INFLUENCE OF THE CARBONIC MACERATION WINEMAKING METHOD ON THE PHYSICOCHEMICAL,
COLOUR, AROMATIC AND MICROBIOLOGICAL FEATURES OF TEMPRANILLO RED WINES

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23 ABSTRACT

24 The aim of the study was to determine the differences in the microbiological, physical-chemical,
25 aromatic and phenolic composition between two winemaking methods: carbonic maceration and
26 the standard method of destemming and crushing. We analysed 84 commercial Rioja wines made
27 from the Tempranillo grape variety during the 2017 vintage, 40 had been made by carbonic
28 maceration and 44 by destemming and crushing. Despite the heterogeneity within the two groups
29 of wines, it was possible to differentiate between them. Wines made by carbonic maceration
30 presented higher aromatic quality due to their higher total content of esters and acetates, as well
31 as a greater colour intensity due to a higher phenolic content and higher rates of ionization and
32 polymerization. In addition, it was observed that the antioxidant activity, the content in coumaroyl
33 derivatives of anthocyanins and the vitisins A and B were considerably greater in wines made by
34 carbonic maceration.

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36 *Key words:* carbonic maceration, colour, aroma, microbiological populations, anthocyanin

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1. INTRODUCTION

The optimization and promotion of traditional winemaking methods, such as carbonic maceration (CM), could be an interesting tool in order to diversify production by offering quality products with very particular characteristics. The Spanish Rioja Qualified Designation of Origin (D.O.Ca. Rioja) is a winemaking area in which this traditional vinification system is fairly widespread. During recent years, winemaking using CM methods has been growing, and these wines are increasingly demanded by consumers (Tesniere & Flanzy, 2011).

Vinification by CM involves the process whereby the grapes are subjected to anaerobic conditions (Flanzy, 2003). So, the intact grape clusters, without destemming or crushing, are placed into tanks and kept under a carbon dioxide atmosphere. Under anaerobic conditions, intracellular fermentation (IF) occurs inside the whole grapes triggering the production of alcohol, the degradation of malic acid, pectolytic and proteolytic phenomena, the formation of volatile compounds and the diffusion of phenolic compounds from the skin to the pulp (Tesniere and Flanzy, 2011). Via these processes, the grapes, at a certain moment, split open and release their juice to the bottom of the tank, where it is fermented by yeasts. Therefore, IF of the grapes and alcoholic fermentation (AF) by the yeasts of must occur simultaneously in the tank. After this first phase, racking is done by drawing off a free-run, partly fermented wine, and the grapes that remain whole are pressed releasing a higher-density press-wine. Then, a second phase begins when both wines - mixed or separate- complete their AF by yeast and the malolactic fermentation by lactic acid bacteria (LAB).

Traditionally, it has been thought that CM wines are very different to those produced by destemming and crushing (DC), being described as light red wines with low tannins, less colour intensity and more intense fruit flavours, which have a shorter life and should be consumed early (Castillo-Sánchez, Mejuto, Garrido & García-Falcón, 2006; Etaio, Elortondo, Albisu, Gaston and

69 Schich, 2009). They are also characterized by lower glycerol content, lower level of fixed acidity
70 and a higher pH, which makes them very round wines, which should be consumed quickly (Etaio,
71 Elortondo, Albisu, Gaston, Ojeda & Schich, 2008).

72 Although CM wines are recognized as high quality young wines, recent works that describe in
73 depth their physicochemical and microbiological characteristics are scarce. In addition, there are
74 many contradictory results between different research findings about CM wines. It has been
75 extensively suggested that the total content of phenolic compounds, the colour intensity and the
76 concentration of tannins are generally lower in CM wines when compared to those made by DC,
77 giving them a very attractive colour and to be less astringent in the tasting (Spranger et al. 2004;
78 Etaio, Meillon, Perez-Elortondo & Schlich, 2016). However, Gómez-Míguez & Heredia (2004)
79 observed a higher concentration of anthocyanins in CM wines, which gave them an intense colour
80 with a tendency to purple, and Sun, Spranger, Roque-do-Vale, Leandro, C. & Belchior (2001) found
81 that wines made by CM contained the highest amounts of both catechins and oligomeric and
82 polymeric proanthocyanidins. With respect to the aroma, these wines have been described as
83 having a specific and rich floral aroma related to different volatile compounds, although without
84 conclusive criteria with which to differentiate these wines. They are thought to have the following
85 features; a higher content of free and combined terpenes, more esters (Antalick, Perello, & de
86 Revel, 2014), fewer traces of aldehydes and C6 alcohols responsible for herbaceous scents
87 (Spranger et al., 2004), and higher concentrations of ethyl cinnamate and benzaldehyde (Flanzy,
88 Flanzy & Bernard, 1987).

89 The contradictory results could be due to a wide range of causes such as the grape variety, the
90 grape ripeness level, the vintage or the winemaking conditions (AF temperature, maceration time,
91 shape and size of tanks, CO₂ addition, additives employed...). All these factors could for instance
92 affect the colour, aroma and composition of the final wines.

93 Therefore, the aim of this work was to extend our knowledge of the general differences between
94 commercial wines produced by DC or CM methods. Our starting hypothesis for this work was that
95 CM wines have physicochemical, aromatic and colour features, which lead them to being
96 consumed quickly. For this purpose, we analysed 84 bottled wines produced in the same region
97 (D.O.Ca. Rioja, Spain), in the same vintage (2017), with the same variety (Tempranillo) and stored
98 under similar conditions (six months after winemaking, 3-4 of them in the bottle, and without oak
99 ageing). However, the wines had been made in different wineries so they were not made in
100 identical conditions, allowing us to define the main characteristics that distinguish CM wines once
101 they are in the marketplace and available to consumers.

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103 2. MATERIAL AND METHODS

104 2.1 Wine origin

105 Eighty-four wines from the D.O.Ca. Rioja were purchased on the local market in April 2018. The
106 wines were all made from the same grape cultivar (cv Tempranillo) and in the same vintage
107 (2017). 40 wines had been made using CM while 44 had been made via the standard destemming
108 and crushing method.

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110 2.2 Physicochemical analysis of the wines

111 The wines were characterized by measuring the alcoholic strength, pH, total acidity, acetic acid,
112 free and total sulphur, colour intensity (CI) and tonality according to the official European
113 Community methods (ECC, 1990). The malic and lactic acids, reducing sugars, acetaldehyde and
114 free amino nitrogen (FAN) were determined by enzymatic methods carried out via an automated
115 analyser (Miura One, TDI, Spain). Total phenolics were determined as the total polyphenol index

116 by spectrophotometric absorbance at 280 nm after the dilution of the samples (Ribereau-Gayón,
117 Peynaud, Sudraud & Ribereau-Gayón 1980). Total anthocyanins were measured by decolouring
118 using SO₂. The ionization index was determined according to Glories (1978), the polymerization
119 index according to Ruiz Hernández (1999), copigmentation according to Boulton (2001) and total
120 tannins according to Riberau-Gayón et al. (1980). Total antioxidant activity in the wines was
121 determined according to the DPPH method as described by Nixdorf & Hermosin-Gutiérrez (2010).

