

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1509279> since 2015-10-19T09:59:25Z

Published version:

DOI:10.1021/acs.jafc.5b00275

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Questa è la versione dell'autore dell'opera:

J. Agric. Food Chem. 2015, 63, 7663–7669,

[dx.doi.org/ 10.1021/acs.jafc.5b00275](https://doi.org/10.1021/acs.jafc.5b00275)

The definitive version is available at:

La versione definitiva è disponibile alla URL:

[<http://pubs.acs.org/doi/abs/10.1021/acs.jafc.5b00275>]

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

Ignacio García-Estévez^a, Paula Andrés-García^a, Cristina Alcalde-Eon^a, Simone Giacosa^b, Luca Rolle^b, Julián C. Rivas-Gonzalo^a, Natalia Quijada-Morín^a and M. Teresa Escribano-Bailón^{a*}

^a*Grupo de Investigación en Polifenoles (GIP), Unidad de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain*

^b*Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Grugliasco (TO), Italy*

**corresponding author: M. Teresa Escribano-Bailón: Tel: +34 923294537; Fax: + 34 923294515; E-mail: escriban@usal.es*

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 MSⁿ, grapevine vigor, mechanical properties

17 **Introduction**

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

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

41 Kennedy¹² 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 ripening¹³ 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 workers¹⁹ 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 below
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 (m²) 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 leaflets 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 60°C and the weight of dried wood was determined.

151 °Brix 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

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 (°Brix) 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

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-workers¹⁷ 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-workers⁴⁰ 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 gallic catechin 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-workers³⁰ 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-workers⁴⁵ 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* ($\beta=-0.741$), 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 ($R^2=0.829$),

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>.

377 **References**

- 378 1. Moreno-Arribas, M. V.; Polo, M. C. *Wine Chemistry and Biochemistry*. Springer
379 Science: New York (NY, USA), 2009.
- 380 2. Alcalde-Eon, C.; Escribano-Bailón, M. T.; Santos-Buelga, C.; Rivas-Gonzalo, J. C.
381 Changes in the detailed pigment composition of red wine during maturity and ageing -
382 A comprehensive study. *Anal. Chim. Acta* **2006**, *563*, 238-254.
- 383 3. Alcalde-Eon, C.; García-Estévez, I.; Ferreras-Charro, R.; Rivas-Gonzalo, J. C.;
384 Ferrer-Gallego, R.; Escribano-Bailón, M. T. Adding oenological tannin vs. overripe
385 grapes: Effect on the phenolic composition of red wines. *J. Food Comp. Anal.* **2014**, *34*,
386 99-113.
- 387 4. He, F.; Liang, N.-N.; Mu, L.; Pan, Q.-H.; Wang, J.; Reeves, M. J.; Duan, C.-Q.
388 Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their
389 color expression. *Molecules* **2012**, *17*, 1571-1601.
- 390 5. Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect.
391 *Phytochemistry* **1993**, *34*, 119-124.
- 392 6. Gawel, R. Red wine astringency: a review. *Aust. J. Grape Wine Res.* **1998**, *4*, 74-95.
- 393 7. Somers, T. C. Polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175-
394 2186.
- 395 8. Kennedy, J. A.; Hayasaka, Y.; Vidal, S.; Waters, E. J.; Jones, G. P. Composition of
396 grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food*
397 *Chem.* **2001**, *49*, 5348-5355.

