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(Article begins on next page)

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Relationship between agronomic parameters, phenolic composition of grape skin and texture properties of *Vitis vinifera* L. cv. Tempranillo

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1　**Abstract**

2　The relationship between the agronomic parameters of grapevine and the phenolic

3　composition of skin of *Vitis vinifera* L. cv. Tempranillo grapes was assessed. Physical

4　and mechanical properties of berries and their skins were also determined and correlated

5　to the chemical composition. Results showed a significant negative correlation between

6　grapevine vigor-related parameters (such as leaf area and bunch weight) and

7　anthocyanin composition, whereas the percentage (*w/w*) of seeds was negatively

8　correlated with the amount of flavanols of grape skins. Texture properties of grape skins

9　also showed an important relationship with chemical composition. Berry hardness

10　showed a negative correlation with the coumaroyl-anthocyanin derivatives but it was

11　positively correlated to skin flavanic composition. Moreover, significant regressions

12　with high coefficients of determination were found between phenolic composition and

13　grapevine vigor-related and texture variables, thus pointing out that these parameters

14　might be useful for estimating phenolic composition of grape skins.

15　**Keywords:** phenolics, anthocyanins, flavanols, Tempranillo red grapes, HPLC-DAD-

16　MS^n, grapevine vigor, mechanical properties

Introduction

Important wine organoleptic properties as color, bitterness and astringency are strongly influenced by the phenolic composition of grapes, which, in turn, also provides important information about the ageing potential of wines.[1] Anthocyanins, which are extracted from grape skins, are the main responsibles for wine color. In grapes, not only the monoglucosides of anthocyanidins are present, but also the acetyl, caffeoyl and *p*-coumaroyl derivatives and even other unusual glycoside-derivatives, such as galactosides.[2] In Tempranillo cultivar, monoglucosides are the main anthocyanins and acetic acid and *p*-coumaric acid are the most common acids esterifying the glucose moiety.[3] Although monoglucosides of anthocyanidins are the major pigments, acyl derivatives can play an important role in wine color stability since acylation can be related to an increase of the anthocyanidin stability against light, temperature or pH changes.[4] Moreover, the presence of a cinnamic acid, such as *p*-coumaric or caffeic acid, in the structure can favor intramolecular copigmentation processes, and, as a consequence, changes in anthocyanin color in comparison with the original non-acylated pigment.[5]

Flavanols are related to wine astringency and bitterness,[6] although they can also play an important role in long-term color stability.[7] Grape flavanols slightly differ in their structure and in their organoleptic properties according to their origin. Flavanols from grape seed derive from (epi)catechin and show higher levels of galloylation, whereas grape skin contain both catechins and gallocatechins and the corresponding derived proanthocyanidins.[8, 9] Furthermore, flavanol galloylation has been associated with more tannic and coarse notes in wine,[10] whereas higher levels of prodelphinidins in wines have as a consequence a reduction of these negative perceptions.[11] Moreover,

41   Kennedy[12] has pointed out that winemakers prefer winemaking procedures leading to an

42   increase of flavanol levels from skins and to a less extraction from seeds.

43   Accumulation of phenolic compounds in red grapes takes place gradually during

44   ripening[13] and their content at harvest time considerably depends on cultivar,

45   agronomical practices, canopy microclimate, and bunch exposure.[14-16] It has been

46   reported in literature that as vine vigor decreased, total soluble solid in grapes, total

47   phenolics and anthocyanin content in wines increased.[17, 18] In particular, Cortell and co-

48   workers[19] have reported greater anthocyanin accumulation in the low-vigor grapevines

49   and significant increases in skin flavanol contents in berries harvested from zones with a

50   reduction in vine vigor. However, it seems that vine vigor has not a significant influence

51   on the flavanol concentration in seeds.[20] Furthermore, although grapevine vigor is

52   mainly related to climatic conditions, it has been documented the occurrence of

53   important differences in grapevine vigor even for an established vineyard with identical

54   grape variety, age, and vineyard management practices. These differences have been

55   related to variations in topography, physical and chemical characteristics of the soil.[20-22]

56   As a result, it could be found within the same vineyard important differences on the

57   levels of acids, anthocyanins, and phenolics that can lead to variations on composition

58   and quality of wines.[23, 24]

59   The numerous physiological and chemical changes that grape berries undergo during

60   grape ripening induce not only modifications on their chemical composition but also in

61   their texture features.[25] These textural modifications have been studied through the

62   evaluation of the grape mechanical properties, which in turn, have been correlated to

63   grape quality.[26, 27] A strong relationship between texture parameters and phenolic

64   ripeness degree and grape variety has been reported.[28-30] In addition, these textural

65   parameters have been demonstrated to be an useful tool to study phenolic extractability

66   from grape skins.[31] However, the studies in literature about the relationship between

67   grapevine-related characteristics, berry mechanical properties and phenolic composition

68   of grapes are scarce.

