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1 **Optimization of a wood-grape mix maceration process. Influence of chips dose and**

2 **maceration time.**

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22 **ABSTRACT**

23 Wood-related phenolics are able to modify the composition of red wine and modulate
24 the colour stability. In this study, the effect of two maceration techniques, traditional
25 and wood-grape mix process, on the phenolic composition and colour of Syrah red
26 wines from warm climate was studied. Two doses of oak chips (3 and 6 g/L) at two
27 maceration times (5 and 10 days) during fermentation was considered. Changes on
28 phenolic composition (HPLC-DAD-MS), copigmentation/polymerisation
29 (spectrophotometry), and colour (Tristimulus and Differential Colorimetry) were
30 assessed by multivariate statistical techniques. The addition of oak chips at shorter
31 maceration times promoted higher phenolic extraction, colour enhancement and
32 stabilisation than traditional maceration. On contrast, increasing chips dose in extended
33 maceration time resulted in wines with lighter and less stable colour. Results open the
34 possibility of optimize alternative technological applications to traditional grape
35 maceration for avoiding the common loss of colour of wines from warm climate.

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37

38 **KEYWORDS:** Wood-grape maceration; American oak chips; Syrah red wine; warm
39 climate; colour stability.

40

41 **1. INTRODUCTION**

42 Phenolic compounds are the main chemical substances responsible for the sensorial
43 characteristics of wines such as colour, astringency and bitterness (Monagas, Bartolomé
44 & Gómez-Cordovés, 2005). Among them, colour is one of the most important attribute
45 defining the quality of wines since it is the first characteristic perceived, and therefore, it
46 influences the acceptability by consumers.

47 In traditional winemaking, anthocyanins and other phenolic compounds are extracted
48 from grapes and diffused into the must and wine along the fermentative maceration
49 process (Busse-Valverde, Gómez-Plaza, López-Roca, Gil-Muñoz, & Bautista-Ortín,
50 2011). While anthocyanins are the pigment accounting directly for the colour of red
51 wine, colourless phenolics such as benzoic or hydroxycinnamic acids, flavanols or
52 flavonols are involved in the stabilization of anthocyanins through copigmentation and
53 polymerisation reactions so, they plays also a key role in the colour stability over time
54 (Boulton, 2001; Escribano-Bailón & Santos-Buelga, 2012). Given the importance of
55 phenolic compounds for wine colour, studying and controlling the processing factors
56 that influence their extraction and content during the maceration and fermentation of
57 grapes is one of the main objectives to produce quality wines, especially in terms of full
58 body-structure and stable colour (Sacchi, Bisson, & Adams, 2005). In these regard,
59 different alternative maceration techniques have been developed to enhance the
60 extraction of grape components responsible for the colour, resulting in wines with a
61 different phenolic composition from those produced by traditional methods (Darías-
62 Martín, Carrillo, Díaz, & Boulton, 2001; Pérez-Lamela, García-Falcón, Simal-Gándara
63 & Orriols-Fernánez, 2007; Canals, Llaudy, Canals & Zamora, 2008; Gordillo et al.,
64 2014; Soto-Vázquez, Río-Segade, & Orriols-Fernández, 2010; Gonzalez-Neves,
65 Barreiro & Fabre, 2010; Ivanova et al., 2011; Añon et al., 2014).

66 In particular, the use of wood fragments during winemaking is an approved oenological
67 practice (OIV, 2012) increasingly applied by oenologists worldwide. Wood fragments
68 obtained from barrels are a natural source of phenolic compounds like benzoic and
69 cinnamic acids, and ellagitanins (among others) that are able to modify the wine
70 composition and its sensory perception (Tao, García, & Da-Wen, 2014). In most cases,
71 wood fragments are applied after the fermentative stage of winemaking to accelerate the
72 aging process artificially and to obtain wines with more complex structure in a short
73 aging period (Gómez García-Carpintero, Gómez Gallego, Sánchez-Palomo, & González
74 Viñas, 2012; Del Barrio-Galán, Medel-Marabolí, & Peña-Neira, 2015). Apart from their
75 recognized implication in the aroma, astringency, and bitterness, wood-related phenolics
76 can also influence the colour stability of wine by participating in copigmentation
77 reactions with anthocyanins (Alañón et al., 2013); especially if used in the initial stages
78 of vinification when the main mechanisms of colour stabilization occur. In fact, the
79 simultaneous maceration of grapes with wood fragments from barrels (wood-grape mix
80 maceration process) has been proved to be an interesting alternative to traditional
81 maceration in red wines from warm climate, where colour fall is a typical problem
82 (Gordillo et al., 2014). In that preliminary study, the colour stabilization was improved
83 due to the combined protective effect of phenolics derived from grape and wood.
84 However, other authors have shown inconsistent effects of wood-related compounds
85 among vineyards or even controversial depending on the conditions applied including
86 the chip dose, wood origin, toasting degree or maceration time without improving the
87 phenolic potential or sensorial characteristics of wines (Zimman, Joslin, Lyon, Meier, &
88 Waterhouse., 2002; Soto-Vazquez et al., 2010; González-Saiz et al., 2014). On the other
89 hand, studies focused on the optimization of the wood-grape mix maceration processes
90 by modifying the maceration conditions are still scarce. Thus, the main objective of this

91 work is to evaluate the impact of applying two proportion of oak chips (3 and 6 g/L) at
92 two maceration times (5 and 9 days) during the fermentative maceration of young Syrah
93 wine from warm climate, and compared the phenolic composition and colour
94 characteristics with a traditionally macerated red wine.

95 **2. MATERIAL AND METHODS**

96 **2.1. Winemaking**

97 Red wines were elaborated from grapes *Vitis vinifera* . Sy h w “C
98 H ” D O gin (DO), in the southwest of Spain (warm climate). About
99 2700 kg of grapes were harvested in 2014 vintage at optimum technological maturity
100 (density of 1.100 g/mL, total acidity of 6.7 g/L and a pH of 3.65) and in good sanitary
101 conditions.

102 Grapes were destemmed and crushed and the must was distributed in stainless steel
103 tanks of 220 L. Wines were elaborated under different maceration conditions by
104 applying two proportion of chips (3 and 6 g/L) and two maceration times (5 and 10
105 days), compared with a traditional macerated red wine. American oak (*Quercus alba*)
106 low-toasted chips of 1 cm^2 average size (Tonelería Martín y Vázquez, Logroño, Spain)
107 were used. All maceration treatments were made in triplicate as follows:

108 *Traditional maceration*: 3 tanks were submitted to traditional grape maceration (without
109 oak chips) for 5 maceration days (C5 wines); and 3 tanks for 10 maceration days (C10
110 wines). Both C5 and C10 wines were considered as control wines.

111 *Wood-grape maceration at 3 g/L of oak chips*: 3 tanks were submitted to the addition of
112 3 g/L of oak chips to the fermentation mash for 5 maceration days (S5 wines); and 3
113 tanks were submitted to the addition of 3 g/L of oak chips to the fermentation mash for
114 10 maceration days (S10 wines).

115 *Wood-grape maceration at 6 g/L of oak chips*: 3 tanks were submitted to the addition of
116 6 g/L of oak chips to the fermentation mash for 5 maceration days (D5 wines); and 3
117 tanks were submitted to the addition of 6 g/L of oak chips to the fermentation mash for
118 10 maceration days (D10 wines).

119 An identical red winemaking procedure was used for all assays. Oenological treatments
120 were adjusted at the same levels for all of the assays: 60 mg/L total sulphur dioxide and
121 7 g/L of total titratable acidity by adding tartaric acid. For all wines, alcoholic
122 fermentation was spontaneously developed. Fermentation caps were punched down
123 once a day during the maceration period. After this, the mash was drawn off to remove
124 the skins and other solid parts, and the free run musts were left to finish the
125 fermentation under the same conditions. Subsequently, the malolactic fermentation was
126 induced by inoculation of *Oenococcus oeni* lactic acid bacteria (>10^{10} CFU *O. oeni*/ml,
127 VINIFERM Oe 104, Agrovin, Spain) at the rate of 14 mL/hL at the end of alcoholic
128 fermentation. When fermentative processes were finished, the wines were racked in 50
129 L stainless steel tanks and stored at 10-15 °C for a stabilisation period of 6 months.