122

123 **2.3 Analysis of grape anthocyanins by liquid chromatography**

124 Anthocyanins were determined by HPLC-DAD using an Agilent 1260 Infinity chromatograph
125 (Agilent, Palo Alto, CA, USA) equipped with a Licrospher® 100 RP-18 reversed-phase column
126 (250 × 4.0 mm; 5 µm packing; Agilent) with a Licrospher® 100 RP-18 pre-column (4 × 4 mm; 5 µm
127 packing; Agilent), both thermostated at 40 °C. The chromatographic procedure for anthocyanin
128 analysis was the same as that described by Portu, López-Alfaro, Gómez-Alonso, López & Garde-
129 Cerdán (2015). 10 µL of wine samples which had previously been filtered (0.22 µm, Easyprep.
130 Quebec, Canada) were injected into the system with a flow rate of 0.630 mL min⁻¹. The eluents
131 used were: acetonitrile–water–formic acid (3:88.5:8.5, v/v/v) (eluent A) and acetonitrile–water–
132 formic acid (50:41.5:8.5, v/v/v) (eluent B) and the following linear solvent gradient was
133 established: 0 min, 6% B; 15 min, 30% B; 30 min: 50% B; 35 min, 60% B; 38 min, 60% B; 46 min, 6%
134 B. Anthocyanins were identified according to the retention times of pure compounds and the UV-
135 Vis characteristics obtained from authentic standards. Quantification was made using the DAD
136 chromatograms recorded at 520 nm and calibration graph of the malvidin-3-O-glucoside.

137

138 **2.4 Analysis of volatile compounds by gas chromatography**

139 The analysis of fermentative volatile compounds in the wine was performed using the method
140 described by Ortega, López, Cacho, & Ferreira (2001) with modifications. The extraction was
141 carried out by mixing 4 mL of sample, 9 mL of $(\text{NH}_4)_2\text{SO}_4$ saturated solution, 40 μL of internal
142 standard solution (2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, 2-octanol,
143 and heptanoic acid, 40 mg of each /100 mL of ethanol) and 300 μL of dichloromethane in 20 mL
144 tubes. The tubes were shaken for 1 h at 400 rpm and then centrifuged at 4000 rpm for 10 min.
145 Once the phases were separated, the dichloromethane phase was recovered. 2 μL of this extract
146 were injected in split mode onto a Hewlett-Packard (Palo Alto, CA) 6890 series II gas
147 chromatograph equipped with an automatic injector and a Hewlett-Packard FID detector.
148 Separation was carried out with a DB-Wax capillary column (60 m x 0.32 mm I.D. x 0.5 μm film
149 thickness; J&W Scientific, Folsom, CA, USA). The temperature program was as follows: 40 $^{\circ}\text{C}$ for 5
150 min, then raised up to 220 $^{\circ}\text{C}$ at a rate of 3 $^{\circ}\text{C}/\text{min}$. The carrier gas was nitrogen at a flow rate of 3
151 mL/min. The injector temperature was 220 $^{\circ}\text{C}$ and the detector temperature was 280 $^{\circ}\text{C}$.
152 Identification of compounds was carried out by comparison of their retention times with those of
153 pure reference standards using a Hewlett-Packard GCD Series II Gas Chromatograph Electron
154 Ionization Detector with the same chromatographic conditions. Quantification of volatile
155 compounds was performed using an internal standard method and was based on calibration
156 curves. We employed different dilutions of a standard solution (12% ethanol and pH 3.6)
157 containing all chemical standards of volatile compounds analysed. These standard solutions
158 underwent the same extraction and analysis process as the wine samples. The calibration curves
159 were prepared analysing the extracts containing all the chemical standards and the internal
160 standards by the GC.

161

162 2.5 Microbiological analysis of the wines

163 The microbial population (yeast and bacteria) present in each wine was analysed. For this purpose,
164 serial dilutions of the wines were plated onto different culture media. Total yeasts were measured
165 by seeding onto a GYP culture medium (20 g/L glucose, 5 g/L yeast extract, 5 g/L peptone, 100
166 mg/L chloramphenicol and 0.1 g/plate biphenyl) and incubation at 28 °C for 48 h. The presence of
167 *Dekkera/Brettanomyces* yeasts was determined by seeding onto a DBDM culture medium (6.7 g/L
168 YNB, 20 g/L agar, 60 mL/L ethanol, 10 mg/L cycloheximide, 100 mg/L p-coumaric, 22 mg/L
169 bromocresol green and 0.1 g/plate biphenyl) and incubation at 25 °C for 21 days in anaerobic
170 conditions (Gas Pak System, Oxoid Ltd., Basingstoke, England). LAB were measured by seeding
171 onto an MRS medium (52 g/L MRS broth, 20g/L agar, 50 mg/L pyrimaricine and 0.1 g/plate
172 biphenyl) and incubation at 30 °C under strict anaerobic conditions for at least ten days. Acetic
173 acid bacteria were determined by seeding onto a Mann culture medium (25 g/L D-mannitol, 3 g/L
174 peptone, 5 g/L yeast extract, 20 g/L agar, 3 U/mL penicillin, 50 mg/L pyrimaricine and 0.1 g/plate
175 biphenyl) and incubation at 28 °C for 48 h. When the incubation periods finished, the plates were
176 examined and colony forming units per millilitre (CFU/mL) were counted in plates with counts
177 between 30 and 300 colonies and expressed in logarithmic units (log).

178

179 2.6 Statistical analysis of the results

180 Analysis of variance (ANOVA) was carried out for all the analytical results determined for the
181 different wines with the IBM® SPSS® Statistic version 23. Significant differences were established
182 by using the Tukey post-hoc test ($p < 0.05$ -0.01).

183

184 3. RESULTS AND DISCUSSION

185

186 3.1 Physicochemical analysis of the wines

187 Significant differences were not found in any of the physicochemical parameters of the wines
188 elaborated by CM and those elaborated by DC. The average values of these parameters are shown
189 in Table 1, which shows that there were hardly any differences in the average values of the
190 chemical parameters of the two types of wines. Although Etaio et al. (2008) indicated that CM
191 wines have a lower level of fixed acidity and a higher pH, by contrast, studying all the wines as a
192 whole, in the current study no differences in these parameters were observed, which is probably
193 due to the fact that these parameters are usually adjusted in wineries. In addition, the variability
194 in all parameters within each group of wines was very large, which is evidenced by the values of
195 the standard deviation (SD) and the coefficients of variation (CV). It is curious that for both types
196 of wines, the highest CV are found in the same parameters; reducing sugars and free and total
197 SO₂. In both cases, the average values obtained are low and below the maximum legal limit. The
198 residual sugar is an intrinsic value of each winemaking process, but in the case of SO₂ content, the
199 wineries adjust them at the time of bottling irrespective of the winemaking method, which would
200 explain the lack of difference between the two types of wines. So, with regard to the parameters
201 studied, the differences would not be directly related to the winemaking method used, but rather
202 to the implicit properties of the wines (winery, location, maturity of the grapes, etc.).