- 398 9. Escribano-Bailón, M. T.; Guerra, M. T.; Rivas-Gonzalo, J. C.; Santos-Buelga, C.
399 Proanthocyanidins in skins from different grape varieties. *Z. Lebensm. Unters. For.*
400 **1995**, *200*, 221-224.
- 401 10. Ferrer-Gallego, R.; García-Marino, M.; Miguel Hernández-Hierro, J.; Rivas-
402 Gonzalo, J. C.; Escribano-Bailón, M. T. Statistical correlation between flavanolic
403 composition, colour and sensorial parameters in grape seed during ripening. *Anal. Chim.*
404 *Acta* **2010**, *660*, 22-28.
- 405 11. Bindon, K. A.; Smith, P. A.; Holt, H.; Kennedy, J. A. Interaction between Grape-
406 Derived Proanthocyanidins and Cell Wall Material. 2. Implications for Vinification. *J.*
407 *Agric. Food Chem.* **2010**, *58*, 10736-10746.
- 408 12. Kennedy, J. A. Grape and wine phenolics: Observations and recent findings. *Cien.*
409 *Investig. Agrar.* **2008**, *35*, 107-120.
- 410 13. Fernández-López, J. A.; Hidalgo, V.; Almela, L.; Roca, J. M. L. Quantitative
411 changes in anthocyanin pigments of *Vitis Vinifera* cv Monastrell during maturation. *J.*
412 *Sci. Food Agric.* **1992**, *58*, 153-155.
- 413 14. Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J. C. Separation of sunlight and
414 temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol.*
415 *Vitic.* **2002**, *53*, 171-182.
- 416 15. Chorti, E.; Guidoni, S.; Ferrandino, A.; Novello, V. Effect of Different Cluster
417 Sunlight Exposure Levels on Ripening and Anthocyanin Accumulation in Nebbiolo
418 Grapes. *Am. J. Enol. Vitic.* **2010**, *61*, 23-30.
- 419 16. Guidoni, S.; Allara, P.; Schubert, A. Effect of cluster thinning on berry skin
420 anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *Am. J. Enol. Vitic.* **2002**, *53*,
421 224-226.
- 422 17. Song, J.; Smart, R. E.; Dambergs, R. G.; Sparrow, A. M.; Wells, R. B.; Wang, H.;
423 Qian, M. C. Pinot Noir wine composition from different vine vigour zones classified by
424 remote imaging technology. *Food Chem.* **2014**, *153*, 52-59.
- 425 18. King, P. D.; Smart, R. E.; McClellan, D. J. Within-vineyard variability in vine
426 vegetative growth, yield, and fruit and wine composition of Cabernet Sauvignon in
427 Hawke's Bay, New Zealand. *Aust. J. Grape Wine Res.* **2014**, *20*, 234-246.
- 428 19. Cortell, J. M.; Halbleib, M.; Gallagher, A. V.; Righetti, T. L.; Kennedy, J. A.
429 Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot Noir) anthocyanins. 1.
430 Anthocyanin concentration and composition in fruit. *J. Agric. Food Chem.* **2007**, *55*,
431 6575-6584.
- 432 20. Cortell, J. M.; Halbleib, M.; Gallagher, A. V.; Righetti, T. L.; Kennedy, J. A.
433 Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine
434 proanthocyanidins. *J. Agric. Food Chem.* **2005**, *53*, 5798-5808.
- 435 21. Dry, P. R.; Loveys, B. R. Factors influencing grapevine vigour and the potential for
436 control with partial rootzone drying. *Aust. J. Grape Wine Res.* **1998**, *4*, 140-148.
- 437 22. Johnson, L. F.; Bosch, D. F.; Williams, D. C.; Lobitz, B. M. Remote sensing of
438 vineyard management zones: Implications for wine quality. *Appl. Eng. Agric.* **2001**, *17*,
439 557-560.
- 440 23. Lamb, D. W.; Weedon, M. M.; Bramley, R. G. V. Using remote sensing to predict
441 grape phenolics and colour at harvest in a Cabernet Sauvignon vineyard: Timing