130 Must and wine samples (100 mL) were taken at the initial point or grape crushing (1
131 day), at the middle of the fermentative alcoholic maceration (3 days), just after the skin
132 removal (5 and 10 days), and 3 and 6 months during stabilisation period. A total of 108
133 samples were analysed in triplicate.

134 **2.2. HPLC-DAD-ESI/MS analysis of phenolic compounds**

135 HPLC separation, identification and quantification of anthocyanin and flavonols was
136 performed in an Agilent 1200 chromatographic system equipped with a quaternary
137 pump, an UV-vis diode-array detector, an automatic injector, and ChemStation software
138 (Palo Alto, CA, USA). Prior direct injection, the samples were filtered through a 0.45
139 lm Nylon filter (E0034, Análisis Vínicos, Spain). All analyses were performed in

140 triplicate. The anthocyanin and flavonols identification was carried out following the
141 method proposed by Gordillo, Cejudo-Bastante, Rodríguez-Pulido, Lourdes González-
142 Miret and Heredia (2013). Phenolic compounds were separated using a Zorbax C18
143 column (250μm 4.6 mm, 5 μm particle size) maintained at 38 ºC. Acetonitrile-formic
144 acid-water (3:10:87) as solvent A and acetonitrile-formic acid-water (50:10:40) as
145 solvent B were used. The elution profile was as follows: 0-10 min 94%A; 10-15 min
146 70%A; 15-25 min 60%A; 25-35 min 55%A; 35-40 min 50%A; 40-42 min 40%A; 42-43
147 min 94%A. The flow rate was 0.8 mL/min and the injection volume was 50 μL. UV-Vis
148 spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm. The
149 quantification was made at 525 and 360 nm (anthocyanin and flavonols, respectively)
150 using the calibration curves obtained in the same chromatographic conditions for
151 malvidin 3-glucoside and quercetin standards. The concentration of phenolic
152 compounds was expressed as mg/L.

153 For flavan-3-ol and phenolic acid analysis, samples were fractionated prior to
154 chromatographic analysis previously described by - S -
155 -Alonso, Rivas-Gonzalo and - , (2006). Briefly, Oasis® MCX
156 (Waters Corporation Mildford, MA, USA) cartridges were used for the separation of
157 flavan-3-ols and phenolic acids. 1 mL of each wine was diluted (1:1) with 0.1M HCl
158 and eluted through previously conditioned cartridges. Anthocyanins and flavonols were
159 retained in the cartridges while flavan-3-ols and phenolic acids were eluted with 8 mL
160 of methanol. A small volume of water was added to the eluate and concentrated under
161 vacuum at lower than 30 ºC until complete elimination of methanol. The volume of the
162 aqueous residue was adjusted to 0.5 mL with ultrapure water, filtered (0.45 μm) and
163 analysed by HPLC-DAD–MS as previously described. The abovementioned HPLC
164 system was coupled to a hybrid triple quadrupole/linear ion trap (QqLIT) mass

165 spectrometer API 3200 QTrap (Applied Biosystems, Foster City, CA, USA) equipped
166 with a Turbo V ionization source and controlled by Analyst software (version 1.5;
167 Applied Biosystems) via the DAD cell outlet. Phenolic acids and flavan-3-ol
168 chromatographic separation was performed on a reversed-phase column Spherisorb
169 ODS-2 (150 x 4.6 mm, 3 µm) from Waters (Milford, MA, USA) maintained at 25ºC.
170 0.25% acetic acid in water (A) and acetonitrile (B) were used as the mobile phases The
171 following linear gradient was used to achieve the chromatographic separation: from 0 to
172 10% B in 5 min, from 10 to 14.5% B in 35 min, from 14.5 to 19% B in 5 min, from 19
173 to 55% B in 5 min, hold at 55%B for 5 min, from 55 to 80% B in 5 min, hold at 80% B
174 for 3 min, from 80 to 0% B in 2 min and hold at 0% B for 5 min. The flow rate was set
175 at 0.5 mL min^{-1} and the injection volume was 100 µL. UV-vis spectra were recorded
176 from 200 to 600 nm, while acquiring at the selected wavelengths of 280 nm for flavan-
177 3-ols quantification and 330 nm for phenolic acids.
178 The mass spectrometer was operated in the negative electrospray ionization (ESI) mode
179 under the following specific conditions: IS: -4500 V; source temperature (TEM),
180 400°C; CUR: 20 psi; GS1: 40 psi; GS2: 30 psi; DP: -40 V; EP: -7 V; and CE: -20 eV.
181 Nitrogen (>99.98%) was employed as curtain, ion source and collision gas. The
182 detection was accomplished in the enhanced MS (EMS) full-scan mode, from *m/z* 100
183 to 1700, and in the enhanced product ion (EPI) mode, to obtain the corresponding full-
184 scan MS/MS spectra.
185 Phenolic compounds were identified by comparison of their retention time, UV-vis
186 spectra and mass spectra features with data reported in the literature and the previously
187 recorded in our laboratory. The phenolic acids quantification was made at 330 nm using
188 external calibration curves of purchased standards, using gallic acid for gallic acid
189 quantification and *p*-coumaric acid for the rest of identified phenolic acids. Flavan-3-ols

190 were quantified using external calibration curves recorded at 280 nm of its
191 corresponding purchased standard. Phenolic compounds concentration was expressed as
192 mg/L.

193 **2.3. Colorimetric analysis**

194 The whole visible spectrum (380-770 nm) of samples was recorded at constant intervals
195 ($\Delta\lambda$=2 m) w h H w -Packard UV-vis HP8452 spectrophotometer (Palo Alto, CA),
196 using 2 mm path length glass cells and distilled water as a reference. The CIELAB
197 parameters (L*, a*, b*, C^*_{ab}, and h_{ab}) were determined by using the original software
198 CromaLab© (Heredia, Álvarez, González-Miret, & Ramírez, 2004), following the
199 C mm L’ ’ , CIE, recommendations (CIE, 2004): the
200 CIE 1964 10° Standard Observer and the CIE Standard Illuminant D65.

201 Also, the colou (Δ $*_{ab}$) were calculated between the samples to state the
202 implications of the maceration treatments on the colour of the final wines, as well as to
203 assess the colour stability. It was calculated as the Euclidean distance between two
204 points in the three dimensional y L* * *: Δ $*_{ab}$ = $[(\Delta L^*)^2$ +
205 $(\Delta\ *)^2 + (\Delta\ *)^2]^{1/2}$.

206 **2.4. Copigmented and Polymerized Anthocyanin Determination**

207 The contribution of copigmented anthocyanins to the total wine colour at pH 3.6 (%
208 Copigmentation) and the degree of anthocyanin polymerisation (% Polymerisation)
209 were determined following the method proposed by Boulton (1996). The pH values of
210 the wine sample were previously adjusted to pH 3.6 using 1 M NaOH or HCl.

211 **2.5. Statistical Analysis**

212 Statistical analysis was carried out by using Statistica version 8.0 software (Statistica,
213 2007). In order to study significant differences between the different types of wines in
214 terms of phenolic composition and colour characteristics, a multifactorial analysis of

215 variance was carried out using the general linear model procedure (GLM). Tukey test
216 was used to evaluate the significance of the analysis.

