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Relationship between skin cell wall composition and anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo at different physiological stages and contents of soluble solids

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Abstract. The relationship between cell wall composition and extractability of anthocyanins from red grape skins was assessed in nine Tempranillo grape samples harvested at three stages of ripening (pre-harvest, harvest and over-ripening) and three different contents of soluble solids within each stage (22, 24 and 26°Brix). Principal components analysis was applied to the obtained data set in order to establish relationship between cell wall composition and the extractability of anthocyanins.
Results showed the influence of physiological stage and °Brix on cell wall composition. Furthermore, total insoluble material exhibits the biggest opposition to anthocyanin extraction, while the highest amounts of cellulose, rhamnogalacturonans-II (RG-II) and polyphenols were positively correlated with anthocyanin extraction.

Introduction. Anthocyanins are responsible for the colour of red wines and their interactions with other phenolic compounds largely determine the colour changes observed during ageing. These compounds, mainly located in grape skins, are released to the maceration media during winemaking. It is generally accepted that extractability of anthocyanins from grape skins increases throughout grape ripening as consequence of the degradation of the cellular wall by pectolytic enzymes [1]. In addition, differences on contents of polysaccharides based on galactose and arabinose, together with changes on the cellulose content and degree of methylation of pectins might also be responsible for the different extractabilities of these phenolic compounds [2]. Cell wall composition is also responsible for its physical properties which are also linked to the anthocyanin extractability.
The aim of this study was to establish relationship between cell wall composition and the extractability of anthocyanins. Furthermore, the influence of different physiological stages and soluble solids heterogeneity within each stage on the cell wall composition was also evaluated.

Materials and Methods. Three different physiological stages (pre-harvest, harvest and over-ripening) and three soluble solids contents (22, 24 and 26 °Brix) within each stage were taken into account using *Vitis vinifera* L. cv. Tempranillo which is the most often used variety to produce quality red wines in Spain. Grape skins were separated manually from the whole grapes and subsamples were taken from each sample. Cell wall material was isolated from grape skins as the 70% ethanol residue following a modification of de Vries *et al.* procedure [3] adapted for grape skins as described elsewhere in Apolinar-Valiente *et al.* [4]. The isolated cell wall material was analyzed in order to ascertain its composition in each case. Cellulose was determined as glucose in accordance with Lurie *et al.* [5] using the phenol method proposed by Doubois *et al.* for its spectrophotometric determination [6]. Lignin was gravimetrically determined as Klason lignin [7, 8]. Non-cellulosic polysaccharides were obtained using an iterative calculation methodology [9] from monosaccharide profiles determined by GC/MS following a modification of Guadalupe *et al.* procedure [10]. Moreover, protein (Bradford method), total polyphenols index (Folin-Ciocalteau method) and the degree of esterification of pectins (i.e. the percentage of total uronic acids that are esterified) were also determined [6, 8]. Anthocyanin extractabilities previously obtained as described elsewhere in Hernández-Hierro *et al.* [11] were used. Anthocyanin extractability was calculated as percentages using the ratio between hydroalcoholic and exhaustive methanolic extractions both determined by RRLC. The maximum percentages of extraction were achieved at the third day of maceration and these

values were used for the subsequent data analysis. The unsupervised pattern recognition method used for data analysis was principal components analysis (PCA), which was applied to the correlation matrix of the original variables. The SPSS 13.0 for Windows software package (SPSS, Inc., Chicago, IL) was used for data processing.

Results and discussion. Results showed some influence of physiological stage and ºBrix on cell wall composition. Generally, the most important factor was the physiological stage even though there was not a clear pattern for any component. **Figure 1** shows the loading plot of the PCA. Total insoluble material exhibits the biggest opposition to both anthocyanin extractions, slightly higher for non-acylated anthocyanin extraction, while the highest amounts of cellulose, rhamnogalacturonans-II (RG-II) and polyphenols are positively correlated with anthocyanin extraction. This analysis also reveals the existence of more slightly oppositions to anthocyanin extraction for the rest of the studied parameters (i.e. the remaining polysaccharides, protein, lignin and degree of esterification of pectins). It is also noteworthy that almost all polysaccharides present the same trend in the PCA loading plot with the exception of RG II, which is the most branched polysaccharide.

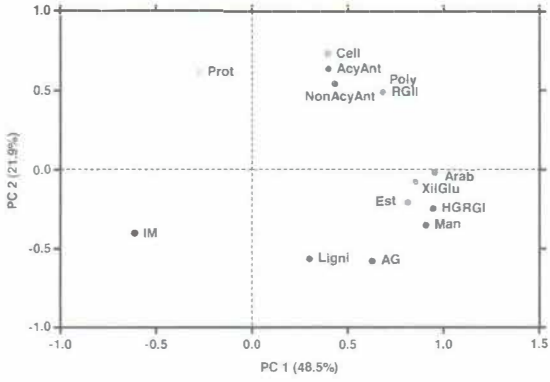

Figure 1. Loading plot of the PCA. IM (insoluble material); Prot (protein); Poly (polyphenols); Ligni (lignine); Cell (cellulose); AG (arabinogalactans); Man (mannan); HG (homogalacturonans); XilGlu (xiloglucans); Arab (arabinans); RGI (rhamnogalacturonans-I); RGII (rhamnogalacturonans-II); ester (degree of esterification of pectins); AcyAnt (acylated anthocyanins); NonAcyAnt (non-acylated anthocyanins).

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