203 The lack of differences in the values of FAN (amino acids except proline and ammonia) and lactic
204 acid content is surprising. Taking into account the proteolytic processes taking place in the grapes
205 during the IF of CM, a higher concentration of amino acids in CM wines was expected (Antalick et
206 al., 2014), which might be observed as an increase of FAN, but it was not the case. A lower content
207 of lactic acid was also expected in CM wines, since malic acid is consumed during IF without
208 generating lactic acid, but this was not found either.

209

210 3.2 Colour parameters and the phenolic composition of the wines

211 Contrary to the homogeneity observed in the physical-chemical parameters of the two types of
212 wines, important differences were found between them in several parameters related to the wine
213 colour and phenolic content (Table 2). The phenolic composition of the wine is responsible for the
214 colour, bitterness, astringency, antioxidant properties and aging behaviour. Moreover, the wine's
215 colour is one the most important parameters that influences consumer choice and this depends
216 on anthocyanin extraction from the grape skins and its stabilization in wine in coloured forms. In
217 addition, red wines with high phenolic content are more attractive due to their likely beneficial
218 effect on health.

219 The wines made by CM presented greater colour intensity and greater concentration of total
220 phenolic compounds than those made by DC. These results correlated well with the higher
221 concentration of tannins and the higher values of the ionization and polymerization indexes of CM
222 wines (Table 2). In this respect, the ionization index measures the quantity of anthocyanins that
223 are in coloured forms, thus explaining the greater colour intensity of CM wines. The
224 polymerization index measures the amount of combined anthocyanins which indicates the wine
225 colour's stability. Sun et al. (2001) also found that wines made by CM contained higher amounts of
226 catechins and oligomeric and polymeric proanthocyanidins and explained it in base on the
227 presence of stems in the tanks. La Notte, Liuzzi, & Gambacorta (1993), explained that CM wines,
228 despite having lower amounts of monomeric anthocyanins, showed similar colour densities to
229 conventional ones, due to a higher degree of ionization of the pigments. These results are
230 therefore in agreement with our current study. However, they are in contrast with the generalized
231 ideas that vinification by CM leads to wines with a lower average content of total phenolic and
232 anthocyanins (Carnacini, Zironi, Potentini, Antonelli, Palotta & Amati (1991); Pellegrini, Simonetti,

233 Gardana, Brenna, Brighenti & Pietta (2000)) and that these wines are light-bodied red wines with
234 low tannin and intense colour (Spranger et al. 2004; Castillo-Sánchez et al., 2006).

235 No differences were found in the total content of anthocyanins (Table 2), although Gómez-Míguez
236 & Heredia (2004) observed a greater concentration of anthocyanins in CM wines. Some authors
237 have indicated that the factors affecting the anthocyanin extractability under CM vinification are
238 the ethanol formed inside the berries and, especially, the toughness of the grape skin, which also
239 depends on the grape variety (Pace, Giascosa, Torchio, Río Segade, Cagnasso & Rolle, 2014).

240 The fact that in our work the wines had been made with the same grape variety could explain the
241 absence of significant differences in this parameter. In addition, there were no differences in the
242 fraction of these compounds that are in free form (X_{FA}), forming copigments (X_C), or in the form of
243 polymeric pigments (X_{PP}). It is worth mentioning the absence of significant differences on the
244 proportion of copigmented anthocyanins. Ramos, Fleuriet, Rascalou & Macheix (1993) indicated
245 that during the CM process, a greater release of *p*-coumaric was produced since the IF is
246 characterized by a deep degradation of *p*-coumaroyltartaric acid ester. These authors found that
247 anthocyanins and hydroxycinnamoyl tartaric acid esters present in the skins of grape berries
248 decreased gradually during anaerobic treatment with CO₂. The released coumaric acid can have a
249 great impact when reacting with anthocyanins forming copigments that contribute to the colour
250 stability of young wines (Boulton, 2001). However, our results could not confirm this point since
251 no differences were observed in the proportion of copigmented anthocyanins

252 On another note, the results of the determination of wine antioxidant activity are shown in Table
253 2. Phenolic compounds, in addition to their influence on wine colour, are also important
254 contributors to the wine's antioxidant properties. Generally, previous authors have shown that
255 there are no important differences between CM and wines made via destemming and crushing
256 with respect to this parameter. For instance, Pellegrini et al. (2000) found that the average total

257 antioxidant activity of the CM wines and control wines was similar. Clare, Skurray & Shalliker
258 (2004) did not find differences between CM and control wines in the concentration of resveratrol,
259 one of the main compounds involved in wine antioxidant activity. Moreover, Sun et al. (2001)
260 observed that CM wines had a smaller amount of resveratrol than those made with skins in AF.
261 Nonetheless, other authors have described different results depending on the grape variety. On
262 account of this, Olejar, Fedrizzi & Kilmartin (2015) observed that the CM technique could
263 significantly increase the antioxidant activity in white Sauvignon Blanc wines, but not in
264 Chardonnay ones (Olejar, Fedrizzi & Kilmartin (2016). Our results clearly showed that Tempranillo
265 wines made by CM had higher antioxidant activity than wines made by destemming and crushing.
266 In our case, this correlates well with the results previously described regarding the total phenolic
267 index and total tannin content.

268 The HPLC analysis of the wines allowed the identification of 17 anthocyanins and two
269 pyranoanthocyanins (vitisins A and B) (Table 3). As can be seen, there were no differences in the
270 proportion of the different anthocyanins, which was the expected result, since the anthocyanin
271 profile is closely related to the grape variety. Moreover, the HPLC analysis showed no significant
272 differences in the total anthocyanin content, which coincides well with the global analysis of
273 anthocyanins in Table 2.

274 However, regarding their individual concentration, 10 of the 17 anthocyanins were found at higher
275 levels in the wines made by CM (Table 3). Significant differences especially concerned
276 coumarylated anthocyanins, since all of them were found at higher concentrations in CM wines.
277 Moreover, malvidin-3-caffeoylglucoside, as well as the acetylated derivatives of cyanidin and
278 petunidin and the non-acylated form of cyanidin, were also found in higher amounts in CM wines
279 when compared to the standard destemming and crushing method. These results suggest that CM

280 could have a significant impact on the extraction of acylated anthocyanins, especially on the
281 coumarylated forms, which could be a specific feature of this vinification method.