217 **3. RESULTS AND DISCUSSION**

218 **3.1. Changes in phenolic composition**

219 The extraction of phenolic compounds under different conditions of maceration time (5
220 and 10 days) and chips dose (0, 3 and 6 g/L) was studied to establish which of these
221 factors have a greater impact in the quality of Syrah wines during winemaking when a
222 wood-grape mix maceration process is applied respect to traditional maceration. In the
223 qualitative analysis of phenolic composition, 27 compounds belonging to diverse
224 phenolic families were identified by HPLC-MS: 10 anthocyanins (non-acylated,
225 acetated, and *p*-coumaroylated derivatives of delphinidin, petunidin, peonidin and
226 malvidin 3-glucoside), 6 phenolic acids (gallic, ellagic, trans-caftaric-protocatehuic, cis-
227 coutaric, trans-coutaric, fertaric, coumaric-hexose and caffeic), 4 flavan-3-ols
228 (gallocatechin, procyanidin B3, (+)-catechin and (-)-epicatechin), and 6 flavonols
229 (myricetin 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, kaempferol 3-
230 glucoside, isorhamnetin 3-glucoside and syringetin 3-glucoside).

231 Table 1 shows the mean concentration (mg/L±SD, n=3) of compounds identified in the
232 wine samples at the end of the fermentative maceration period (skin removal), grouped
233 according to their maceration time and chips dose applied. The data were subjected to a
234 multifactor analysis of variance by using the general linear model procedure (GLM,
235 Tukey Test) for testing the significance of the effects of the factors on the phenolic
236 composition. In addition, new dependent variables were calculated as the sum of
237 individual phenolic compounds identified for each phenolic family (anthocyanins and
238 their derivatives; phenolic, benzoic and hydroxycinnamic acids; flavan-3-ols and
239 flavonols). They were included in the multifactorial analysis to know whether a

240 combination of phenolics grouped into phenolic families varies as a function of the
241 assayed factors.
242 The results indicated that the maceration time and the proportion of chips applied during
243 the alcoholic fermentative maceration had a significant influence (p-values of 0.018 and
244 0.02, respectively) on the extraction of the different phenolic families. The interaction
245 effects between the factors were significant for the global content of anthocyanins and
246 flavan-3-ols, which are major phenolic compounds in red grapes and wood, respectively
247 (Waterhouse, 2002). Although there was no interaction effect on the global content of
248 phenolic acids, it was significant for benzoic acids, which are also grape/wood-related
249 phenolic compounds (Watherhouse, 2002; Cabrita, Barrocas-Diaz, & Costa-Freitas,
250 2011). In particular, the interaction effects showed the stronger influence (higher level
251 of significance, $p<0.001$) of the maceration time on most of the individual anthocyanins
252 and phenolic acids at skin removal, while the chip dose stronger influenced the benzoic
253 acids and flavan-3-ol extraction.
254 In can be observed that shorter maceration times (5 days) led to wines with higher
255 contents of anthocyanins (mainly glucosides and acetates) and phenolic acids (mainly
256 gallic, *t*-caftaric-protocatechuic, and *t*-coutaric acids) than longer maceration time (10
257 days), which were in contrast richer flava-*3*-ols (mainly GC, (+)-catechin and
258 epicatechin). The decrease of anthocyanins in extended macerations is in agreement
259 with the results reported by other authors (Sacchi et al., 2005; Cheynier et al., 2006;
260 Gonzalez-Neves et al., 2010; Ivanova et al., 2011; Gonzalez-Sainz et al., 2014). This
261 effect could be explained by the different reactions involving anthocyanins and other
262 phenolic compounds easily diffused from the skin and pulp into the must in the first
263 days of maceration. These competing processes such as oxidation, hydrolysis,
264 condensation or polymerisation make them to be degraded or transformed progressively

265 into new polymeric compounds (Gonzalez-Neves et al., 2010). At the same time, wood
266 fragments and solid parts of grapes might adsorb these compounds making them slowly
267 decrease from the earlier stages of vinification, especially when maceration is extended
268 over time (Gordillo et al., 2014; Del Barrio Galán et al., 2015). On contrast, longer
269 maceration time (10 days) positively affected the extraction of some flavan-3-ols whose
270 diffusion from skins and seeds into the must is favoured with higher alcohol content,
271 sulfur dioxide, temperature, and contact time (Canals et al., 2008; Jensen; Blachez,
272 Egebo, & Meyer, 2007; Quijada-Morín et al., 2015).
273 Regarding the chips treatment, it seemed that the effect on the extraction of phenolic
274 compounds varied according to the maceration time applied. In shorter maceration times
275 (5 days), wines elaborated in contact with wood (S5 and D5) had higher global contents
276 of anthocyanins, benzoic acids and flavan-3-ols than traditional macerated wines (C5).
277 However, the proportion of chips differently affected the content of each phenolic
278 family. When the chip dose was increased (D5 wines, 6g/L chips), the global levels of
279 anthocyanins and flavan-3-ols were slightly decreased while the levels of benzoic acids
280 increased. Thus, considering in the data analysis only shorter macerated wines, S5
281 wines stated for their significant highest content in all classes of pigments as well as
282 GC, procyanidin B3 and (+)-catechin; and D5 wines for being the richest in gallic and
283 ellagic acids.
284 In the case of longer maceration times, wines elaborated in contact with oak chips (S10
285 and D10) had significant higher content on flavan-3-ols and benzoic acids than
286 traditional macerated wines (C10), but lower anthocyanin content. Other authors have
287 also observed similar results in wines from different varieties macerated with oak chips
288 during fermentation for some of the non-coloured phenolics identified (Zimman et al.,
289 2002; Gordillo et al., 2014). Notwithstanding, the differences for the anthocyanins

290 content were only significant respect to D10 wines, that is, at the higher chips dose
291 applied. Again, with the exception of ellagic and coumaric acids, most of phenolic
292 compounds were slightly decreased with the increase of chip dose in the fermentation
293 mash. Thus, lower chip doses combined with higher maceration times led to wines (S5)
294 with similar pigment content that traditional macerated wines but significantly richer in
295 some copigments such as GC, procyanidin B3, (-)-epicatechin, gallic and ellagic acids.
296 Finally, the maceration time and the chips dose applied did not influence the extraction
297 of the individual flavonol compounds and so their global content in wines.
298 Fig. 1 and 2 shows the evolution of the total anthocyanin content (mg/L±SD, n=3) in 5
299 and 10 days macerated wines (respectively), and the percentage of copigmentation and
300 polymerisation, during 6 months of storage. After pressing, a marked decrease of
301 anthocyanins was observed in all wines but the pigment stability was influenced by the
302 interaction of the maceration time and chip dose. It can be observed that the lowest
303 pigment loss corresponded to S5 wines (30%), that is, when the lower maceration wine
304 was combined with the lower chip dose (Fig. 1A). On the contrary, the highest
305 decreases in total anthocyanins corresponded to T9 and D9 wines (45% and 40% of
306 global pigment loss, respectively), that is, when the higher maceration time and chips
307 dose was applied (Fig. 2A).
308 With regard to the contribution of different group of pigments to the total colour
309 (copigmented and polymeric pigments), the effect varied with the maceration time. In
310 shorter maceration time (5 days), wines elaborated in contact with oak chips (S5 and
311 D5) reached higher levels of percentage of copigmentation and polymerisation than
312 traditional macerated wines (C5), which confirm the positive effect of a wood-grape
313 mix maceration in the phenolic structure of wines (Fig. 1B and 1C). This fact is in
314 accordance with the higher extraction of specific colourless wood-related compounds

315 that can act as good copigments (Table 1), as previously reported by other authors with
316 similar maceration time and chip dose applied (Zimman, 2002; Gordillo et al., 2014). In
317 longer macerated time (10 days), wines elaborated in contact with wood (S10 and D10)
318 reached again higher degree of copigmentation than traditional macerated wines, C10
319 (Fig. 2B). However, during the stabilisation period, wines elaborated with higher doses
320 of chips (D10) showed the significant (p<0.05) lowest degree of polymerisation and
321 therefore, the lower pigment stability (Fig. 2C). This finding could be related with the
322 higher adsorption of pigment and copigments extracted during the maceration stage
323 respect to C10 and S10 wines.

