Influence of oak wood chips-grape mix maceration on the extraction of anthocyanins from low-extractable anthocyanin content red grapes

Berta Baca-Bocanegra[1], Julio Nogales-Bueno[1], Francisco José Heredia[1], José Miguel Hernández-Hierro[1,*]

[1] Food Colour and Quality Laboratory, Área de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain.

*** Corresponding author:** José Miguel Hernández-Hierro

Phone: +34 9545 56495

E-mail: jmhhierro@us.es

Abstract

Wine color depends not only on the amount of anthocyanin present in the grapes, but also on the amount of them that may be extracted from grapes and their interactions with other phenolic compounds. Color stabilization is also specially important in poor color wines. The main goal of this study was to evaluate the effect of the addition of oak wood chips on the extractability of anthocyanins from homogeneous grapes which were previously classified by hyperspectral image analysis.

Ten *Vitis vinifera* L. cv. Syrah grape skins from grapes previously classified as low extractable anthocyanin content by hyperspectral image analysis were underwent to simulated maceration in wine-like solution, with or without French oak (*Quercus petraea* L.) wood light toasted chips. For each sample, anthocyanin composition of the extracts was measured at the third day of maceration by HPLC chromatographic analysis and after that the obtained data were submitted to chemometric analysis.

The presence of oak wood chips in the extraction media did not cause significant changes on the anthocyanin extractability for all samples in the aforesaid homogeneous created group. The use of this technique might allow wine producers obtaining red wines that may present high color quality and stability due to the copigmentation and color preservation procedure. Hyperspectral image analysis was a crucial non-destructive tool in this study that allows to sort the berries and then use the same samples for other destructive analyses such as the evaluation of influence of oak chips-grape mix maceration on the anthocyanin extraction from grape skins.

Keywords: Anthocyanins, Chemical Composition, Chemometrics, HPLC, Grapes.

Introduction

Color is one of the main characteristic defining the quality of wines and usually the first attribute perceived by consumers. Deep color and hue are usually demanded by them. Thus, wine color is a very important parameter for winemakers who want to get high quality wines. Phenolic compounds participate in astringency, bitterness and color. Among them anthocyanins extracted from grape skin are the principal compounds involved in the color of red wines and their interactions with other phenolic compounds (called copigments), normally colorless, allow improving the color stabilization of aged wines by copigmentation reactions [1,2]. Factors such as cultivar, growing region, climate, and growth conditions may influence the levels of anthocyanins [3-7].

The expression of the color in red wines depends not only on the amount of anthocyanin present in the grapes, but also on the amount of them that may be extracted from grapes. Several factors have been shown to affect the extraction of phenolic compounds into the must [8]. Due to their vacuolar location, the diffusion of anthocyanins into the must requires the break of their skin cell wall. Riper grapes have higher cell wall degradation hence they have higher extraction degree [8,9]. This parameter is also linked to grape maceration conditions [10]. Soluble solids content of grape must and different stages of ripening also affect the amount of extractable phenols from grape skins [11-14].

The stressful climate conditions typical of the warm regions make difficult to obtain high quality red wines, with high intensity and stable color especially when they are subjected to ageing process. This event normally occurred since the phenolic and technological maturity of the grapes do not happen at the same time [5], and so, at the moment of harvesting the grapes have high sugar content but phenolic unripe [15]. Wines made from these grapes, low in pigments and cofactors, are not able to form

much copigmentation [1] and as a result, the color stabilization does not correctly develop.

Taking into account these considerations, other alternatives winemaking techniques have been used aimed at implementing the production of high quality red wines in the last years. The procedures intended to enhance the extraction of grape components responsible for the color of wine or added other which allow improving the color stabilization such as copigments.

It is well known that the presence of copigments in red wines improve and stabilize its color by copigmentation reaction [7,16,17]. Wood is a natural source of phenols [18] that could be implied into copigmentation reactions. So adding oak wood chips as a source of copigments such as flavanols or ellagitannin could be a good alternative to resolve the problem of wine color stabilization. Anthocyanins have demonstrated to react with flavanols, involving acetaldehyde or not, to generate either acetaldehyde-derived or direct flavanol-anthocyanin condensation product [19-21]. The formation of most of anthocyanin-derived pigments is directly related to the presence in the wine of compounds such as proanthocyanidins or acetaldehyde. Oak ellagitannins have demonstrated to indirectly influence the formation of some of these derivative pigments. Their structure allow them to participate in oxidation reactions acting as consumers of oxygen and then protecting other compounds such as anthocyanins against oxidation, favoring the formation of acetaldehyde from ethanol in the medium [22] and favoring several polymerization reactions [22,23]. In this way, the ellagitannins have the ability to modify the color of the red wine both indirectly as just indicated as directly, through the products derived from the direct reaction between anthocyanins and ellagitannins. So, anthocyanins can react with *C*-glucosidic ellagitannins to generate anthocyanin-ellagitannin hybrid pigments such as A-type and B-type vitisins [24,25]. These