282 In this respect, anthocyanins in grape and wine can be found to be acylated by aliphatic (i.e. acetic
283 acid) or aromatic acids (i.e. hydroxycinnamic acids like *p*-coumaric and caffeic acid). The aromatic
284 acylation of anthocyanins has been described to shift their colour toward blue and increase their
285 stability to pH changes, high temperatures or SO₂ (He et al., 2012; Giusti & Wrolstad, 2003).
286 Moreover, anthocyanin acylation with cinnamic acids has been reported to increase their
287 antioxidant activity (Stintzing, Stintzing, Carle, Frei & Wrolstad, 2002), which could support the
288 foregoing results regarding this parameter.

289 These data are also in agreement with Pellegrini et al. (2000) who indicated that CM wines showed
290 a different phenolic pattern, with anthocyanins more abundant in CM than in the control.
291 However, our data contradict those indicated by most of the authors. For instance, Spranger et al.
292 (2004), found that the CM wines contained lower amounts of anthocyanins and suggested that the
293 higher temperature of CM might increase the degradation of anthocyanins. Similarity, Gomez-
294 Míguez & Heredia (2004) found that CM wines contained the lowest amount of all individual
295 anthocyanins and concluded that IF does not favour the extraction or stabilization of
296 monoglucoside anthocyanins because the degree of skin tissue degradation was insufficient. Some
297 authors have related the smaller quantity of individual anthocyanins to a higher quantity of
298 polymeric pigments (Sun et al. 2001). The contradiction between different results could be due to
299 the grape variety studied (enzymatic endowment, content of the different anthocyanins, and
300 resistance to breakage). Gomez-Míguez & Heredia (2004) also observed a constant increase in
301 individual anthocyanin fractions during the whole maceration period in CM, reaching it maximum
302 just before pressing and a marked decrease after pressing, which could be due to the mix between
303 pressed and free-run wine. Therefore, the intensity of the IF within the grapes will depend on their

304 resistance to rupture under pressure, so that the effect of CM vinification might be highly
305 conditioned by the hardness of the grapes (Giascosa et al., 2013). For this reason, some
306 importance has been attributed to the resistance to compression of the grapes in CM vinification,
307 as this is a variety dependent characteristic. In our case every wine was made with the same
308 variety and then the differences found could be directly related to the winemaking process.

309 Moreover, vitisins A and B were also higher in CM wines (Table 3). A study carried out by Chinnici,
310 Sonni, Natali, Galassi & Riponi (2009) also pointed to a higher content in vitisins in these wines.
311 This was due to a deviation of the metabolism of the berry that significantly affects the production
312 of organic acids (decrease of malic acid, greater stability of tartaric and citric acids and increase of
313 fumaric, shikimic and succinic acids) during CM. This increase in succinic acid formation improves
314 the formation of vitisin A and its derived compounds (acetyl and coumaryl vitisin A). The content
315 of vitisin A is related to the content in pyruvic acid and it could become the main pigment in older
316 wines because of its slow degradation rate (Boido, Alcalde-Eon, Carrau, Della Cassa & Rivas-
317 Gonzalo, 2006). B-type vitisins could be found in high amounts in CM wines, probably because of
318 the high amounts of acetaldehyde coming from anaerobic metabolism (Amati, Donati, Galassi &
319 Pallotta (1973). Chinnici et al. (2009) indicated that this could be a specific feature of CM wines
320 and its magnitude may depend on the actual percentage of CM in blended final products.

321 Winemaking techniques influence the phenolic content of red wines and anthocyanin stabilization,
322 determining the quality of the wine. The quantity of phenolic compounds that are transferred
323 from grapes into wine during maceration varies according to the conditions of the process
324 (Tahmaz & Soylemezoglu, 2017). Some metabolites produced during winemaking may condense
325 with grape anthocyanins to produce highly stable pyranoanthocyanin adducts, such as vitisin A
326 and B respectively (Morata, Calderón, González, Gómez-Cordovés & Suárez, 2007). This reaction
327 can lead to the stabilization of anthocyanins during wine aging. Probably during the CM process

328 more carbonyl compounds are synthesized which directly impacts on the formation of stable
329 pigments, as they act as precursors of pyranoanthocyanins. Stabilization of anthocyanins can also
330 occur through reaction between anthocyanins and tannins to form pigmented tannins and
331 through copigmentation of anthocyanins. In the CM wines, we found a greater quantity of tannins
332 which, through direct or acetaldehyde bridged condensation adducts with anthocyanins, could
333 lead to a greater stabilization of the colour. Taking into account all these results, it could be
334 suggested that the intensity of colour of CM wines is not necessarily lower or less stable than in
335 standard wines made by destemming and crushing.

336

337 **3.3 Aromatic composition of wines**

338 The analysis of the aromatic composition of wines by gas chromatography allowed the
339 identification of 37 volatile compounds. The overall results are shown in Figure 2, where it can be
340 seen that there were no significant differences in the total content of alcohols, or in short chain
341 acids between the two types of wines. However, CM wines showed a significantly higher total
342 concentration in ethyl esters and acetates of higher alcohols. Taking into account that these two
343 types of compounds have fruity and floral aromas, CM wines would have a fruitier character than
344 those made by DC. Antalick et al. (2014) also found higher concentrations of alcohol acetates in
345 CM wines and related it to the lack of oxygen during vinification and the higher levels of amino
346 acids released in anaerobic metabolism. However, we did not find differences in the content of
347 FAN between the two types of wine (Table 1), perhaps because these had been converted into
348 alcohols and later into esters, which would justify the higher concentration of the latter
349 compounds in CM wines. The formation of esters differs widely depending on various factors such
350 as AF temperature, nutrient availability, pH, unsaturated fatty acids, sterol levels and oxygen levels
351 (Styger, Prior & Bauer, 2011).

Analysing the acetates of higher alcohols (Table 4) individually, significant differences were detected in all the compounds except hexyl acetate. Regarding the ethyl esters, although in the total sum there was a significant difference, individually only butyrate and ethyl isobutyrate showed differences. Antalick et al. (2014) found exactly the same results with respect to these compounds: higher concentration of ethyl butyrate in CM wines and ethyl isobutyrate in DC wines.

Although the sum of higher alcohols and acids showed no significant differences, some were found individually in certain compounds (Table 4). 1 Propanol and 1 Butanol were higher in CM wines, and methionol and hexanol in DC wines. Rizzon, Miele, Meneguzzo & Zanuz (1999) observed higher amounts of higher alcohols in the DC wines than in the CM wines, while Yang, Kakuda & Subden (2006) found hardly any differences between the types of wine and indicated that the sensory contribution of higher alcohols in CM wines was inconsequential.

The hexanol level was much higher in DC wines. Similar results were found by Salinas, Alonso, Navarro, Pardo, Jimeno & Huerta (1996) and Spranger et al. (2004) who indicated that extended pomace contact can increase the formation of this compound and perhaps the less intense maceration of the grapes in CM winemaking could explain the lower content of hexanol. Significant differences were also found in several acids individually (propanoic, butyric, octanoic and decanoic), where the higher content was found in CM wines, except in the case of propanoic acid (Table 4).