324 **3.2. Changes in wine colour**

325 The changes in the colour parameters (L*, C^*_{ab}, and h_{ab}; mean±SD, n=3) during
326 winemaking in 5 and 10 days macerated wines, grouped by the chips dose, are shown in
327 Fig. 3 and 4, respectively. As can be seen, the maceration treatments applied induced
328 notable differences in the colour characteristics of wines and their stability over time.
329 At skin removal, the colour extraction was different for each maceration treatment but
330 was coherent with the pigment extraction. As can be seen in Table 1, the interaction
331 effects between the factors were significant for all the colorimetric parameters, except to
332 the lightness (L*). Results showed the stronger influence (p<0.001) of the maceration
333 time in both on quantitative (L*, a*, C^*_{ab}) and qualitative (b*, h_{ab}) parameters, while the
334 chips dose only influenced the quantitative ones (a*, C^*_{ab}). As expected, the higher
335 pigment extraction during fermentative maceration in shorter maceration wines, the
336 significant higher values of chroma (C^*_{ab}) and lower of hue (h_{ab}) respect to longer
337 maceration wines. Also, shorter macerated wines showed slightly lower values of
338 lightness (L*), but the differences were not significant for all wines. These results imply

339 darker and more saturated bluish colour at skin removal respect to the extended
340 maceration time (Ivanova et al., 2011).
341 On the other hand, the combination of increasing chips dose with shorter maceration
342 time led to wines (S5 and D5) with higher values of C^*_{ab} and lower of hue respect to
343 traditional macerated wines (C5). However, these colorimetric differences were only
344 significant for the quantitative colour attribute chroma (C^*_{ab}) between C5 and S5 wines.
345 On contrast, the opposite effect was observed when longer maceration times were
346 combined with chips dose, since lower values of chroma and higher of lightness were
347 observed in S10 and D10 wines respect to traditional macerated wines (C10). These
348 results were in accordance with the lower pigment extraction of wines macerated in
349 contact with oak wood, especially those elaborated with higher chips dose (D10).
350 The evolution of colour parameters over time was in agreement with the behavior of the
351 anthocyanin content of wines, as well as with the contribution of the different group of
352 pigments to the total colour (copigmented and polymeric pigments). For shorter
353 maceration times (5 days), S5 wines with significant higher anthocyanin content,
354 proportion of copigments and copigmentation degree showed a more vivid bluish colour
355 (higher chroma values and lower of hue) than traditional macerated wines or with 6g/L
356 chips (Fig. 3B and 3C). At longer maceration time, wines summited to a wood-grape
357 maceration process (S10 and D10 wines) showed lighter and less intense colour than
358 traditional macerated wines, C10 (Fig. 4A and 4B). These differences were more
359 marked when higher proportion of oak chips were applied (D10 wines). Although D10
360 wines showed higher level of copigmentation than C10 wines (Fig. 1E), it seemed that
361 those wines had higher difficulty to convert the earlier copigmentation complexes into
362 more stable pigments despite having higher amounts of some copigments. In fact,

363 higher pigment loss and lower degree of polymerisation (Fig. 2A and 2C) resulted in a
364 net loss in colour in final wines.
365 Differential Tristimulus Colorimetry was applied to objectively asses the colour
366 extraction and colour stability of each wine during vinification, and compare them
367 according to the maceration time and the chips doses applied. For this purpose, colour,
368 lightness, chroma and hue differences (ΔE^*_{ab} ΔL^* ΔC^*_{ab}, Δh_{ab}, respectively) of wines
369 were calculated during the extraction stage (from the grape crushing to the skin
370 removal) and from the skin removal to the end of stabilisation period (6 months).
371 Results obtained are shown in Table 2.
372 During the extraction period, wines submitted to shorter maceration times showed
373 higher ΔE^*_{ab} values than longer macerated wines, indicating higher colour variation.
374 The negative values obtained for ΔL^* and Δh_{ab} but positive for ΔC^*_{ab} are indicative of
375 an increase of the quantity of colour of wines and to a displacement towards blue hue of
376 wines, which is characteristic of the pigment extraction from grapes to wine. As
377 observed, S5 wine presented the highest values of ΔE^*_{ab} (65.0) and ΔC^*_{ab} (+52
378 CIELAB u.) but the lowest of Δh_{ab} (-15.4°). This observation means higher colour
379 extraction, colour intensity and bluish tonality; which is in accordance with the colour
380 parameters obtained at skin removal (Table 1), as well as the higher phenolic extraction
381 (pigments and copigments) and degree of copigmentation in S5 wines.
382 During the stabilization period, according to the sign of ΔL^*, ΔC^*_{ab} and Δh_{ab}, higher
383 ΔE^*_{ab} values are indicative of lower colour stability. In this sense, the combination of
384 shorter maceration times with chips doses increased the colour stability of wines respect
385 of those elaborated with traditional grape maceration (lower ΔE^*_{ab} in S5 and D5 than
386 C5 wines). This positive effect is supported by the higher degree of polymerisation
387 reached at the end of the storage period in wines macerated with oak chips (%

388 Polymerisation = 54% and 57.8% in S5 and D5 versus 50% in C5 wines). However, the
389 opposite effect was observed when longer maceration time was applied. The
390 combination of increasing chips dose in extended maceration time resulted in higher
391 ΔE^*_{ab} values; and therefore, lower colour stability respect to traditional macerated
392 wines. As previously explained, lower degree of polymerisation was observed in final
393 S10 and D10 wines in comparison to C10 wines (% Polymerisation = 51% and 46% in
394 S5 and D5 versus 53% in C5 wines).

395 **4. Conclusions**

396 The application of a wood-grape mix maceration process during winemaking at the rates
397 studied (3 and 6 g/L) increased the content of some phenolic compounds having high
398 copigmentation power (flavanols and phenolic acids) in wines respect to the traditional
399 grape maceration process. However, the effect on the pigment extraction depended on
400 the maceration time applied, with important consequences on the colour quality and
401 stability. The application of oak chips at shorter maceration time (5 days) improved the
402 anthocyanin extraction in young wines contributing to better preserve their colour
403 characteristics during stabilisation than traditional macerated wines, especially at lower
404 chips proportion (3 g/L). At longer maceration time (10 days), the addition of chips did
405 not improved the extraction of pigments and colour probably due to a higher adsorption
406 of compounds by the fermentation mash, resulting in lower colour stability in final
407 wines respect to traditional macerated wines.

408 **Acknowledgments**

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413

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514

515 **Figure Captions**

516 **Fig. 1.** Evolution of the (A) Total Anthocyanins, (B) Percentage of Copigmentation, and
517 (C) Percentage of Polymerisation, in 5 days macerated wines during winemaking (C5: 0
518 g/L, S5: 3 g/L, D5: 6 g/L of oak chips).

519 **Fig. 2.** Evolution of the (A) Total Anthocyanins (mg/L± SD, n=3), (B) Percentage of
520 Copigmentation, and (C) Percentage of Polymerisation, in 10 days macerated wines
521 during winemaking (C10: 0 g/L, S10: 3 g/L , D10: 6 g/L of oak chips).

522 **Fig. 3**. Changes in the colour parameters (mean±SD, n=3) in 5 days macerated wines
523 during winemaking: (A) L*, lightness; (B) C^*_{ab}, chroma; (C) h_{ab}, hue angle (C5: 0 g/L,
524 S5:3 g/L, D5: 6 g/L of oak chips).

525 **Fig. 4**. Changes in the colour parameters (means±SD, n=3) in 10 days macerated wines
526 during winemaking: (A) L*, lightness; (B) C^*_{ab}, chroma; (C) h_{ab}, hue angle (C10: 0 g/L,
527 S10:3 g/L, D10: 6 g/L of oak chips).

528

529

Table1

Table 1. Phenolic composition (mg/L±SD, n=3) and CIELAB colour parameters (mean±SD; n=3) of Syrah wines according to the maceration time (5 and 10 days) and chips dose (Control: 0 g/L; Simple: 3 g/L; Double: 6 g/L of oak chips), at the end of the maceration period (skin removal).