pigments are more stable against hydration than their anthocyanin precursor [25]. The traditional source of ellagitannins is the oak barrels where the wine is kept during the ageing process [26,27]. Nevertheless, they can also be released from the chips or staves. The main goal of this study is to evaluate the effect of the addition of oak wood chips as copigment sources on the anthocyanin extraction from grape skins. Those grapes were previously classified by hyperspectral image analysis according to the amount of anthocyanins transferred to the extraction media.

Material and methods

Samples

Grapes of two red *V. vinifera* L. varieties (cv. Syrah and Tempranillo) were collected from two vineyards located in the Condado de Huelva Designation of Origin D.O. (Andalusia, Spain), which is under warm climatic conditions influence [7]. Grapes were collected when the vineyards were harvested (August 27 and 12, 2013 respectively). One hundred single berries were collected for each variety. In order to achieve representative grape samples, these were collected from both sides (sunlight and shade) of vines located in different rows within the vineyard. Edge rows and the first two vines in a row were avoided. Samples were collected from the top, middle, and bottom of the different clusters. After that, samples were refrigerated and they were immediately carried to the laboratory, tempered and subjected to the hyperspectral analysis.

Sample selection by hyperspectral analysis

Hyperspectral imaging was used to classify the grapes according to the amount of anthocyanins transferred to the extraction media [28]. Hyperspectral imaging device (Infaimon S.L., Barcelona, Spain) comprised a Xenics® XEVA-USB InGaAs camera

(320 x 256 pixels; Xenics Infrared Solutions, Inc., Leuven, Belgium), a spectrograph (Specim ImSpector N17E Enhanced; Spectral Imaging Ltd., Oulu, Finland) covering the spectral range between 900 and 1700 nm (spectral resolution of 3.25 nm). The individual hyperspectral image of each grape was recorded. After calibration and segmentation processes, the average spectral profile for each grape was saved. Noisy wavebands at both extremes of the spectral range were removed. Thus, only spectral data in the resulting effective wavelength of 950−1650 nm regions were used in data analysis because of reduced efficiency outside this range in the used device. Gapes were classified as high or low extractable anthocyanin content according to the values predicted by the hyperspectral analysis.

Determination of extractable anthocyanin content

Grape skins were separated manually from the whole grapes and they were weighted, then grape skins were immediately frozen and stored an -20 ºC until analysis were performed. Selected Syrah grape skins was split into two parts, then were underwent to simulated maceration in wine-like solution (12.5% ethanol, 4 g L^{-1} of tartaric acid adjusted at pH 3.6 with NaOH 0.5 M), with or without oak wood chips as a control. French oak (*Quercus petraea* L.) wood light-toasted chips of 1 cm^2 average size (Tonelería Martín y Vázquez, Logroño, Spain) were added to the wine-like solution in a 4 g L^{-1} ratio. Grape skins were added to extraction media in a 1:20 ratio. For each sample, anthocyanin composition of the extracts was measured at the third day of maceration at ambient temperature and without agitation. In order to measure the extractable anthocyanin content, the supernatant was diluted 1:2 with 0.1M HCl, filtered through 0.45 µm pore size filters and directly injected into the chromatographic system. Anthocyanin compounds were identified by HPLC analysis at 520 nm and comparing their retention times and spectral characteristics with data reported by relevant literature

references [29,30]. All analyses were performed in duplicate. Fig. 1 shows the whole analytical workflow process.

Statistical analysis

Statistical analysis was carried out using Statistica version 8.0 software (Statistica, 2007). Principal component analysis was used as an unsupervised pattern recognition method in order to obtain a general overview about the samples taking into account individual, acylated, non acylated and total anthocyanins as studied variables. Furthermore, univariate analysis of variance (ANOVA) was applied to discriminate among the means of chemical data (i.e. individual, acylated, non acylated and total anthocyanins) taking into account pairs of control wines and wines with addition of oak wood chips. The statistically significant level was considered at $\alpha=0.05$.