The concentration of ethyl lactate (Table 4) was lower in CM wines, which would indicate less accumulation of lactic acid during this vinification process, although as we have indicated previously, there were no significant differences in the lactic acid content between the two types of wine (Table 1). The ethyl acetate content was higher in CM wines and methanol content was higher in DC wines. Similar results in these two parameters were detected by Rizzon et al. (1999) in French Cabernet wines made by both methods. The lower content of methanol in the CM wines

377 could be due to the effect of anaerobic conditions on the activity of pectolytic enzymes, which
378 favour the hydrolysis of pectins and the release of methanol.

379 Overall, aromatic compounds would indicate slight differences between the two types of wines.
380 This could be due to the type of compounds analysed in this work, many of them originated by the
381 microbial metabolism. Dourtoglou, Yannovits, Tychopoulos & Vamvakias (1994) indicated that the
382 typical volatiles of CM wines giving the fruity-floral aroma were formed during the stage of storage
383 under CO₂ atmosphere. They found 114 volatile compounds in the CO₂ treated sample against 60
384 in the control sample. Ducruet et al. (1983) concluded that the typical volatiles of CM wines were
385 phenols, ethyl and methyl vainillate and ethyl cinnamate, all of them formed during the first phase
386 of vinification when the whole grapes are kept under CO₂ atmosphere.

387

388 **3.4 Microorganisms in wines**

389 The shelf life of a young wine is directly related to the presence of spoiling microorganisms. The
390 microbiological load of CM wines, favoured by the vinification conditions (high temperature, low
391 dose or absence of sulphite, higher pH due to degradation of acids, increase in the amount of
392 assimilable nitrogen), can make them more susceptible to microbial alterations during storage.
393 However, according to the data of the microbiological study of the wines (Figure 2), there were no
394 significant differences between the types of wine for any microorganism studied. It could be
395 explained by the absence of differences in important physicochemical parameters for microbial
396 population development, such as pH, alcohol, sulphur and FAN.

397 Although without significance, in CM wines the content of LAB was higher than in DC ones. In
398 addition, in two of the 40 wines produced by CM, *Brettanomyces* yeasts were isolated, which were
399 not detected in wines made by DC. In Figure 2, it can also be observed that in the wines made by
400 CM a smaller number of yeast colonies was detected and that acetic bacteria were not isolated in

any of the analysed wines. The variability found in the number of microorganisms among wines made with the same technique was so high that it did not allow a clear correspondence between the microbial population and the processing technique. This variability could be linked to the different treatments of CM and DC wines applied by the wineries prior to bottling and releasing for sale.

406

407 4. CONCLUSIONS

408

Despite the heterogeneity within the two groups of samples studied, determined by the particular way of processing in each winery (degree of ripeness and integrity of the grapes, time of maceration, temperature), it has been possible to differentiate between the two types of wine mainly from their polyphenolic and aromatic composition. However, no significant differences were observed in the physical-chemical composition or in the microbial load between the types of wine.

The wines made by CM showed higher total content of esters and acetates, compounds which could have an effect on fruit-like aroma. CM wines also had greater colour intensity due to their higher concentration in phenolic compounds, and higher rates of ionization and polymerization. Traditionally, it has been thought that CM wines evolve negatively during storage due to the low level of monomeric pigments that can react to give stable condensed products with a lower tendency to oxidation. However, our results seemed to be in contrast to this idea, since the anthocyanin content of CM wines was similar to the wines produced by destemming and crushing, but CM wines had higher levels of compounds that contribute to wine colour stability.

The results from the current work have also shown that wine anthocyanins, in particular the coumaroylated derivatives, as well as wine antioxidant activity and the amount of vitisins A and B,

425 were considerably influenced by vinification by CM. These compounds could be a specific feature
426 of this kind of vinification. Nevertheless, it is necessary to confirm this hypothesis with new tests
427 carried out in the same winery, starting from the same grapes and produced via both methods,
428 controlling during the whole winemaking process.

429

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436

437 **Conflict of interest**

438 The authors declare that they have no conflict of interest

439

440 **5. REFERENCES**

441 Amati, A., Donati, A.M., Galassi, S. & Pallotta, U. (1973). Esperimenti di vinificazione traite
442 macerazione carbonica di uve Romagnole. Caratteristiche chimiche e fisico-chimiche di vini
443 Sangiovese, Merlot et Cagnina ottenuti mediante macerazione carbonica. *Scienza e Tecnologia*
444 *degli Alimenti* 3 (6), 357-361.

445

446 Antalick, G., Perello, M.C. & de Revel, G. (2014) Esters in Wines: New Insight through the
447 Establishment of a Database of French Wines. *American Journal of Enology and Viticulture* 65, 293
448 -304. <https://doi:10.5344/ajev.2014.13133>.

449

450 Boido, E., Alcalde-Eon, C., Carrau, F., Della Cassa, E. & Rivas-Gonzalo, J.C. (2006). Aging effect on
451 the pigment composition and colour of *Vitis vinífera* L. cv. Tannat wines. Contribution of the main
452 pigment families to wine colour. *Journal of Agricultural and Food Chemistry* 54, 6692-6704.
453 <https://doi.org/10.1021/jf061240m>.

454

455 Boulton, R. (2001). The copigmentation of anthocyanins and its role in the colour of red wine: A
456 critical review *American Journal of Enology and Viticulture* 52, 2, 67-87.

457

458 Carnacini, A., Zironi, R., Potentini, G., Antonelli, A., Palotta, U. & Amati, A. (1991). Influence of the
459 conservation conditions and aging on composition of wines obtained by carbonic maceration from
460 grapes cv. Montepulciano. *Wein-Wissenschaft46, Viticulture and Enology Sciences*, 93-99.

461

462 Castillo-Sánchez, J.J., Mejuto, J.C., Garrido, J. & García-Falcón, S. (2006). Influence of wine-making
463 protocol and fining agents on the evolution of the anthocyanin content, colour and general
464 organoleptic quality of Vinhao wines. *Food Chemistry*, 97, 130-136.
465 <https://doi:10.1016/j.foodchem.2005.03.030>.

466

467 Clare, S., Skurray, G. & Shalliker, R.A. (2004). Effect of Pomace-Contacting method on the
 468 concentration of cis- and trans-resveratrol and resveratrol glucoside isomers in Wine. *American*
 469 *Journal of Enology and Viticulture* 55, 401-406.

470

471 Chinnici, F., Sonni, F., Natali, N., Galassi, S. & Riponi, C. (2009). Colour features and pigment
 472 composition of Italian carbonic macerated red wines. *Food Chemistry* 113, 651-657.
 473 <http://doi:10.1016/j.foodchem.2008.07.055>.