69   Due to the importance of phenolic compounds for wine organoleptic properties,

70   phenolic composition has to be taken into account for the selection of harvest date.

71   However, the harvest date is traditionally and chiefly selected based on the

72   technological maturity of grapes, which is related to sugar concentration of grapes and

73   therefore determines the alcohol content of wine. Nevertheless, the environmental and

74   climatic conditions may cause technological maturity to be reached before phenolic

75   maturity, and it seems that global climate change is going to increase this delay,[32]

76   making even more difficult to choose the appropriate harvest date in order to obtain

77   high quality wines. For this reason, the knowledge about detailed phenolic composition

78   of grapes can be helpful to establish strategies for harvest planning.

79   The purpose of this study was to evaluate the usefulness of parameters related to

80   grapevine vigor and grape texture as indicative tools of the grape skin phenolic

81   composition. Specifically, the main objective of this work was to study the relationship

82   between the phenolic composition of *Vitis vinifera* L. cv. Tempranillo grape skins and

83   the vigor-related grapevine characteristics. In addition, the relation between texture

84   properties of the berries and their phenolic composition has also been assessed.

85   **Materials and Methods**

86   *Samples*

87   Thirteen different locations of a vineyard (100 ha) located in Zamora, Spain

88   (coordinates 41°18'26"N 5°21'45"W), were selected based on different orographic

89   terrain features, such as orientation, altitude and slope. For each location, all the grapes

90   (*Vitis vinifera* L. cv Tempranillo) from two different grapevines were collected. All

91   grape samples were collected in the same day at harvest time. Grape samples consisted

92   of 300 berries randomly-selected from all collected grapes.

93   *Analysis of phenolic composition*

94   Skins were manually separated from berries and extracted following Ferrer-Gallego

95   and co-workers.[33] The detailed phenolic composition of grape skins (mg/g of skin) was

96   analyzed by means of HPLC-DAD-MS. Grape-skin extracts were directly analyzed for

97   determining anthocyanin composition whereas it was fractionated as explained bellow

98   before analysis of flavanols. In both cases, HPLC analyses were performed in a

99   Hewlett–Packard 1200 Series HPLC (Agilent Technologies, Waldbronn, Germany).

100   Mass spectrometry was carried out using an API 3200 Qtrap equipped with an ESI

101   source and a triple-quadrupole linear ion trap mass analyzer that was controlled by

102   Analyst 5.1 software (Applied Biosystems, Darmstadt, Germany). All the analyses were

103   performed in triplicate.

104   Anthocyanin composition was determined by using the methodology described by

105   Alcalde-Eon and co-workers.[3] Twenty-three different anthocyanins were identified and

106   quantified, and grouped into eleven variables depending on the type of anthocyanidin

107   and on the type of anthocyanin derivative (see **Table 1**). Quantification was performed

108   by HPLC-DAD using external calibration curves of standards of 3-*O*-glucosides of

109   delphinidin, cyanidin, petunidin, peonidin and malvidin, purchased from Extrasynthèse

110   (Lyon, France). Each determined anthocyanin was quantified using the calibration curve

111   of the corresponding anthocyanin monoglucoside.

112   In order to analyze flavanols and phenolic acids, grape-skin extracts were

113   fractionated prior to HPLC-DAD-MS analysis with the objective of eliminating the

114   anthocyanins. Fractionation was carried out according to the procedure described by

115   González-Manzano and co-workers for wine samples.[34] Chromatographic analysis was

116   performed following the methodology reported by Ferrer-Gallego and co-workers.[10]

117   Detection was carried out at 280 nm (proanthocyanidins) and 330 nm (phenolic acids)

118   as the preferred wavelengths. Quantification was performed by HPLC-DAD using

119   external calibration curves of purchased standards, unless standards of dimeric and

120   trimeric procyanidins which were isolated in our laboratory as described by González-

121   Manzano and co-workers.[34] Nineteen different flavanols were determined and grouped

122   into twelve variables depending on the type of flavanol and the polymerization degree

123   (see Table 1). The calibration curves of catechin, dimeric procyanidin and trimeric

124   procyanidin were employed for quantifying catechin and epicatechin, dimeric

125   procyanidins and trimers and tetramers of procyanidins respectively. Galloylated

126   procyanidins were quantified using the epicatechin 3-*O*-gallate calibration curve,

127   whereas gallocatechins and prodelphinidins were quantified using the gallocatechin

128   calibration curve. Two hydroxybenzoic acids and eleven hydroxycinnamic acids and

129   their tartaric esters or glucosidic derivatives were determined and grouped into seven

130   variables (see Table 1). Hydroxybenzoic acids and hydroxycinnamic acids were

131   quantified using the gallic acid and *p*-coumaric acid calibration curves respectively.