							Effect		
	C5	**S5**	**D5**	**C10**	**S10**	**D10**	**Maceration time**	**Chips dose**	**Interaction**
Phenolic compounds									
Sum of anthocyanins	1429.5±3.6a	1636.0±0.3b	1500.2±27.7ab	1239.3.5±42.6c	1170.9±37.4cd	1081.6±89.4d	***	*	**
Sum of glucosides	952.8±3.2a	1094.3±0.9b	1013.2±13.7ab	828.0±28.8c	775.6±19.9cd	710.0±54.8d	***	**	***
Sum of acetates	325.5±4.2a	365.4±0.3b	331.2±5.9a	279.1±6.8c	270.5±8.8c	254.2±17.8c	***	**	***
Sum of coumaroylated	151.2±0.2ab	176.3±0.6a	155.7±7.7ab	132.2±7.2bc	124.8±9.1c	117.4±17.6c	***	ns	ns
Sum of phenolic acids	181.1±2.0a	181.2±3.8a	184.8±5.2a	107.3±9.2b	123.2±0.3b	112.7±0.8b	***	ns	ns
Sum of flavan-*3*-ols	127.7±2.3a	148.7±1.4b	135.3±5.3ab	148.8±0.5ab	184.9±4.8c	169.9±0.1c	***	***	*
Sum of benzoic acids	73.2±0.2a	73.4±0.5a	78.2±0.1b	33.0±0.5c	48.1±0.3d	44.1±0.1e	***	***	***
Sum of hydroxycinnamic acids	107.9±2.1a	107.8±4.3a	106.6±5.1a	74.3±9.2b	75.2±0.8b	68.1±0.9b	***	ns	ns
Sum of flavonols	34.5±2.1a	34.8±3.6a	33.3±1.4a	36.1±7.8a	30.3±2.2a	29.1±5.9a	ns	ns	ns
Anthocyanins									
Delphinidin 3-glucoside	55.9±1.8a	70.4±0.1b	63.8±1.6b	46.6±1.8c	40.9±1.5c	34.1±4.6d	***	**	***
Petunidin 3-glucoside	99.9±1.9a	123.2±1.2b	110.5±1.1a	83.5±3.9c	78.5±2.1c	66.7±7.6d	***	***	***
Peonidin 3-glucoside	78.6±1.9a	98.7±0.4b	96.5±6.5b	76.7±2.6ac	72.3±2.3ac	64.9±8.5c	***	*	***
Malvidin 3- glucoside	718.4±11.4a	802.1±6.1b	724.4±17.4ab	621.3±20.9c	583.8±15.3cd	544.3±34.8d	***	**	***
Petunidin 3-acetyl-glucoside	24.9±1.1a	29.2±0.4b	25.4±0.7a	21.5±3.7c	20.4±0.6c	17.5±1.4d	***	***	***
Peonidin 3- acetyl-glucoside	41.1±1.1a	48.7±0.6b	45.0±0.1ab	36.4±0.7c	35.9±0.9c	33.5±2.8c	***	**	***
Malvidin 3- acetyl-glucoside	259.6±2.1a	287.4±0.1b	261.0±6.7a	221.3±5.5c	214.2±7.5c	203.2±13.8c	***	**	***
Petunidin 3-*p*-coumaroil-glucoside	16.7±0.3a	18.3±0.6a	16.8±1.4a	13.9±0.6b	12.7±0.6b	11.3±1.7b	***	ns	ns
Peonidin 3- *p*-coumaroil-glucoside	41.1±0.8ab	47.9±0.7a	40.8±2.9ab	34.5±2.5bc	32.8±2.9bc	31.3±4.8c	***	ns	ns
Malvidin 3- *p*-coumaroil-glucoside	93.3±0.3a	110.3±0.6b	98.2±3.4ab	83.8±4.2ac	79.3±5.8ac	74.8±11.1c	***	ns	*
Benzoic acids									
Gallic acid	73.2±0.1a	72.7±0.4a	77.2±0.1b	33.0±0.1c	46.8±0.4d	42.5±0.2e	***	***	***
Ellagic acid	tr	0.7±0.2a	1.03±0.1b	tr	1.3±0.2b	1.6±0.1c	***	***	**
Hydroxycinn. acids									
t-caftaric-protocatechuic acid	54.7±0.1a	51.2±0.6a	49.7±0.7a	37.6±4.5b	37.3±0.7b	33.1±1.1b	***	**	ns

	C5	S5	D5	C10	S10	D10	Effect		
							Maceration time	Chips dose	Interaction
c-coutaric acid	3.5±2.1a	3.2±1.7a	2.2±0.1a	2.9±0.9a	3.6±0.3a	3.4±0.1a	ns	ns	ns
t-coutaric acid	32.3±0.1a	30.4±0.2ab	34.7±1.0a	20.6±2.8bc	20.9±0.3bc	17.7±0.3c	***	ns	ns
Fertaric acid	8.2±0.3a	7.7±0.7a	7.1±0.1a	6.2±1.2a	6.5±0.3a	6.3±0.1a	**	ns	ns
Coumaric hexose acid	4.7±5.5a	10.3±1.7b	9.1±2.2b	3.8±0.1a	3.5±0.2a	4.3±0.4a	***	**	**
Caffeic acid	4.4±2.1a	5.0±0.1a	3.8±0.1ab	3.1±0.3b	3.2±0.1b	3.3±0.1b	***	ns	ns
Flavan-3-ols									
Galocatechin(GC)	101.5±0.7a	122.7±0.9ac	109.4±4.2ac	101.3±6.5a	125.8±1.5b	118.1±0.7bc	**	***	ns
Procyanindin B3	11.1±0.3a	18.7±0.1b	11.1±0.1a	10.6±0.2a	13.4±0.2c	18.5±0.6b	ns	***	***
(+)- Catechin	9.8±0.6ab	11.7±0.4b	9.2±0.2a	17.8±0.2c	19.7±0.9c	14.9±0.4d	***	***	*
(-)- Epicatechin	5.2±0.4a	5.4±0.9a	5.6±0.7a	15.0±1.2b	25.8±2.2c	15.5±2.9b	***	**	**
Flavonols									
Myricetin 3-glucuronide	8.9±0.6a	8.8±0.8a	8.5±0.4a	8.9±0.2a	7.8±0.5a	7.6±0.8a	**	*	ns
Quercetin 3-glucuronide	6.7±0.6a	6.7±0.7a	6.4±0.1a	8.2±0.3a	7.2±0.7a	7.1±1.3a	ns	ns	ns
Quercetin 3-glucoside	12.1±0.4a	12.0±1.1a	11.5±0.5a	10.5±0.3a	9.6±0.6a	10.8±1.3a	**	ns	ns
Kaempferol 3-glucoside	0.5±0.1a	0.6±0.2a	0.5±0.2a	0.3±0.1a	0.3±0.1a	0.2±0.1a	***	ns	ns
Isorhamnetin 3-glucoside	3.9±0.4a	3.4±0.1a	3.8±0.5a	4.5±0.1a	3.9±0.2a	4.0±0.4a	ns	ns	ns
Syringetin 3-glucoside	2.3±0.2a	2.6±0.3a	2.5±0.1a	3.3±0.1a	2.8±0.1a	2.7±0.3a	**	*	**
Colour Data									
L*	58.9±0.3a	58.8±0.9a	59.3±0.3a	60.7.5±0.3ab	61.5±0.2ab	64.5±2.9b	**	ns	ns
a*	50.1±0.2a	53.5±1.1b	50.9±1.3a	44.4±0.2c	43.3±0.3c	39.6±2.8d	***	***	*
b*	-5.3±0.1a	-5.7±0.1a	-5.3±0.5a	-3.7±0.1b	-3.3±0.1bc	-4.3±0.4c	***	ns	**
C^*_{ab}	50.2±0.3a	53.8±1.1b	51.1±1.3a	44.6±0.3c	43.4±0.3c	39.8±2.7d	***	***	***
h_{ab}	-6.0±0.1a	-6.3±0.4a	-5.9±0.4a	-4.7±0.2b	-4.4±0.1b	-6.1±1.1b	***	ns	**

tr:traces; Different letters in the same row mean significant differences (ns: no significant; *$p<0.05$; ** $p<0.01$; *** $p<0.001$)