- 442 observations against vine phenology and optimising image resolution. *Aust. J. Grape*
443 *Wine Res.* **2004**, *10*, 46-54.
- 444 24. Bramley, R. G. V.; Ouzman, J.; Boss, P. K. Variation in vine vigour, grape yield and
445 vineyard soils and topography as indicators of variation in the chemical composition of
446 grapes, wine and wine sensory attributes. *Aust. J. Grape Wine Res.* **2011**, *17*, 217-229.
- 447 25. Rolle, L.; Siret, R.; Segade, S. R.; Maury, C.; Gerbi, V.; Jourjon, F. Instrumental
448 Texture Analysis Parameters as Markers of Table-Grape and Winegrape Quality: A
449 Review. *Am. J. Enol. Vitic.* **2012**, *63*, 11-28.
- 450 26. Rolle, L.; Gerbi, V.; Schneider, A.; Spanna, F.; Segade, S. R. Varietal Relationship
451 between Instrumental Skin Hardness and Climate for Grapevines (*Vitis vinifera* L.). *J.*
452 *Agric. Food Chem.* **2011**, *59*, 10624-10634.
- 453 27. Giordano, M.; Zecca, O.; Belviso, S.; Reinotti, M.; Gerbi, V.; Rolle, L. Volatile
454 fingerprint and physico-mechanical properties of 'muscat blanc' grapes grown in
455 mountain area: A first evidence of the influence of water regimes. *Ital. J. Food Sci.*
456 **2013**, *25*, 329-338.
- 457 28. Maury, C.; Madieta, E.; Le Moigne, M.; Mehinagic, E.; Siret, R.; Jourjon, F.
458 Development of a mechanical texture test to evaluate the ripening process of Cabernet
459 Franc grapes. *J. Texture Stud.* **2009**, *40*, 511-535.
- 460 29. Río Segade, S.; Orriols, I.; Giacosa, S.; Rolle, L. Instrumental texture analysis
461 parameters as winegrapes varietal markers and ripeness predictors. *Int. J. Food Prop.*
462 **2011**, *14*, 1318-1329.
- 463 30. Río Segade, S.; Rolle, L.; Gerbi, V.; Orriols, I. Phenolic ripeness assessment of
464 grape skin by texture analysis. *J. Food Comp. Anal.* **2008**, *21*, 644-649.
- 465 31. Rolle, L.; Torchio, F.; Ferrandino, A.; Guidoni, S. Influence of wine-grape skin
466 hardness on the kinetics of anthocyanin extraction. *Int. J. Food Prop.* **2012**, *15*, 249-
467 261.
- 468 32. Mira de Orduña, R. Climate change associated effects on grape and wine quality and
469 production. *Food Res. Int.* **2010**, *43*, 1844-1855.
- 470 33. Ferrer-Gallego, R.; Hernández-Hierro, J. M.; Rivas-Gonzalo, J. C.; Escribano-
471 Bailón, M. T. Determination of phenolic compounds of grape skins during ripening by
472 NIR spectroscopy. *LWT-Food Sci. Technol.* **2011**, *44*, 847-853.
- 473 34. González-Manzano, S.; Santos-Buelga, C.; Pérez-Alonso, J. J.; Rivas-Gonzalo, J.
474 C.; Escribano-Bailón, M. T. Characterization of the mean degree of polymerization of
475 proanthocyanidins in red wines using liquid chromatography-mass spectrometry (LC-
476 MS). *J. Agric. Food Chem.* **2006**, *54*, 4326-4332.
- 477 35. OIV. Recueil international des méthodes d'analyse des vins et des moûts. In
478 Organisation Internationale de la Vigne et du Vin: Paris, 2008.
- 479 36. Letaief, H.; Rolle, L.; Gerbi, V. Mechanical behavior of winegrapes under
480 compression tests. *Am. J. Enol. Vitic.* **2008**, *59*, 323-329.
- 481 37. Ferrer, M.; Echeverria, G.; Carbonneau, A. Effect of Berry Weight and its
482 Components on the Contents of Sugars and Anthocyanins of Three Varieties of *Vitis*
483 *vinifera* L. under Different Water Supply Conditions. *S. Afr. J. Enol. Vitic.* **2014**, *35*,
484 103-113.