132   *Biophysical and technological variables*

133   Eight different biophysical variables were studied (see Table 1), which were also

134   determined at harvest time for each grapevine selected. Data are the average of the

135   values determined for the two grapevines of the same location. Leaf area (m^2) was the

136   total leaf area of grapevine. In order to calculate this value, the number of long,

137   medium-length and short vine shoot of each grapevine was determined. Considering

138   that long vine shoots have in average 20 knots with 4 big-size leafs each one, whereas

139   medium-long ones have 12 knots with 3 medium-size leafs each one and short vine

140   shoots have 8 knots with 2 small-size leafs each one, the total number of leafs of each

141 size could be calculated. The average area of each kind of leaf was determined from the

142 area of 10 leafs of each size, which was used to calculate the total leaf area. The grape

143 production (kg) was the total weight of bunches of each grapevine. The average weight

144 of bunches was calculated as the average of the weight of all bunches collected from the

145 same grapevine. The average weight of berries was calculated from the weight of 50

146 different berries collected from the same grapevine. Moreover, the percentage (*w/w*)

147 that skin and seeds represented in berry weight was also measured after manual

148 separation of skin and seeds from berries. Grapevines were also pruned after leaf fall

149 allowing us to calculate the weight of fresh wood. The pruned wood was then dried for

150 72 h at 60ºC and the weight of dried wood was determined.

151 ºBrix and pH were directly measured in the grape must by using an optical

152 refractometer and a pH-meter, respectively. Titratable acidity was calculated after acid-

153 base titration of must employing NaOH 0.1 M and expressed as tartaric acid equivalents

154 (g/L).[35]

155 *Instrumental mechanical properties*

156 The mechanical properties of the berries were assessed following Letaief and co-

157 workers methods.[36] A whole-berry texture profile analysis (TPA) double-compression

158 test was carried out at a test speed of 1 mm/s until 25% of sample deformation (2

159 seconds waiting time between compressions), with the hardness (N), gumminess (N)

160 and chewiness (mJ) parameters calculated from the force-distance curve.[36] Berry skin

161 break force (F_{sk}, N) was evaluated with a puncture test on the intact berry performed at

162 a test speed of 1 mm/s until 3 mm of sample deformation,[36] while the berry skin

163 thickness (Sp_{sk}, μm) was assessed with a 0.2 mm/s compression of a piece of skin using

164 a 2-mm flat cylindrical probe.[36] These parameters were determined analyzing 30

165 randomly selected berries collected from the two grapevines of each location.

166   *Statistical analysis*

167   Principal component analysis (PCA) was used for data analysis as unsupervised

168   pattern recognition method. The data matrix was constituted by the values determined

169   for all the 46 variables described in Table 1 for each selected location. Correlation

170   analyses were carried out and Pearson's coefficient and the two-tailed *p*-value were

171   obtained. Backward stepwise multiple linear regression (MLR) was performed in order

172   to assess the relation between phenolic composition and the rest of variables. The

173   coefficient of determination (R^2) and the signification (*p*-value, bilateral) of the built

174   models were studied. The software package IBM® SPSS® Statistics v. 21.0 (IBM,

175   Armonk (NY), USA) was used for data processing.

176   **Results and Discussion**

177   *Study of correlations*

178   Principal component analysis was conducted as unsupervised pattern recognition in

179   order to observe relationships between biophysical, technological and texture variables

180   and those related to phenolic composition. Fig. 1 shows the projection of the samples on

181   the plane defined by the first and second principal components and also the

182   corresponding loadings plot. The first principal component (PC1) describes 44.15% of

183   the variability and the second principal component (PC2) describes 16.93% of the

184   variability. As can be seen in Fig. 1a, the distribution of samples into the score plot did

185   not show any important grouping, thus pointing out to the important differences among

186   the selected grapevines (see also Table 1 in Supporting Information), which will allow

187   us to study possible correlations between the variables employed. Fig. 1b shows the

188   variables on the loadings plot. It can be observed that there is a strong opposition along

189   PC1 between flavanol composition of grape skins and some of the biophysical variables

190   studied, such as leaf area (*Leaf_area*), the average weight of bunch (*Bunch_weight*), the

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191   weight of fresh (*Fresh_wood*) and dry wood (*Dry_wood*) and the percentage (*w/w*) of