Table2

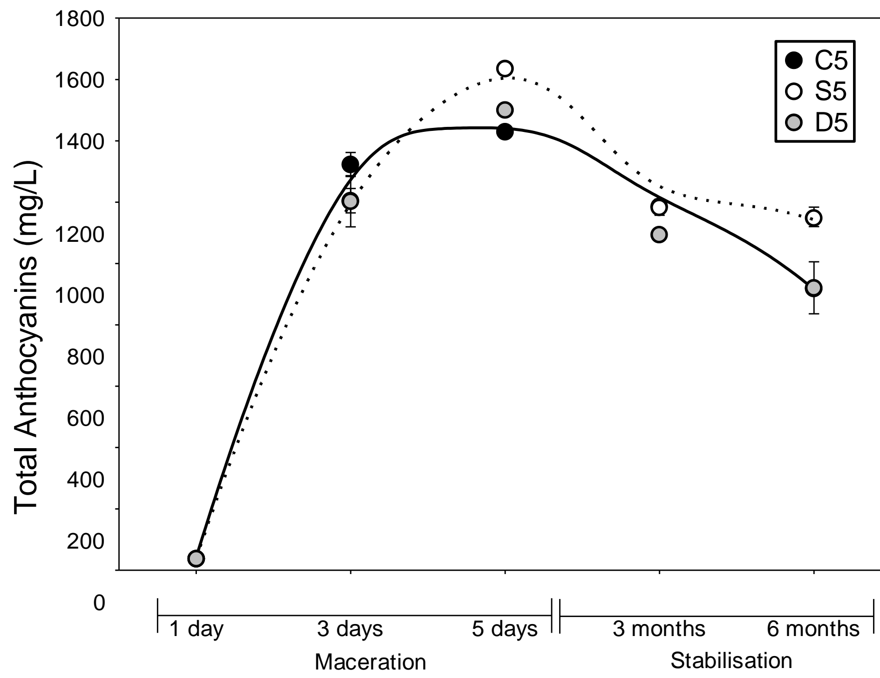
Table 2. Colour, lightness, chroma and hue differences (ΔE^*_{ab}, ΔL^*, ΔC^*_{ab}, Δh_{ab}) calculated for each wine from the beginning and the end of the maceration and stabilisation period, according to the maceration time (5 and 10 days) and chips dose (Control: 0 g/L; Simple: 3 g/L; Double: 6 g/L of oak chips).

		C5	**S5**	**D5**	**C10**	**S10**	**D10**
Maceration period	ΔE^*_{ab}	62.7	65.0	63.1	57.3	55.8	52.1
	ΔL^*	- 39.7	- 38.9	- 39.4	- 37.9	- 37.2	- 34.2
	ΔC^*_{ab}	+ 48.4	+ 52.0	+ 49.2	+ 42.7	+ 41.6	+ 39.3
	Δh_{ab}	- 15.2	- 15.4	- 15.0	- 13.8	- 13.5	- 15.2
Stabilisation period	ΔE^*_{ab}	33.8	23.14	29.4	20.2	24.0	26.4
	ΔL^*	+ 19.2	+ 9.1	+ 15.2	+ 11.6	+ 14.4	+ 15.7
	ΔC^*_{ab}	- 27.1	- 20.8	- 24.6	- 16.3	- 19.0	- 20.7
	Δh_{ab}	+ 9.6	+ 6.2	+ 8.7	+ 4.3	+ 5.0	+ 5.7

Figure1

Fig. 1

A)



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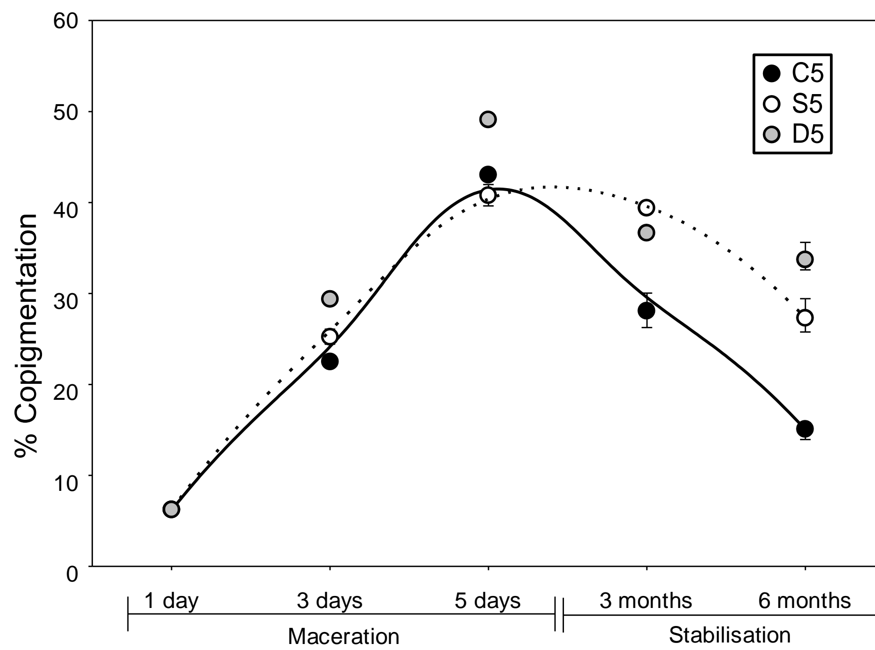


C)

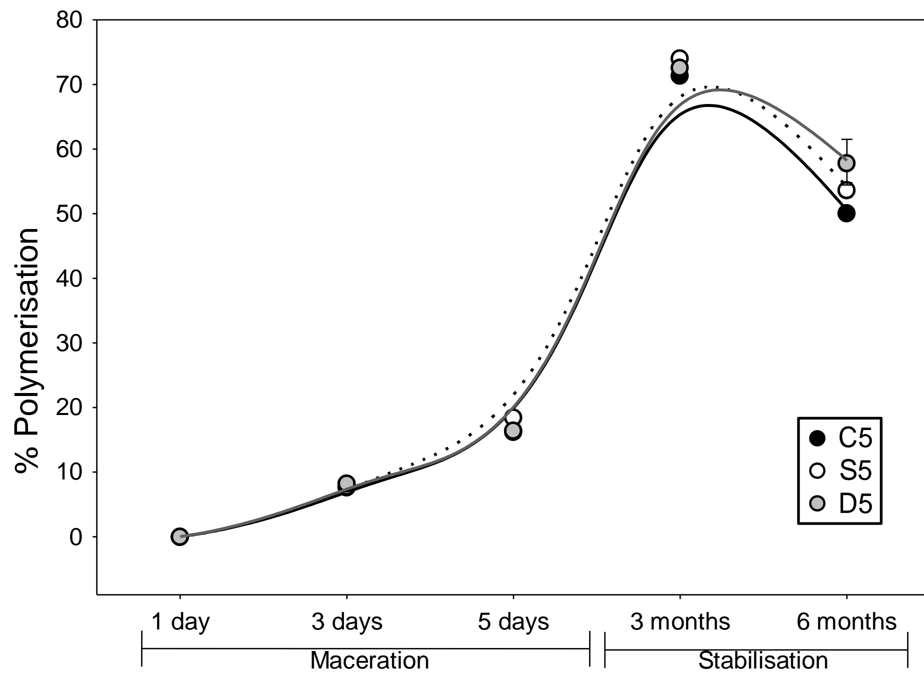
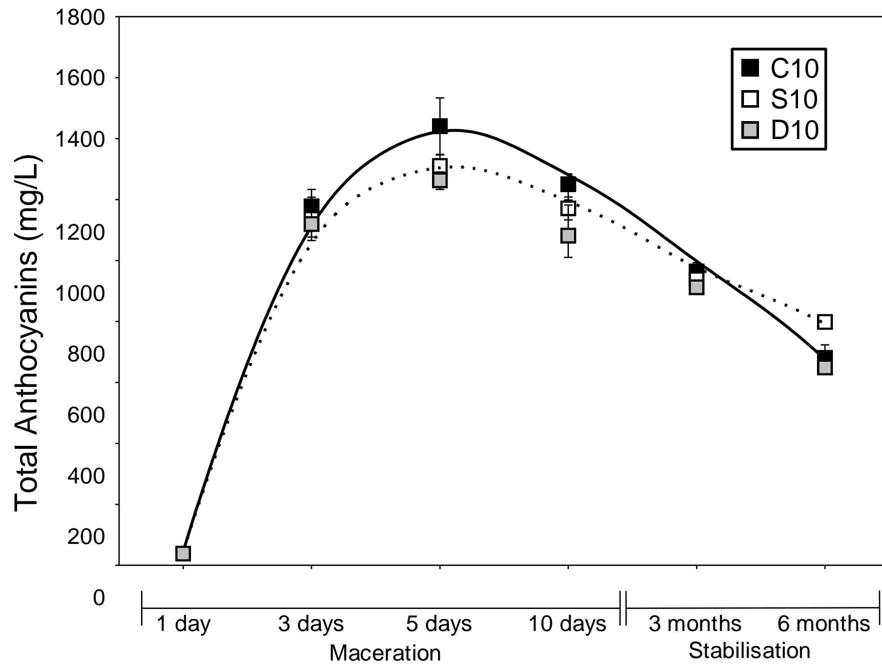