- 485 38. Barbagallo, M. G.; Guidoni, S.; Hunter, J. J. Berry Size and Qualitative
486 Characteristics of *Vitis vinifera* L. cv. Syrah. *S. Afr. J. Enol. Vitic.* **2011**, *32*, 129-136.
- 487 39. Gray, J. D.; Coombe, B. G. Variation in Shiraz berry size originates before fruitset
488 but harvest is a point of resynchronisation for berry development after flowering. *Aust.*
489 *J. Grape Wine Res.* **2009**, *15*, 156-165.
- 490 40. Giacosa, S.; Marengo, F.; Guidoni, S.; Rolle, L.; Hunter, J. J. Anthocyanin yield and
491 skin softening during maceration, as affected by vineyard row orientation and grape
492 ripeness of *Vitis vinifera* L. cv. Shiraz. *Food Chem.* **2015**, *174*, 8-15.
- 493 41. Hernández-Hierro, J. M.; Quijada-Morín, N.; Martínez-Lapuente, L.; Guadalupe, Z.;
494 Ayestaran, B.; Rivas-Gonzalo, J. C.; Escribano-Bailón, M. T. Relationship between skin
495 cell wall composition and anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo
496 at different grape ripeness degree. *Food Chem.* **2014**, *146*, 41-47.
- 497 42. Hanlin, R. L.; Hrmova, M.; Harbertson, J. F.; Downey, M. O. Review: Condensed
498 tannin and grape cell wall interactions and their impact on tannin extractability into
499 wine. *Aust. J. Grape Wine Res.* **2010**, *16*, 173-188.
- 500 43. Ortega-Regules, A.; Romero-Cascales, I.; Ros-García, J. M.; López-Roca, J. M.;
501 Gómez-Plaza, E. A first approach towards the relationship between grape skin cell-wall
502 composition and anthocyanin extractability. *Anal. Chim. Acta* **2006**, *563*, 26-32.
- 503 44. Ortega-Regules, A.; Ros-García, J. M.; Bautista-Ortín, A. B.; López-Roca, J. M.;
504 Gómez-Plaza, E. Changes in skin cell wall composition during the maturation of four
505 premium wine grape varieties. *J. Sci. Food Agric.* **2008**, *88*, 420-428.
- 506 45. Ruiz-García, Y.; Smith, P. A.; Bindon, K. A. Selective extraction of polysaccharide
507 affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydr. Polym.* **2014**,
508 *114*, 102-114.

509
510 Thanks are due to the Spanish MICINN and FEDER (Projects ref. AGL2011-30254-
511 C02-01 and AGL2014-58486-C2-1-R) for financial support.

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
<i>Anthocyanins (mg/g of skin)</i>		<i>Phenolic acids (mg/g of skin)</i>	
Dp	Total delphinidin derivatives	a_cafataric	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
Monogl	Total anthocyanin monoglucosides	HC	Total hydroxycinnamic acids
Acet	Total anthocyanin acetylglucosides	HB	Total hydroxybenzoic acids
Coumar	Total anthocyanin coumaroylglucosides	<i>Agronomic, biophysical and technological variables</i>	
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)
<i>Flavanols (mg/g of skin)</i>		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	°Brix of grape must
PC_nongal	Total of non-galloylated procyanidins	pH	pH of grape must
PC	Total of catechins and procyanidins	Titrateable_ac	Titrateable acidity of must (g/L of tartaric acid)
GCs	Gallocatechin and epigallocatechin	<i>Mechanical properties variables</i>	
PD_dimer	Dimers of prodelfinidins	Hardness	Berry hardness by TPA test (N)
PD_trimers	Trimers of prodelfinidins	Gumminess	Berry gumminess by TPA test (N)
PD	Total of gallocatechins and prodelfinidins	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
Monogluc	-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, $n=26$)

Table 3. Results Of The MLR Carried Out Using The Level Of Coumaroyl-Glucoside Anthocyanins (Up) And Of Total Flavanols (Down) As Dependent Variables.