192   seeds in total grape weight (*Perc_seed*). This latter variable also showed a clear

193   negative relationship with the total anthocyanin content (*Anthoc*). Hence, it seems that it

194   might be a negative relationship between the biophysical features of grapevine

195   determined in this work and the phenolic composition of grapes. In the same way, the

196   acyl derivatives of anthocyanins [mainly the coumaroyl derivatives (*Coumar*)] showed

197   high negative values in PC2, in contrast to texture variables and leaf area, which

198   showed high positive values in this PC. Thus, there also may be a negative relationship

199   not only between the composition on anthocyanin acyl derivatives of grapes and their

200   texture properties but also between the levels of these compounds and the biophysical

201   features of grapevine. Moreover, from the low loading values obtained for Brix degree

202   in PC1 and in PC2 (lower than 0.45 and higher than -0.08 respectively), it seems that

203   this variable barely contribute to explain sample variability. This could be related to

204   similarities on the sugar content (ºBrix) of analyzed grapes (see Table 1 in Supporting

205   Information), which would indicate that all samples were collected at a similar status of

206   technological maturity. However, phenolic composition is crucial for samples

207   differentiation, which may point out important differences on the phenolic maturity of

208   collected samples. These results indicate that grapes collected from the same vineyard at

209   a similar status of technological maturity can show important differences on phenolic

210   ripeness. These differences, as it will be explained bellow, can be related to differences

211   on grapevine vigor.

212   In order to assess the significance of these relationships, the correlation between all

213   variables employed in the study was investigated by means of the Pearson's coefficients

214   and its significance. Table 2 shows the most important significant correlations between

215   the phenolic composition of grape skins and the rest of variables employed in this study.

216 The phenolic composition did not show any significant correlations with the percentage

217 (*w/w*) of skins (data not shown). However, they corroborate the negative relationship

218 between the percentage (*w/w*) of seeds in relation to the whole grape (*Perc_seed*) and

219 the flavanic composition of grape skins indicated in the PCA plotting (Fig. 1b). This is

220 in accordance with studies in literature which have reported that skin weight was not a

221 determining factor for anthocyanin potential of the berries, but that seeds weight seemed

222 to significantly affect the grape composition.[37] All variables related to flavanic

223 composition showed high negative coefficients of Pearson with *Perc_seed* variable.

224 Among them, the total content of flavanols (*PAC*), as well as the total content of

225 procyanidins (*PC*) and prodelphinidins (*PD*) showed Pearson's coefficients lower than -

226 0.76. Moreover, these correlations are highly significant ($p<0.01$). Thus, it seems that

227 the heavier the seed, the lower amounts of flavanols in the skins. It might be possible

228 that synthesis of flavanols in seeds and in skin could be competitive, and that the

229 highest weight of the seed reflects higher synthesis rate of flavanols in this part of the

230 berry, at the expense of the synthesis in the grape skin. This negative correlation is also

231 observed between total hydroxybenzoic acids content in grape skin and the percentage

232 (*w/w*) of seeds. Since one of the two hydroxybenzoic acids (the major one) found in the

233 skin is gallic acid, and this acid is also found in grape seeds, this negative correlation

234 might be also due to the same reason that those proposed for flavanols.

235 Total leaf area of grapevine also correlates negatively with phenolic composition of

236 grape skin. Anthocyanin compounds presented the highest negative Pearson's

237 coefficients. Malvidin derivatives (*Mv*) and the acyl-derived anthocyanins (*Acyl*) levels

238 were the most strongly correlated to leaf area. The acyl-derived, and, in particular, the

239 coumaroyl-anthocyanin derivatives (*Coumar*), also showed a strong negative

240 relationship with the weight of wood pruned from the grapevine (*Fresh_wood* and

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241   *Dry_wood*). These two variables, together with leaf area, could be related to vine vigor.

242   Our results are consistent with those recently reported by Song and co-workers[17] that

243   have found that as vine vigor decreased, total soluble solid in grapes and total phenolics

244   and anthocyanins in wines increased, thus pointing out a negative relationship between

245   vine vigor and grape phenolic composition. Moreover, vine vigor could be related to the

246   grapevine water availability that in turn seems to affect the composition of grapes since

247   an excess in water conditions has demonstrated to be more negative for anthocyanin

248   contents than strong deficit conditions.[37]

249   It could also be observed (Table 2) a significant negative relationship between the

250   average weight of bunches (*Bunch_weight*) and the monoglucoside (*Monoglc*) and total

251   anthocyanin (*Antoc_total*) contents. Moreover, the level of anthocyanin caffeoyl