Figure2

Fig. 2

A)



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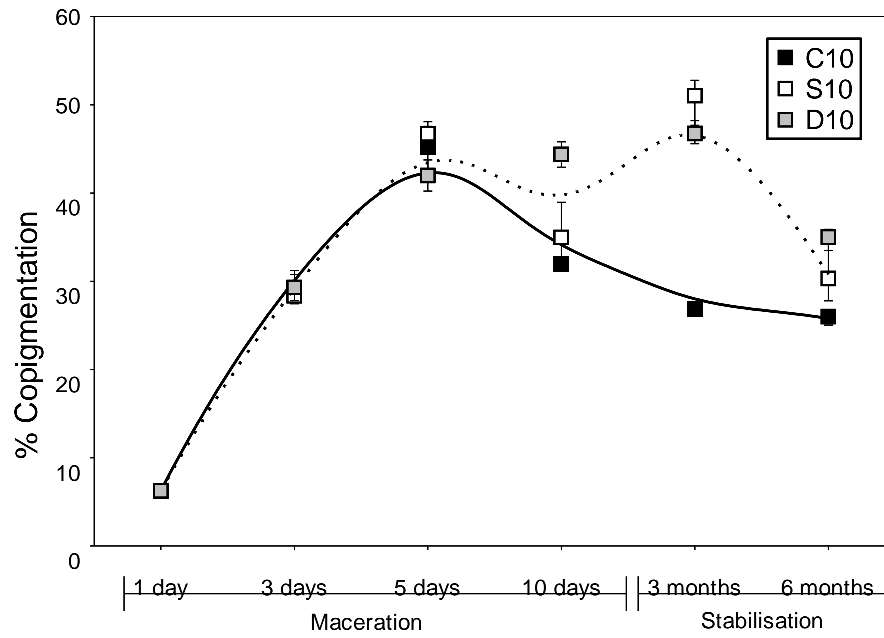


C)

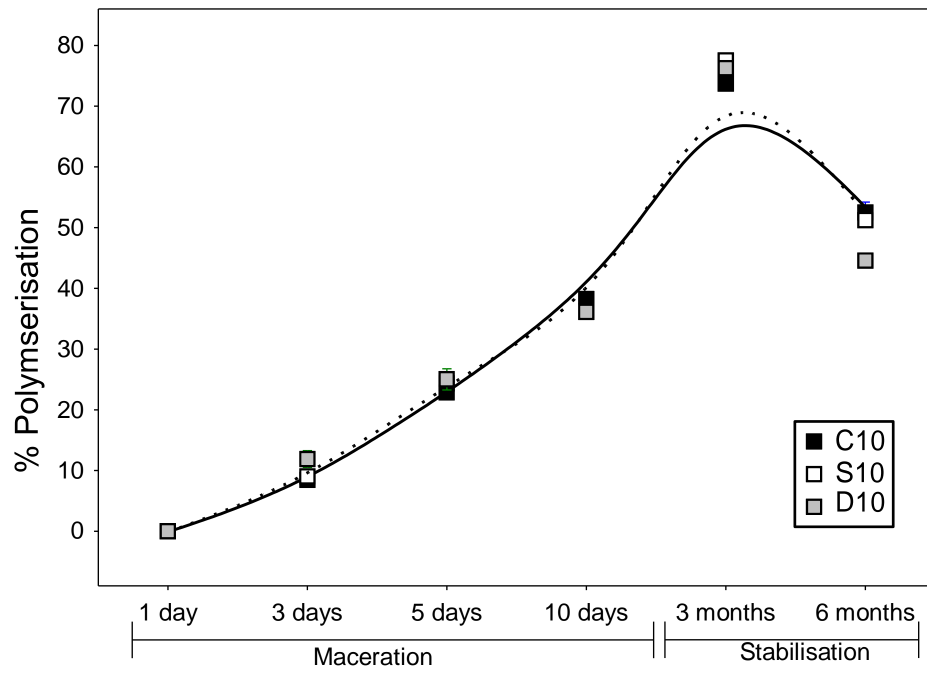
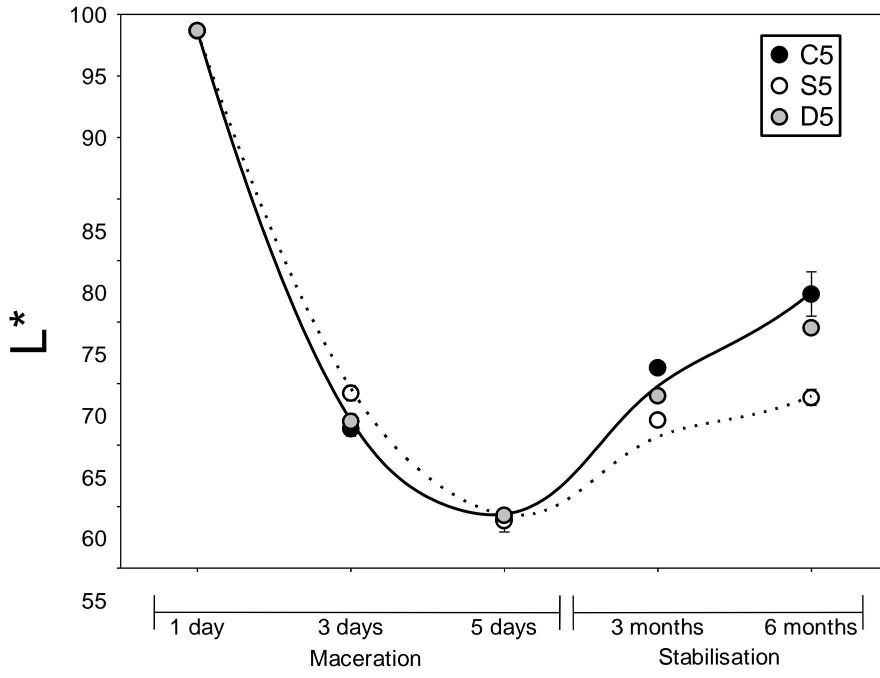