Results and discussion

Sample selection and anthocyanins analysis

Extractable anthocyanin content of Syrah grapes ranged from 0.02 to 3.67 mg g^{-1} of skin grapes expressed as malvidin 3-*O*-glucoside equivalents with a standard deviation value of 0.63 mg g^{-1} of grape skin (n=98). Tempranillo grapes ranged from 0.23 to 3.35 mg g^{-1} of grape skin expressed as malvidin 3-*O*-glucoside equivalents with a standard deviation value of 0.54 mg g^{-1} of grape skin (n=99). The results reveal a wide heterogeneity of values. Levels of red grapes (*Vitis vinifera* L.) phenolic compounds depend on a number of factors including the variety of grape, high or low skin: volume ratio, growing region, climate, and growth conditions [31]. Furthermore, similar heterogeneity can be also found within the same physiological stage. Some studies describe a Gaussian bell-shaped distribution of soluble solids and extractable total phenolic content in a sampling

point [14,32]. Thus, there is substantial variation in levels of phenolic compounds generally.

Since extractable content of anthocyanin is lower in Syrah grapes than Tempranillo grapes the aforesaid cultivar was considered the most interesting variety for the assay. Statistical median value (1.8 mg g^{-1} of grape skin) was used as cut-off value. Ten Syrah grapes classified by the hyperspectral prediction model as low extractable anthocyanin grapes were used in that assay (extractable anthocyanin content less than 1.8 mg g^{-1} of grape skin).

The separation carried out by HPLC allowed the quantification of 15 anthocyanins. Taking into account their basic structure, anthocyanins were also grouped as acetyls anthocyanins (Delphinidin-3-*O*-(6′-acetyl)-glucoside, Cyanidin-3-*O*-(6′-acetyl)-glucoside, Petunidin-3-*O*-(6′-acetyl)glucoside, Peonidin-3-*O*-(6′-acetyl)glucoside, Malvidin-3-*O*-(6′-acetyl)glucoside), coumaroyls anthocyanins (Cyanidin-3-*O*-(6′-*p*-coumaroyl)glucoside, Petunidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*trans*), Malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*cis*), Peonidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*trans*), Malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*trans*)), non-acylated anthocyanins (Delphinidin 3-*O*-glucoside, Cyanidin 3-*O*-glucoside, Petunidin 3-*O*-glucoside, Peonidin 3-*O*-glucoside, Malvidin 3-*O*-glucoside) and acylated anthocyanins as sum of acetyls and coumaroyls anthocyanins. The sum of all them was also expressed as total anthocyanins. The results were expressed as mg of malvidin-3-*O*-glucoside equivalents per gram of grape skin. Fig. 2 shows total extractable anthocyanin content obtained for each sample with and without oak wood chips by HPLC analysis. The results obtained in this assay, taking into account only the samples without oak chips, are in accordance with values previously predicted by the hyperspectral method; only two samples have total extractable anthocyanin content slightly higher than 1.8 mg g^{-1} of grape skin. These

results confirm that the aforementioned hyperspectral method presents a good potential for the classification of grapes according to extractable anthocyanin contents.

Changes in anthocyanin extraction

Principal component analysis was used as an unsupervised pattern recognition method in order to obtain a general trend of the samples. Fig. 3 shows the projection of the samples on the plane defined by the first and second principal component. The first principal component (PC1) describes 54.72 % of the variability in the data and the second (PC2) describes 26.10 %. In the score plot the samples are represented according to the presence or absence of oak wood chips. In this graph it is not possible to observe a trend among extractable anthocyanin contents from samples extracted in model wine solution with or without oak wood chips.

Moreover, a univariate analysis of variance was carried out using extractable anthocyanin contents as dependent variables and presence or absence of oak wood chips in the model wine solutions as independent variable. Results are shown in Table 1. No significant differences were found among wines and for each variable after a maceration process with oak wood chips ($p>0.05$). This univariate analysis confirms the previous general overview obtained in the PCA analysis and highlights that none of the studied variables has been perturbed by presence of oak wood chips.

The addition of oak wood chips to extraction media neither resulted in a significant increase nor a decrease of the extractable anthocyanin content. Therefore it could be reasonable to assume that did not take place adsorption of the anthocyanins on the chips surface. Thus, the anthocyanins present in the media are available to participate in the suitable chemical reactions that lead to new derivatives resulting in changes of wine color. Apart from that, oak derived compounds transferred to the media such as flavanols or ellagitannins can react with anthocyanins to generate anthocyanin-

ellagitannin hybrid pigments more stable than their precursor [24]. Indirectly, ellagitannins also affect the wine color since they protect anthocyanins from oxidation [22] and favoring several polymerization reactions [22,23].