252   derivatives is also strongly correlated ($r=-0.666$, $p<0.05$) to average weight of berries

253   (*Berry_weight*). Therefore it seems that the heavier the bunches and berries were, the

254   lower levels of anthocyanins (both total, monoglucoside and caffeoyl derivatives) the

255   skins and, consequently, the berries showed. These results are in accordance to those

256   reported in literature showing that the total anthocyanin content (mg/berry) and

257   anthocyanin concentration (mg/kg of berries and in mg/g of skin) were dependent on

258   berry mass variation.[38] Likewise, it seems that the berries in which seeds accounted for

259   a higher weight percentage (*Perc_seed*) show lower levels of monoglucosides, since a

260   significant negative correlation ($r=-0.600$, $p<0.05$) between these two variables was

261   observed. It has been reported that berry weight is more related to seed weight than to

262   skin and flesh weight,[37, 38] so this might explain why both *Berry_weight* and *Perc_seed*

263   variables showed a relationship with anthocyanin composition whereas no-relation were

264   found with *Perc_skin* variable. These correlations between physical features of berries

265   and its phenolic composition might be explained because grape development occurs in

266   two main stages. The first stage, comprising the flowering and green berry stages, and

267   maybe even prior to that, during differentiation of the primordia,[39] seems critical in

268   determining berry weight.[38] However, anthocyanin and sugars accumulation takes place

269   in a second stage, from veraison to harvest. Thus, if the first stages were the most

270   important, bunches, berries and seeds could be heavier but grapes may show lower

271   levels of anthocyanins.

272   Finally, it was also observed a strong negative correlation between the texture

273   features of grape and its phenolic composition. In particular the berry skin break force

274   (F_{sk}) and the levels of anthocyanidin-coumaroylglucosides (r=-0.635, $p<0.05$) and of

275   total acyl-derived anthocyanin (r=-0.589, $p<0.05$) are negatively correlated (Table 2).

276   These results are in accordance with those reported by Giacosa and co-workers[40] who

277   have observed on Shiraz grapes significant lower values of F_{sk} in berries showing higher

278   levels of coumaroyl-anthocyanins derivatives in its composition. These results are also

279   consistent with other studies available in literature pointing out to the potential of the

280   mechanical properties of berry skin (such as F_{sk} and Sp_{sk}) to predict the anthocyanin

281   extractability.[29, 31] Moreover, it has also been reported that cell-wall composition affects

282   the anthocyanin extraction, in particular, the presence of higher amounts of glucose,

283   rhamnose, 2-O-methylxylose and lignin in the cell-wall composition would prevent

284   anthocyanin extraction from grape skin.[41] Considering this, there might be a relationship

285   between the cell-wall composition and the levels of coumaroyl-anthocyanin derivatives

286   that may be explained by a possible interaction between the acyl-derived anthocyanins

287   and some components of grape cell-wall, which in turn may determine the texture

288   features of grapes. Further studies about the cell-wall and phenolic composition and

289   texture features of berry skin must be carried out to assess this possibility.

290   Moreover, it has been observed a significant positive correlation between berry

291   hardness and its flavanic composition. It is worth noting the strong correlation between

292   this texture parameter and the level of gallocatechin and epigallocatechin (r=0.699,

293   p<0.01, Table 2). Thus, it seems that berry hardness might be indicative of the levels of

294   flavanols in berry skin. Rio Segade and co-workers[30] has reported that break force and

295   thickness of berry skin can be considered mechanical properties adequate for the

296   estimation of the degradability of the skin cell-wall. Degradation is related to the

297   changes in the structure of cell-wall by depolymerisation and formation of new cross-

298   linking bridges,[42] and to changes in its composition by loss of galactose, and other

299   pectic sugars such as arabinose and rhamnose.[30, 43, 44] Considering that these texture

300   parameters could be related to cell-wall composition, the correlation found between

301   flavanic composition and berry hardness might be explained, as in the case of acyl-

302   derived anthocyanin, by a specific interaction of flavanols with some cell-wall

303   components. In fact, Ruiz-García and co-workers[45] have pointed out that pectic

304   polysaccharides have an important binding-affinity for flavanols, whereas cellulose, due

305   to a low porosity, showed less affinity for these compounds. Thus, both higher levels of

306   flavanols and higher values of hardness of berry might be related to higher levels of

307   cellulose in cell-wall. However, further specific studies about the relationship between

308   cell-wall composition and texture features of berries must be carried out to assess this

309   possibility.

310   *Regression studies*

311   Considering the aforementioned correlations, different multiple linear regressions

312   (MLR) were carried out to assess the influence of biophysical, technological and texture

313   variables employed in this work on the phenolic composition of grape skin. Backward-

314   stepwise strategy was employed for MLR, in which all the considered variables were

315   used at the start of the process and then the least significant one is removed at each step.