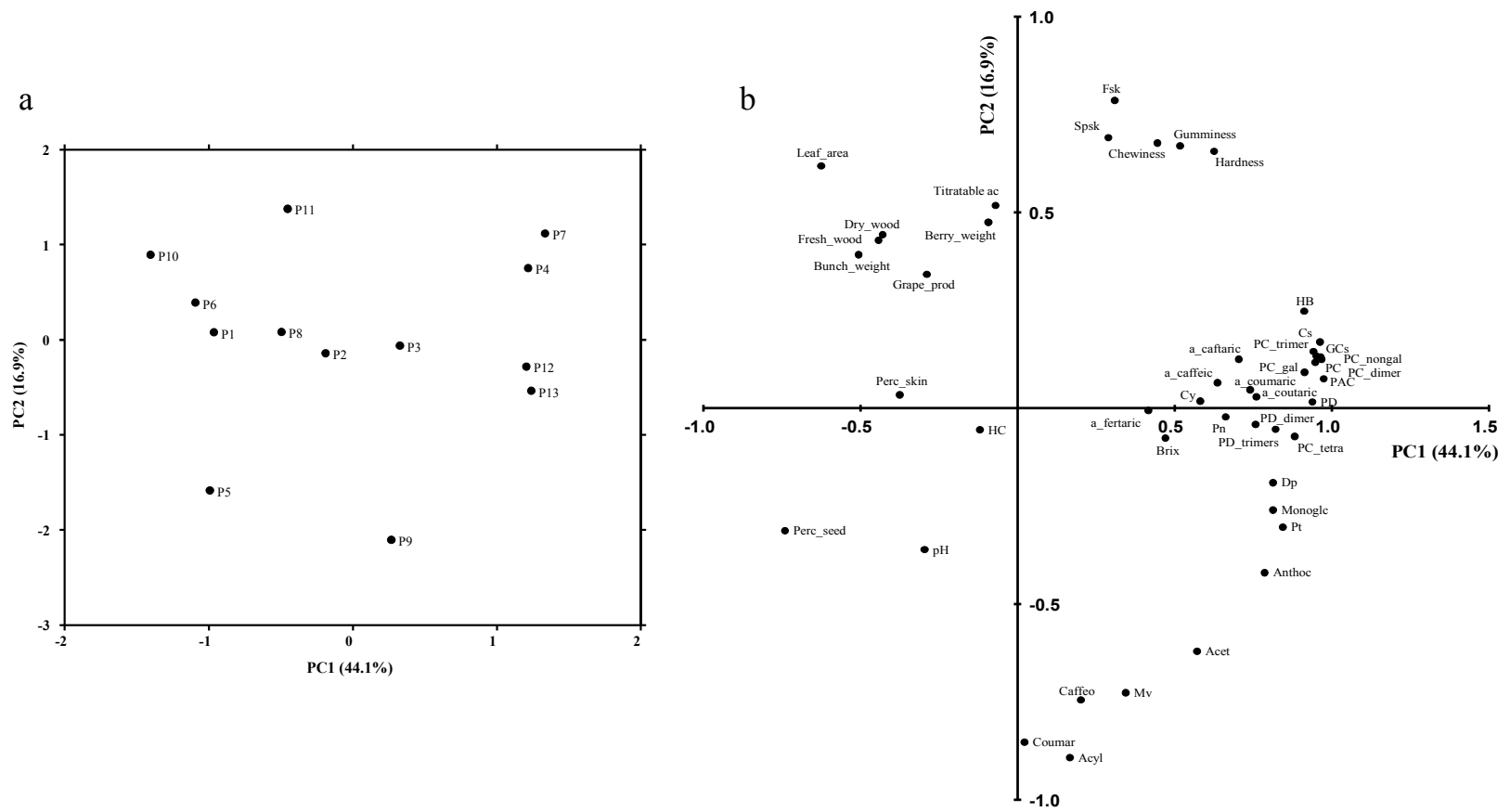
Dependent variable: Coumaroyl-glucoside anthocyanins (Coumar, mg/g of skin)
 $R^2=0.856$

	Non-standardized coefficients (B)	Standardized coefficients (β)	<i>p</i> -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)	<i>p</i> -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