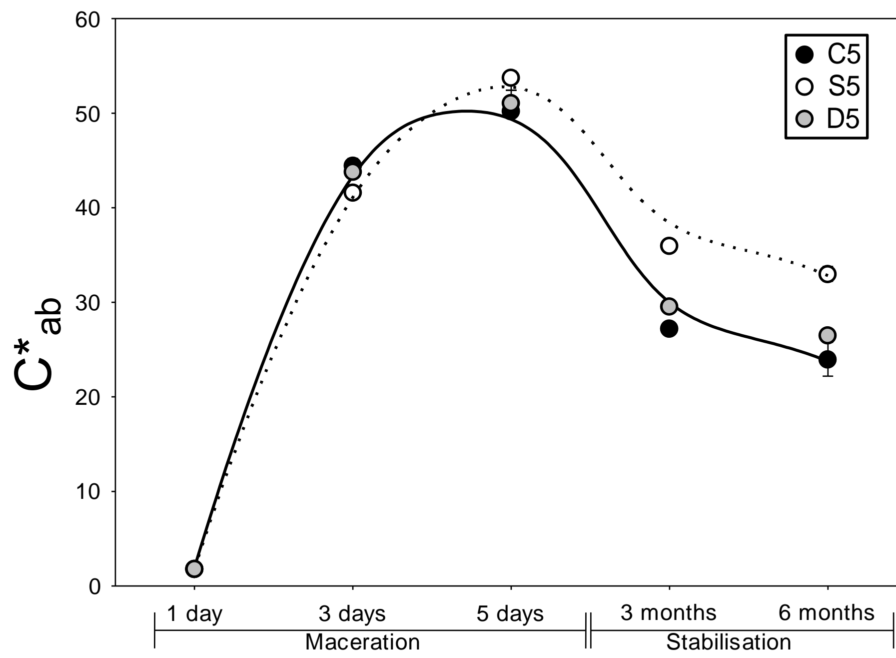
Figure3

Fig.3

A)



B)



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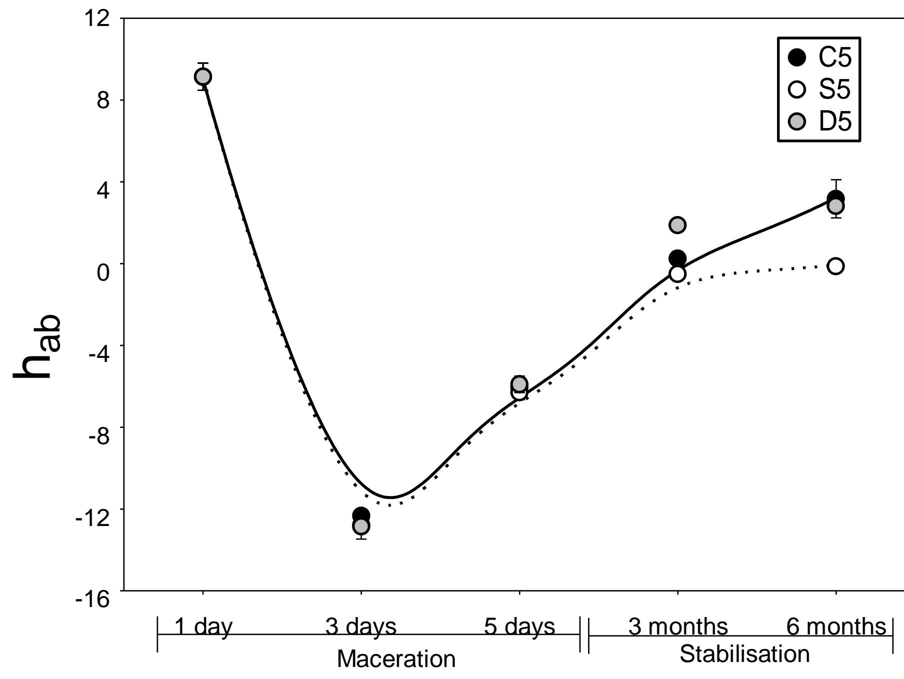
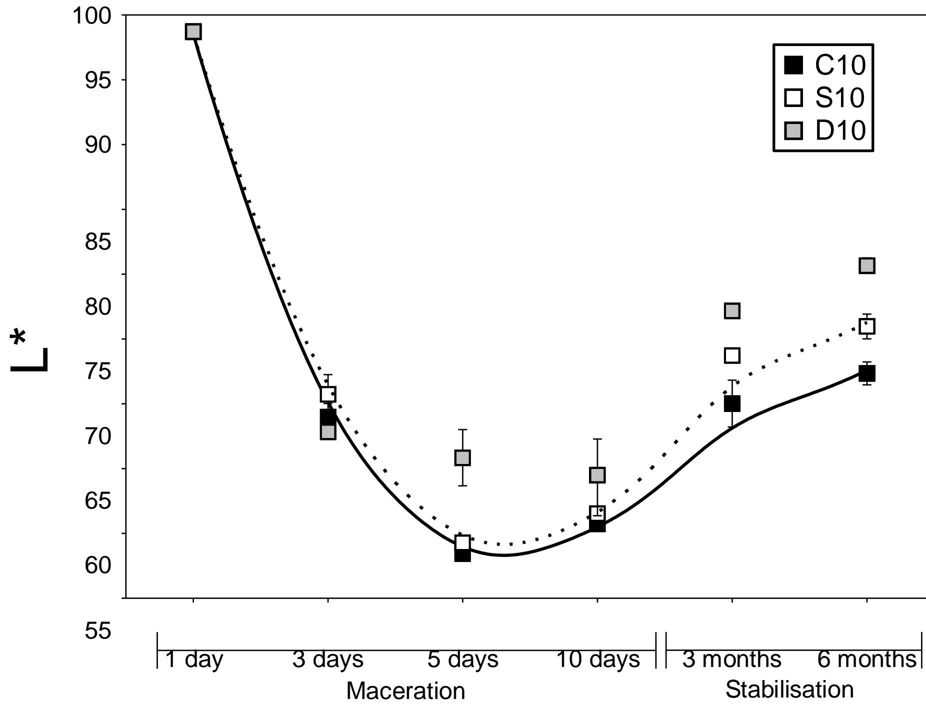


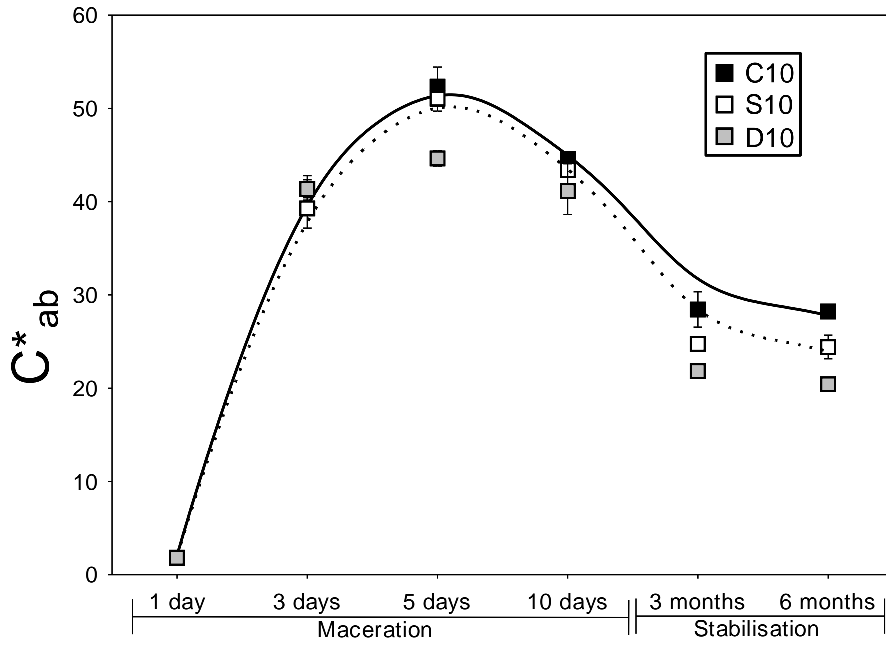
Figure4

Fig. 4

A)



B)



C)