316   The model is refitted after each step including only the most significant variables. First,

317   due to the correlation found between the amount of coumaroyl-anthocyanin derivatives

318   and the texture parameters that pointed out a possible relationship between these

319   compounds and cell-wall composition, the variable *Coumar* was selected as dependent

320   variable whereas the biophysical, technological and texture variables described in Table

321   1 were used as independent variables. Among all the variables considered, only the dry

322   weight of pruned wood (*Dry_wood*), the berry skin break force (F_{sk}) and the berry skin

323   thickness (Sp_{sk}) were considered statistically significant ($p<0.05$) in the fitted final

324   model. The value of the coefficient of determination (R^2), the non-standardized

325   coefficients (B) and the standardized coefficients (β) were obtained. The coefficient of

326   determination ($R^2=0.856$) indicates that the proposed model explains the 85.6% of the

327   variability of the levels of coumaroyl-anthocyanin derivatives, which supposed a good

328   fit to the data. Table 3 shows the values of the regression constant and of the β

329   parameter for each variable, which could be considered the best estimation about its

330   contribution to the model. As can be observed in the study of correlations, these three

331   variables (*Dry_wood*, F_{sk} and Sp_{sk}) showed a negative relationship with the levels of

332   coumaroyl-anthocyanin derivatives. The most important variable in the study was

333   *Dry_wood* (β=-0.741), thus indicating out the importance of grapevine vigor in the

334   levels of these anthocyanin-type compounds in grapes.

335   Considering the important role of flavanols in some organoleptic properties of wines

336   such as astringency or color, MLR was also performed using the levels of total flavanols

337   (*PAC*) as dependent variable and the biophysical, technological and texture variables

338   described in Table 1 as independent variables. Table 3 shows the result of fitting. The

339   proposed model explained 82.9% of the variability of total flavanol levels ($R^2=0.829$),

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340 which indicates the goodness of data fitting. As can be observed in Table 3, the

341 percentage (*w/w*) of seeds and leaf area showed a negative relationship whereas berry

342 hardness showed a positive relationship with flavanol content. The most important

343 variable in this model is the percentage (*w/w*) of seeds, thus pointing out the importance

344 of seed size on the flavanic composition of grape skins.

345 The proposed models indicated that there is a strong relationship between the

346 biophysical parameters of grapevine (mostly vine vigor represented by leaf area, dry

347 weight of pruned wood and seed weight), the texture features (evaluated as instrumental

348 mechanical properties) of berries and the phenolic composition of grape skins. Although

349 this study has been carried out only in one vintage; we have chosen a vineyard large

350 enough to have important differences on orographic terrain features. This could be

351 observed in the PCA and also in the high variability of variables that have been used in

352 this work (see Table 1 in Supporting Information). Thus, the results here presented set

353 an important precedent since they establish the importance of agronomic parameters and

354 texture properties for estimating phenolic composition of grape skins. However further

355 studies involving different vineyards, grape cultivars and different vintages must be

356 done in order to corroborate the quantitative relationship between these variables.

357 In conclusion, the results obtained pointed out an important relationship between

358 phenolic composition of grape skin, biophysical features of grapevines and berry texture

359 properties. Anthocyanin composition showed significant negative correlation with

360 grapevine vigor-related parameters (such as leaf area and bunch weight), whereas the

361 amount of flavanols of grape skins was negatively correlated with the percentage (*w/w*)

362 of seeds. Moreover, the phenolic composition is also correlated to some mechanical

363 properties of grapes. Berry skin break force showed a negative correlation with the

364 coumaroyl-anthocyanin derivatives, whereas berry hardness was positively correlated to

365   flavanic composition. Thus, it could be proposed a relationship between both acyl-

366   derived anthocyanins and flavanols and grape cell-wall composition. A significant

367   regression was found between coumaroyl-anthocyanin derivatives and some biophysical

368   (weight of pruned wood) and texture (berry skin break force and berry skin thickness)

369   variables. Likewise, a significant regression was also found between flavanol levels and

370   the percentage (w/w) of seeds, leaf area and berry hardness. These results pointed out

371   that grapevine vigor-related and texture parameters might be useful for estimating

372   phenolic composition of grape skins.

373   **Supporting Information description**

374   Supporting Information Available: Minimum, maximum, average values and coefficient

375   of variation of all the variables employed in this study. This material is available free of

376   charge via the Internet at http://pubs.acs.org.