474

475 Dourtoglou, V.G., Yannovits, N.G., Tychopoulos, V.G. & Vamvakias, M.M. (1994). Effect of storage
 476 under CO₂ atmosphere on the volatile, amino acid and pigment constituents in red grape (*Vitis*
 477 *vinifera* L. Var. Agiorgitiko). *Journal of Agriculture and Food Chemistry* 42, 338-344.

478

479 Ducruet, V., Flanzy, C., Bourzeix, M. & Chamboy, Y. (1983). Volatiles of young wines obtained by
 480 carbonic maceration. *Sciences des Aliments* 3, 413-426.

481

482 ECC (1990). Commission Regulation Vo. 2676/90 concerning the establishment of common
 483 analytical methods in the sector of wine. *Official Journal of European Communities* L272, 1-192.

484

485 Etaio, I., Elortondo, F.J.P., Albisu, M., Gaston, E., Ojeda, M. & Schich, P. (2008). Effect of
 486 winemaking process and addition of white grapes on the sensory and physicochemical
 487 characteristics of young red wines. *Australian Journal of Grape and Wine Research*, 14, 211-222.
 488 <https://doi:10.1111/j.1755-0238.2008.00024.x>.

489

490 Etaio, I., Elortondo, F.J.P., Albisu, M., Gaston, E., Ojeda, M. & Schich, P. (2009). Sensory attribute
491 evolution in bottled young red wines from Rioja Alavesa. *European Food Research Technology*,
492 228, 695-705. <https://doi:10.1007/s00217-008-0980-1>.

493

494 Etaio, I., Meillon, S., Perez-Elortondo, F.J.P. & Schlich, P. (2016). Dynamic sensory description of
495 Rioja Alavesa red wines made by different winemaking practices by using Temporal Dominance of
496 Sensations. *Journal of Sciences and Food Agriculture* 96, 3492–3499. [https://doi:](https://doi:10.1002/jsfa.7533)
497 10.1002/jsfa.7533.

498

499 Flanzy, C., Flanzy, M. & Bernard, P. (1987). La vinification par maceration carbonique, INRA. Paris,
500 FRA, *INRA Editions*, 126 p. <https://prodinra.inra.fr/record/114214>.

501

502 Flanzy, C. (2003). Vinificación por maceración carbónica. Enología: Fundamentos científicos y
503 tecnológicos, (2d ed.). A. Madrid Vicente Ediciones. Madrid: Mundi Prensa Libros S.A. ISBN 84-
504 8476-074-X 84-89922-74-8.

505

506 Giacosa, S., Torchio F., Segade S.R., Gaiotti, F., Tomasi, D., Lovat, L., Viucenzi, S. & Rolle, L. (2013).
507 Physico-mechanical evaluation of the aptitude of berries of red wine grape varieties to resist the
508 compression in carbonic maceration vinification. *International Journal of Food Science and*
509 *Technology* 48, 817–825. [http://doi: 10.1111/ijfs.12032](http://doi:10.1111/ijfs.12032).

510

511 Giusti, M.M. & Wrolstad, R.E. (2003). Acylated anthocyanins from edible sources and their
 512 applications in food systems. *Biochemical Engineering Journal* 14, 3, 217-225. .
 513 [http://doi: 10.1016/S1369-703X \(02\)00221-8](http://doi:10.1016/S1369-703X(02)00221-8).

514

515 Glories, Y. (1978). Recherches sur la Matière colorantes des vins rouges. Ph.D.Thesis Université de
 516 Bourdeaux II (France).

517

518 Gómez-Miguez, M. & Heredia, F.J. (2004). Effect of the maceration technique on the relationships
 519 between anthocyanin composition and objective color of Syrah wines. *Journal of Agriculture and*
 520 *Food Chemistry* 52, 5117–5123. [https://doi: 10.1021/jf049570z](https://doi:10.1021/jf049570z).

521

522 La Notte, E., Liuzzi, V.A. & Gambacorta, G. (1993). Il colore del vino in relazione alla frazione
 523 antocianica ionizzata. Influenza del sistemadi macerazione. *Rivista di viticoltura e di Enologia* 1, 21-
 524 35.

525 Morata, A., Calderón, F., González, M. C., Gómez-Cordovés, M. C. & Suárez, J. A. (2007). Formation
 526 of the highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic
 527 acid and acetaldehyde. *Food Chemistry*, 100(3), 1144–1152.
 528 <http://doi.org/10.1016/j.foodchem.2005.11.024>.

529

530 Olejar, K.J., Fedrizzi, B., Kilmartin, P.A. (2015). Antioxidant activity and phenolic profiles of
 531 Sauvignon Blanc wines made by various maceration techniques. *Australian Journal of Grape and*
 532 *Wine Research* 21, 57–68. [https://doi: 10.1111/ajgw.12119](https://doi:10.1111/ajgw.12119).

533

534 Olejar, K.J., Fedrizzi, B., Kilmartin, P.A. (2016). Enhancement of Chardonnay antioxidant activity
535 and sensory perception through maceration technique. *LWT-Food Science and Technology* 65,
536 152–157. [https://doi: 10.1016/j.lwt.2015.08.001](https://doi.org/10.1016/j.lwt.2015.08.001).

537

538 Ortega, C., López, R., Cacho, J. & Ferreira, V. (2001). Fast analysis of important wine volatile
539 compounds. Development and validation of a new method based on gas chromatographic-flame
540 ionisation detection analysis of dichlorometane microextracts. *Journal of Chromatography A* 923,
541 205-214. [http://doi:10.1016/S0021-9673\(01\)00972-4](http://doi.org/10.1016/S0021-9673(01)00972-4).

542

543 Pace, C., Giascosa, S., Torchio, F., Río Segade, S., Cagnasso, E. & Rolle, L. (2014). Extraction kinetics
544 of anthocyanins from skin to pulp during carbonic maceration of winegrape berries with different
545 ripeness levels. *Food Chemistry* 165, 77-84. [https://doi: 10.1016/j.foodchem.2014.05.070](https://doi.org/10.1016/j.foodchem.2014.05.070).

546

547 Pellegrini, N., Simonetti, P., Gardana, C., Brenna, O., Brighenti, F. & Pietta, P. (2000). Polyphenol
548 content and total antioxidant activity of vini novelli (young red wines). *Journal of Agriculture and*
549 *Food Chemistry* 48, 732–735. <http://doi.org/10.1021/jf990251v>.

550

551 Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., & Garde-Cerdán, T. (2015). Changes on
552 grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea.
553 *Food Chemistry* 180, 171–180. [https://doi: 10.1016/j.foodchem.2015.02.042](https://doi.org/10.1016/j.foodchem.2015.02.042).

554

555 Ramos, T., Fleuriet, A., Rascalou, M. & Macheix, J.J. (1993). The effect of anaerobic metabolism of
 556 grape Berry skin son phenolic compounds. *American Journal of Enololgy and Viticulture* 44(1), 13-
 557 16.

