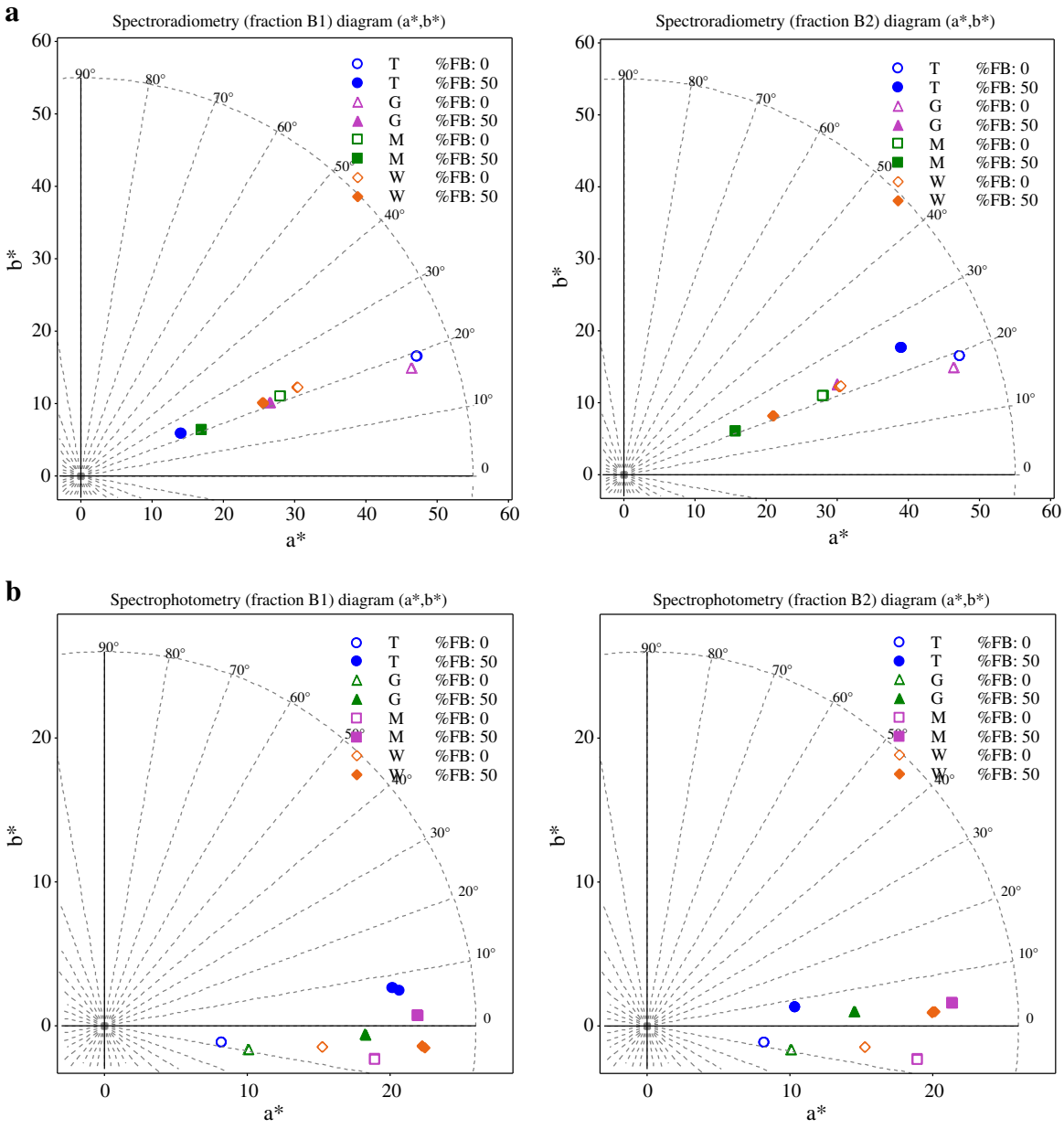

Fig. 8. Localisation area of the blending fractions of the T, G, M and W wines on the (a*,b*) diagram measured by spectroradiometry (a) and spectrophotometry (b). Fraction A–fraction B1 (fraction B1) and fraction A–fraction B2 (fraction B2); %FB: 0, 100% of fraction A, and %FB: 50, fraction A and B (50:50).

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