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512

513   **Figure captions**

514   **Figure 1**. Representation of the samples in the score plot (**a**) and the variables in the

515   loading plot (**b**) on the plane defined by the first and second principal components.

516

Table 1: Variables

Name of variable	Meaning of the variable	Name of variable	Meaning of the variable
Anthocyanins (mg/g of skin)		*Phenolic acids (mg/g of skin)*	
Dp	Total delphinidin derivatives	a_caftaric	Total caftaric acids
Cy	Total cyanidin derivatives	a_coutaric	Total coutaric acids
Pt	Total petunidin derivatives	a_fertaric	Total fertaric acids
Pn	Total peonidin derivatives	a_caffeic	Total caffeic acids
Mv	Total malvidin derivatives	a_coumaric	Total coumaric acids and its glucoside derivatives
Monoglc	Total anthocyanin monoglucosides	HC	Total hydroxycinnamic acids
Acet	Total anthocyanin acetylglucosides	HB	Total hydroxybenzoic acids
Coumar	Total anthocyanin coumaroylglucosides	*Agronomic, biophysical and technological variables*	
Caffeo	Total anthocyanin caffeoylglucosides	Leaf_area	Total leaf area (m^2)
Acyl	Total anthocyanin acylglucosides	Fresh_wood	Total weight of fresh wood (kg)
Anthoc	Total anthocyanins	Dry_wood	Total weight of dry wood (kg)
Flavanols (mg/g of skin)		Grape_prod	Total weight of bunches (kg)
Cs	Catechin and epicatechin	Bunch_weight	Average of the weight of bunches (g)
PC_dimer	Dimers of procyanidins	Berry_weight	Average of the weight of berries (g)
PC_trimer	Trimers of procyanidins	Perc_skin	Percentage (*w/w*) of berry skin
PC_tetra	Tetramers of procyanidins	Perc_seed	Percentage (*w/w*) of berry seeds
PC_gal	Total of galloylated procyanidins	Brix	ºBrix of grape must
PC_nongal	Total of non-galloylated procyanidins	pH	pH of grape must
PC	Total of catechins and procyanidins	Titratable_ac	Titratable acidity of must (g/L of tartaric acid)
GCs	Gallocatechin and epigallocatechin	*Mechanical properties variables*	
PD_dimer	Dimers of prodelphinidins	Hardness	Berry hardness by TPA test (N)
PD_trimers	Trimers of prodelphinidins	Gumminess	Berry gumminess by TPA test (N)
PD	Total of gallocatechins and prodelphinidins	Chewiness	Berry chewiness by TPA test (mJ)
PAC	Total catechins, gallocatechins and proanthocyanidins	F_{sk}	Berry skin break force (N)
		Sp_{sk}	berry skin thickness (μm)

Table 2. Pearson's Coefficients Of The Most Important Significant Correlation Between Phenolic Composition Of Grape Skins And Biophysical, Technological And Texture Variables.

	Perc_seed	Leaf_area	Fresh_wood	Dry_wood	Bunch_weight	Berry_weight	F_{sk}	Hardness
Mv	ns	-0.691**	ns	ns	ns	ns	ns	ns
Monoglc	-0.600*	-0.561*	ns	ns	-0.577*	ns	ns	ns
Coumar	ns	-0.607*	-0.698**	-0.706**	ns	ns	-0.635*	ns
Caffeo	ns	ns	ns	ns	ns	-0.666*	ns	ns
Acyl	ns	-0.660*	-0.682*	-0.692**	ns	ns	-0.589*	ns
Anthoc	ns	-0.652*	ns	ns	-0.586*	ns	ns	ns
GCs	-0.825**	ns	ns	ns	ns	ns	ns	0.699**
PC_gal	-0.616*	-0.563*	ns	ns	ns	ns	ns	0.648*
PC_nongal	-0.792**	ns	ns	ns	ns	ns	ns	0.653*
PC	-0.764**	ns	ns	ns	ns	ns	ns	0.661*
PD	-0.782**	ns	ns	ns	ns	ns	ns	0.630*
PAC	-0.791**	ns	ns	ns	ns	ns	ns	0.660*
HB	-0.723**	ns	ns	ns	ns	ns	ns	0.678*

See Table 1 for further information about variable meaning. ns, * and ** indicate the level of significance (no significant, $p<0.05$ and $p<0.01$, respectively, n=26)