558

559 Ribéreau-Gayon, J., Peynaud, E., Sudraud, P., Ribereau-Gayon, P. (1980). Tratado de Enología:
 560 Ciencias y Técnicas del vino. Tomo I: Análisis y control de los vinos. Ed. Hemisferio sur S.A. Buenos
 561 Aires, Argentina.

562

563 Rizzon, L.A., Miele, A., Meneguzzo, J. & Zanuz, M.C. (1999). Efeito de três procesos de vinificação
 564 sobre a composição química e a qualidade do vino Cabernet franc. *Pesquisa Agropecuária*
 565 *Brasileira* 34, 7, 1285-1293. ISSN 1678-3921 online version.

566

567 Ruiz Hernández, M., 1999. La crianza del vino tinto desde la perspectiva vitícola. AMV Eds., Madrid
 568 Spain. ISBN: 13:9788484760481.

569

570 Salinas, M.R., Alonso, G.L., Navarro, G., Pardo, F., Jimeno, J. & Huerta, M.D. (1996). Evolution of
 571 the aromatic composition of wines undergoing carbonic maceration under different aging
 572 conditions. *American Journal of Enology and Viticulture* 47, 2, 134-144.

573

574 Spranger, I., Clímaco C., Sun, B., Eiriz, N., Fortunato, C., Nunes, A., Leandro, M.C., Alvear, M.L., &
 575 Belchior, P. (2004). Differentiation of red winemaking technologies by phenolic and volatile
 576 composition. *Analytica Chimica Acta* 513, 151–161. [https://doi: 10.1016/j.aca.2004.01.023](https://doi.org/10.1016/j.aca.2004.01.023).

577

578 Stintzing, F.C., Stintzing, A.S., Carle, R., Frei B. & Wrolstad, R.E. (2002). Colour and antioxidant
579 properties of cyanidin-based anthocyanin pigments. *Journal of Agricultural and Food Chemistry* 50,
580 21, 6172-6181. <https://doi.org/10.1021/jf0204811>.

581

582 Styger, G., Prior, B. & Bauer, F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology*
583 *and Biotechnology* 38, 1145-1159. <http://doi: 10.1007/s10295-011-1018-4>.

584

585 Sun, B., Spranger, I., Roque-do-Vale, F., Leandro, C. & Belchior P. (2001). Effect of different
586 winemaking technologies on phenolic composition in Tinta Miúda red wines. *Journal of Agriculture*
587 *and Food Chemistry* 49, 5809-5816. <https://pubs.acs.org/doi/abs/10.1021/jf010661v>.

588

589 Tahmaz, H. & Soylemezoglu, G. (2017). Effects of Vinification Techniques Combined with UV-C
590 Irradiation on Phenolic Contents of Red Wines. *Journal of Food Science*, 82(6), 1351–1356.
591 <http://doi.org/10.1111/1750-3841.13724>.

592

593 Tesniere, C. & Flanzy, C. (2011). Carbonic maceration wines: characteristics and winemaking
594 process. In: Jackson RS (ed) *Advances in Food Nutrition Research* (pp. 1–15). Academic Press
595 Elsevier, Burlington.

596

597 Yang, D.Y., Kakuda, Y. & Subden, R.E. (2006). Higher alcohols, diacetyl, acetoin and 2,3 butanediol
598 biosynthesis in grapes undergoing carbonic maceration. *Food Research International* 39, 112-116.
599 <http://doi.org/10.1016/j.foodres.2005.06.007>.

600

601

Table 1. Average data of physicochemical parameters of wines elaborated by carbonic maceration (n=40) and by destemming and crushing (n=44) with the standard deviation (SD) and the coefficient variation (CV).

Physicochemical parameters	Winemaking processes			
	Carbonic Maceration		Destemming and Crushing	
	Average± SD	CV	Average± SD	CV
Ethanol % (v/v)	13.67± 0.62	4.55	13.57± 0.63	4.65
pH	3.77± 0.11	2.93	3.74± 0.14	3.84
Total acidity (g/L)	4.57± 0.61	13.45	4.62± 0.58	12.61
Reducing sugars (g/L)	2.52± 0.62	24.50	2.53± 0.77	30.36
Acetic acid (g/L)	0.36± 0.09	24.73	0.40± 0.09	23.62
Lactic acid (g/L)	1.76± 0.51	29.16	1.81± 0.39	21.55
Acetaldehyde (g/L)	32± 6	19.73	30± 5	17.42
FAN * (mg/L)	65± 21	33.04	67± 17	25.40
Free SO ₂ (mg/L)	14± 4	26.91	15± 6	42.49
Total SO ₂ (mg/L)	37± 15	40.31	39± 13	34.12

*FAN: free amino nitrogen

Table 2. Average data of colour parameters of wines elaborated by carbonic maceration (n=40) and by destemming and crushing (n=44) $\pm$ the standard deviation.

Colour Parameters		Winemaking processes	
		Carbonic maceration	Destemming and crushing
Color intensity (CI)		9.45 $\pm$ 1.92 b	8.36 $\pm$ 1.68 a
Hue		0.656 $\pm$ 0.062	0.676 $\pm$ 0.66
Total polyphenol index (TPI)		55.13 $\pm$ 6.77 b	51.57 $\pm$ 7.81 a
Ionized anthocyanins (%)		19.90 $\pm$ 4.33	17.37 $\pm$ 3.42
Polimerization index		2.14 $\pm$ 0.45 b	1.91 $\pm$ 0.36 a
Total antocyanins (mg/l)		495.6 $\pm$ 106.3	459.9 $\pm$ 122.7
Total tannins (g/L)		2.67 $\pm$ 0.51 b	2.49 $\pm$ 0.54 a
	X _{FA} ^a	0.548 $\pm$ 0.072	0,528 $\pm$ 0,049
Copigmentation	X _C ^b	0.246 $\pm$ 0.078	0,266 $\pm$ 0,058
	X _{PP} ^c	0.206 $\pm$ 0.036	0,206 $\pm$ 0,043
Total antioxidant activity ^d		0.522 $\pm$ 0.060 b	0.474 $\pm$ 0.065 a

For each row, values with different letters are significantly different between the samples (p $\leq$ 0.05).

^a Fraction of anthocyanins in free form; ^b Fraction of anthocyanins forming copigments; ^c Fraction of anthocyanins in the form of polymeric pigments; ^d As millimoles of Trolox equivalents per litre of wine.

Table 3. Anthocyanin content (mg/L) in wines elaborated by carbonic maceration (n=40) and by destemming and crushing (n=44) $\pm$ the standard deviation.