Conclusions

The hyperspectral method previously described was used here for the selection of samples and presents a good potential for the classification of grapes according to extractable total anthocyanin contents.

Moreover, it can be assumed from the results that the presence of oak wood chips in the extraction media did not cause significant changes in the anthocyanin extractability for all samples. This practice does not promote the extraction of anthocyanins from skin but extraction of specific colorless oak chips compounds which could stabilize wine color protecting anthocyanins against oxidation or interacting with them to generate more stable derivatives.

Taking into account, the addition of oak wood chips may be a good technique to obtain red wines that may present better color quality and stability due to the copigmentation procedure. However, further studies would be necessary in order to assess the effect that other sources of copigments or other amount of them have in the anthocyanin extraction.

Acknowledgments

The Spanish Ministerio de Economía y Competitividad is thanked for project AGL-2014-58486-C2. Universidad de Sevilla is thanked for B. Baca-Bocanegra predoctoral grant (VPPI-II.2) and J. Nogales-Bueno postdoctoral grant (VPPI-II.4). The authors thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

1. Boulton R (2001) The copigmentation of anthocyanins and its role in the color of red wines. A critical review. Am J Enol Vitic 52:67-87

2. Gordillo B, Cejudo-Bastante MJ, Rodriguez-Pulido FJ, Gonzalez-Miret ML, Heredia FJ (2013) Application of the differential colorimetry and polyphenolic profile to the evaluation of the chromatic quality of Tempranillo red wines elaborated in warm climate. Influence of the presence of oak wood chips during fermentation. Food Chem 141:2184-2190

3. Downey MO, Dokoozlian NK, Krstic MP (2006) Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am J Enol Vitic 57:257-268

4. Ferrer-Gallego R, Hernandez-Hierro JM, Rivas-Gonzalo JC, Escribano-Bailon MT (2012) Influence of climatic conditions on the phenolic composition of *Vitis vinifera* L. cv. Graciano. Anal Chim Acta 732:73-77

5. Mira de Orduña R (2010) Climate change associated effects on grape and wine quality and production. Food Res Int 43:1844-1855

6. Ryan JM, Revilla E (2003) Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. J Agric Food Chem 51:3372-3378

7. Gordillo B, Rodriguez-Pulido FJ, Mateus N, Escudero-Gilete ML, Gonzalez-Miret ML, Heredia FJ, de Freitas V (2012) Application of LC-MS and tristimulus colorimetry to assess the ageing aptitude of Syrah wine in the Condado de Huelva D.O. (Spain), a typical warm climate region. Anal Chim Acta 732:162-171

8. Ribéreau-Gayon P, Dubourdieu D, Doneche B, Lonvaud A, Glories Y, Maujean A, Branco JM (2006) Handbook of Enology, The microbiology of wine and vinifications. vol v. 1. J. Wiley & Sons, Chichester, England.

9. Hernandez-Hierro JM, Quijada-Morin N, Martinez-Lapuente L, Guadalupe Z, Ayestaran B, Rivas-Gonzalo JC, Escribano-Bailon MT (2014) Relationship between skin cell wall

composition and anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo at different grape ripeness degree. Food Chem 146:41-47

10. González-Manzano S, Rivas-Gonzalo JC, Santos-Buelga C (2004) Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal Chim Acta 513:283-289

11. Canals R, Llaudy MC, Valls J, Canals JM, Zamora F (2005) Influence of the physiological stage and tehe content of soluble solids on the anthocyanin extractability of *Vitis vinifera* L.cv. Tempranillo grapes. Anal Chim Acta 53:4019-4025

12. Fournand D, Vicens A, Sidhoum L, Souquet JM, Moutounet M, Cheynier V (2006) Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. J Agric Food Chem 54:7331-7338

13. Hernandez-Hierro JM, Quijada-Morin N, Rivas-Gonzalo JC, Escribano-Bailon MT (2012) Influence of the physiological stage and the content of soluble solids on the anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo grapes. Anal Chim Acta 732:26-32

14. Zouid I, Siret R, Jourjon F, Mehinagic E, Rolle L (2013) Impact of grapes heterogeneity according to sugar level on both physical and mechanical merries properties and their anthocyanins extractability at harvest. J Texture Stud 44:95-103

15. Mori K, Sugaya S, Gemma H (2005) Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. Sci Hortic-Amsterdam 105:319-330

16. González-Manzano S, Dueñas M, Rivas-Gonzalo JC, Escribano-Bailón MT, Santos-Buelga C (2009) Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. Food Chem 114:649-656