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Table 3. Results Of The MLR Carried Out Using The Level Of Coumaroyl-Glucoside Anthocyanins (Up) And Of Total Flavanols (Down) As Dependent Variables.
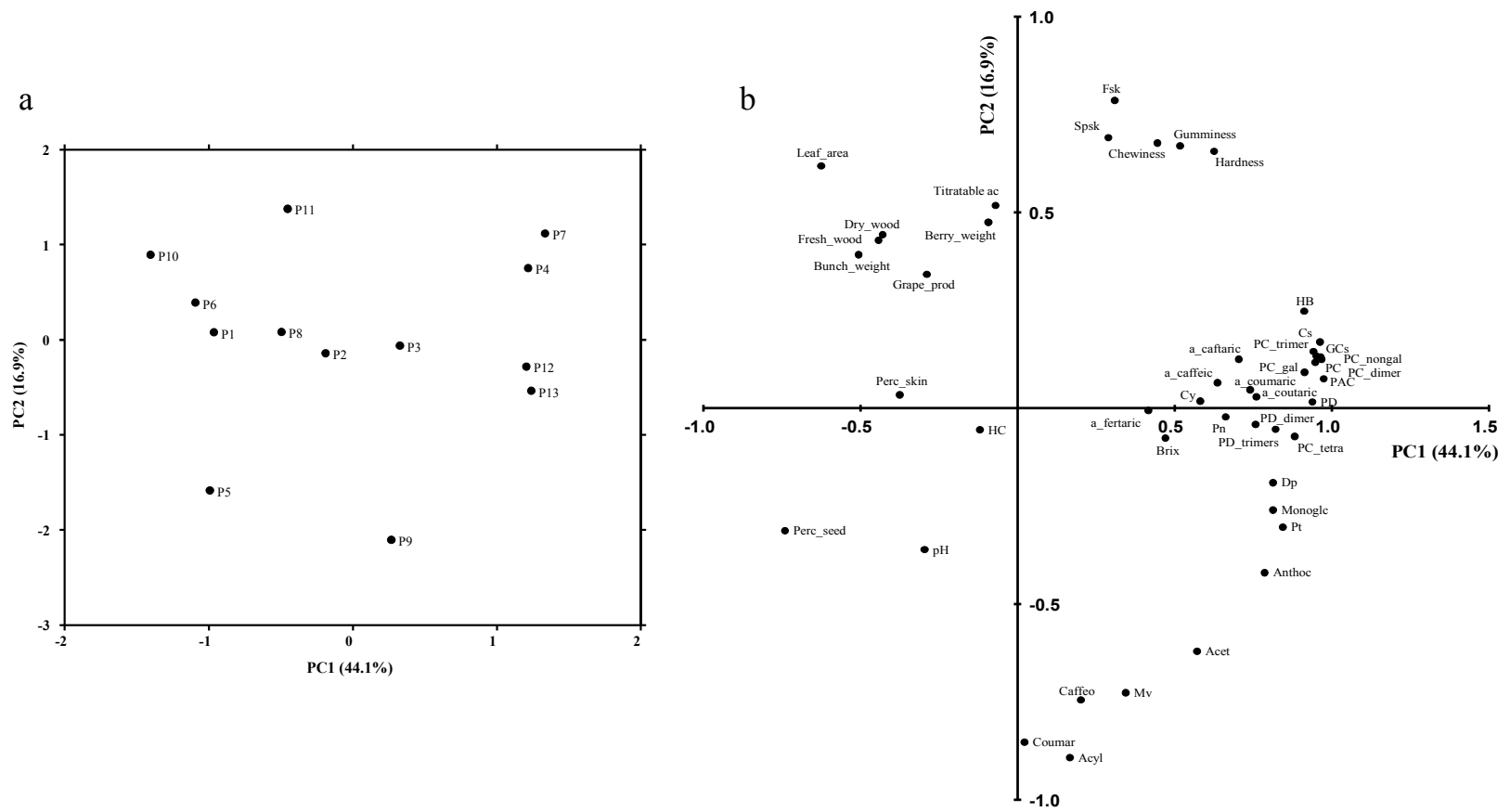
Dependent variable: Coumaroyl-glucoside anthocyanins (Coumar, mg/g of skin)
$R^2=0.856$

	Non-standardized coefficients (B)	Standardized coefficients (β)	*p*-value
Constant	1.934		<0.001
Dry_wood (kg)	-0.333	-0.741	<0.001
F_{sk} (N)	-0.715	-0.300	0.008
Sp_{sk} (mm)	-0.002	-0.352	0.006

Dependent variable: Total Flavanols (PAC, mg/g of skin) $R^2=0.829$

	Non-standardized coefficients (B)	Standardized coefficients (β)	*p*-value
Constant	2.664		0.020
Leaf_area (m^2)	-8.331	-0.406	0.019
Perc_seed	-0.335	-0.507	0.001
Hardness (N)	0.181	0.310	0.009

Figure 1

TOC Graphic