17. Gordillo B, Rodriguez-Pulido FJ, Gonzalez-Miret ML, Quijada-Morin N, Rivas-Gonzalo JC, Garcia-Estevez I, Heredia FJ, Escribano-Bailon MT (2015) Application of differential colorimetry to evaluate anthocyanin-flavonol-flavanol ternary copigmentation interactions in model solutions. J Agric Food Chem 63:7645-7653

18. De Rosso M, Cancian D, Panighel A, Dalla Vedova A, Flamini R (2008) Chemical compounds released from five different woods used to make barrels for aging wines and spirits: volatile compounds and polyphenols. Wood Sci and Technol 43:375-385

19. Escribano-Bailón T, Dangles O, Brouillard R (1996) Coupling reactions between flavylium ions and catechin. Phytochemistry 41:1583-1592

20. Francia-Aricha EM, Guerra MT, Rivas-Gonzalo JC, Santos-Buelga C (1997) New anthocyanin pigments formed after condensation with flavanols. J Agric Food Chem 45:2262-2266

21. Rivas-Gonzalo JC, Bravo-Haro S, Santos-Buelga C (1995) Detection of compounds formed through the reaction of malvidin 3-monoglucoside and catechin in the presence of acetaldehyde. J Agric Food Chem 43:1444-1449

22. Vivas N, Glories Y, Lagune L, Saucier C, Augustin M (1994) Estimation du degré de polymérisation des procyanidines du raisin et du vin par la méthode au p-dimethylaminocinnamaldéhyde. J Int Sci Vigne Vin 28:319-336

23. Timberlake CF, Bridle P (1976) Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. Am J Enol Vitic 27:97-105

24. Quideau S, Jourdes M, Lefeuvre D, Montaudon D, Saucier C, Glories Y, Pardon P, Pourquier P (2005) The chemistry of wine polyphenolic C-glycosidic ellagitannins targeting human topoisomerase II. Chemistry 11:6503-6513

25. Chassaing S, Lefeuvre D, Jacquet R, Jourdes M, Ducasse L, Galland S, Grelard A, Saucier C, Teissedre P-L, Dangles O, Quideau S (2010) Physicochemical studies of new anthocyano-ellagitannin hybrid pigments: about the origin of the influence of oak C-glycosidic ellagitannins on wine color. Eur J Org Chem 1:55-63

26. García-Estévez I, Escribano-Bailón MT, Rivas-Gonzalo JC, Alcalde-Eon C (2012) Validation of a mass spectrometry method to quantify oak ellagitannins in wine samples. J Agric Food Chem 60:1373-1379

27. Cadahia E, Varea S, Muñoz L, Fernandez de Simon B, García-Vallejo MC (2001) Evolution of ellagitannins in Spanish, French, and American oak woods during natural seasoning and toasting. J Agric Food Chem 49:3677-3684

28. Nogales-Bueno J, Baca-Bocanegra B, Rodríguez-Pulido FJ, Heredia FJ, Hernández-Hierro JM (2015) Use of near infrared hyperspectral tools for the screening of extractable polyphenols in red grape skins. Food Chem 172:559-564

29. Garcia-Marino M, Hernandez-Hierro JM, Rivas-Gonzalo JC, Escribano-Bailon MT (2010) Colour and pigment composition of red wines obtained from co-maceration of Tempranillo and Graciano varieties. Anal Chim Acta 660:134-142

30. Hernández-Hierro JM, Nogales-Bueno J, Rodríguez-Pulido FJ, Heredia FJ (2013) Feasibility study on the use of near-infrared hyperspectral imaging for the screening of anthocyanins in intact grapes during ripening. J Agric Food Chem 61:9804-9809

31. Crozier A, Clifford MN, Ashihara H (2006) Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet. Blackwell Publishing, Oxford, England

32. Kontoudakis N, Esteruelas M, Fort F, Canals JM, De Freitas V, Zamora F (2011) Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. Food Chem. 124:767-774

Figure captions

Fig. 1 Schematic representation of the entire process. Hyperspectral screening of the predicted extractable total anthocyanin content, model wines elaboration, macerations and chromatographic analyses of the extractable anthocyanin contents

Fig. 2 Total extractable anthocyanins content (mg g^{-1} of grape skin) for each sample with or without oak wood chips. Horizontal line that intersects the axis "y" in the point 1.8 shows cut-off value to select the assay samples

Fig. 3 Score plot of samples in the space defined by PC1 and PC2 codified as with or without addition of oak wood chips