Anthocyanins	Winemaking processes	
	Carbonic maceration	Destemming and crushing
Delphinidin-3-glc	42.34 $\pm$ 15.76	36.66 $\pm$ 14.43
Cyanidin-3-glc	4.40 $\pm$ 2.00 b	2.75 $\pm$ 1.22 a
Petunidin-3-glc	42.63 $\pm$ 13.44	37.13 $\pm$ 12.77
Peonidinn-3-glc	12.83 $\pm$ 4.84	11.05 $\pm$ 4.63
Malvidin-3-glc	168.5 $\pm$ 41.9	161.7 $\pm$ 41.8
Delphinidin3-acglc	3.46 $\pm$ 0.96	3.52 $\pm$ 1.07
Cyanidin-3-acglc	0.324 $\pm$ 0.104 b	0.236 $\pm$ 0.075 a
Petunidin-3-acglc	3.73 $\pm$ 1.06 b	2.93 $\pm$ 0.94 a
Peonidin-3-acglc	1.28 $\pm$ 0.98	1.13 $\pm$ 0.92
Malvidin-3-acglc	8.97 $\pm$ 3.39	8.90 $\pm$ 3.19
Delphinidin-3-cmglc	4.86 $\pm$ 1.73 b	3.64 $\pm$ 1.46 a
Cyanidin-3-cmglc	1.25 $\pm$ 0.43 b	1.02 $\pm$ 0.39 a
Petunidin-3-cmglc	4.19 $\pm$ 1.55 b	3.03 $\pm$ 1.17 a
Peonidin-3-cmglc	3.27 $\pm$ 1.11 b	2.68 $\pm$ 1.16 a
Malvidin-3- <i>cis</i> -cmglc	1.66 $\pm$ 0.57 b	0.856 $\pm$ 0.239 a
Malvidin-3- <i>trans</i> -cmglc	22.22 $\pm$ 7.57 b	17.22 $\pm$ 5.60 a
Malvidin-3-CfGlc	0.658 $\pm$ 0.216 b	0.434 $\pm$ 0.163 a
Total anthocyanins	326.6 $\pm$ 89,5	294.9 $\pm$ 82.4
Total non-acylated	270.7 $\pm$ 74.0	249.3 $\pm$ 70.1
Total acetylated	17.76 $\pm$ 5.19	16.72 $\pm$ 5.22
Total coumaroylated	37.45 $\pm$ 12.38 b	28.45 $\pm$ 9.31 a
Total acylated	55.87 $\pm$ 17.11 b	45.61 $\pm$ 15.25 a
Vitisin A	2.76 $\pm$ 1.41 b	2.13 $\pm$ 0.67 a
Vitisin B	2.00 $\pm$ 0.5 b	1.50 $\pm$ 0.59 a

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, coumaroylglucoside; cfiglc, caffeoylglucoside. For each row, values with different letters are significantly different between the samples ($p \leq 0.01$).

Table 4. Aromatic compounds (mg/L) in wines elaborated by carbonic maceration (n=40) and by destemming and crushing (n=44) $\pm$ the standard deviation.

Aromatic compounds	Winemaking processes	
	Carbonic maceration	Destemming and crushing
1 propanol	44.2 $\pm$ 25.9 b	33.9 $\pm$ 9.7 a
1 butanol	2.30 $\pm$ 1.37 b	1.56 $\pm$ 0.80 a
Isobutanol	50.5 $\pm$ 13.0	55.5 $\pm$ 11.4
Amylic alcohols	286.2 $\pm$ 45.0	287.4 $\pm$ 39.9
2 phenyl ethanol	44.5 $\pm$ 16.6	46.2 $\pm$ 10.6
1 hexanol	1.13 $\pm$ 0.61 a	1.87 $\pm$ 0.40 b
Benzyl alcohol	0.238 $\pm$ 0.127	0.257 $\pm$ 0.147
Methionol	1.55 $\pm$ 0.68 a	1.88 $\pm$ 0.67 b
cis-3-hexenol	0.183 $\pm$ 0.050	0.242 $\pm$ 0.087
3-etoxi 1 propanol	1.34 $\pm$ 2.40	0.640 $\pm$ 0.457
Total alcohols	432.2$\pm$70.9	429.6$\pm$54.4
Isoamyl acetate	2.78 $\pm$ 1.99 b	1.14 $\pm$ 0.95 a
Hexyl acetate	0.086 $\pm$ 0.131	0.046 $\pm$ 0.049
2-phenyl ethyl acetate	0.244 $\pm$ 0.172 b	0.102 $\pm$ 0.109 a
Total acetates	3.11$\pm$2.13 b	1.29$\pm$1.05 a
Ethyl propionate	0.138 $\pm$ 0.051	0.130 $\pm$ 0.026
Ethyl 3-hydroxibutirate	0.630 $\pm$ 0.271	0.552 $\pm$ 0.166
Ethyl isobutyrate	0.044 $\pm$ 0.021 a	0.053 $\pm$ 0.016 b
Ethyl butyrate	0.293 $\pm$ 0.100 b	0.241 $\pm$ 0.057 a
Ethyl hexanoate	0.357 $\pm$ 0.106	0.339 $\pm$ 0.075
Ethyl octanoate	0.276 $\pm$ 0.124	0.229 $\pm$ 0.100
Ethyl decanoate	0.059 $\pm$ 0.029	0.065 $\pm$ 0.016
Total esters	1.80$\pm$0.52 b	1.61$\pm$0.28 a
Propanoic acid	7.94 $\pm$ 4.39 a	9.86 $\pm$ 3.74 b
Isobutyric acid	2.87 $\pm$ 1.07	3.03 $\pm$ 1.16
Butiric acid	2.44 $\pm$ 0.65 b	2.06 $\pm$ 0.58 a
Isovaleric acid	2.48 $\pm$ 0.75	2.24 $\pm$ 0.57
Hexanoic acid	2.77 $\pm$ 0.74	2.73 $\pm$ 0.59
Octanoic acid	2.47 $\pm$ 0.73 b	2.13 $\pm$ 0.62 a
Decanoic acid	0.525 $\pm$ 0.21 b	0.394 $\pm$ 0.178 a
Total acids	21.5$\pm$5.1	22.5$\pm$4.0
Other volatile compounds		
Methanol	108.3 $\pm$ 45.7 a	173.5 $\pm$ 32.3 b
Ethyl acetate	70.8 $\pm$ 23.4 b	54.2 $\pm$ 9.7 a
Ethyl lactate	81.9 $\pm$ 28.0 a	94.7 $\pm$ 25.9 b
Diethyl succinate	7.01 $\pm$ 2.50	7.91 $\pm$ 2.44
Acetoin	23.0 $\pm$ 15.3	19.8 $\pm$ 15.2
Dyacetil	5.65 $\pm$ 2.14 b	4.39 $\pm$ 2.69 a
Butyrolactone	13.1 $\pm$ 2.9	13.5 $\pm$ 4.6

For each row, values with different letters are significantly different between the samples ($p \leq 0.05$).

Figure 1. Aromatic composition of wines elaborated by carbonic maceration (CM) (n=40) and by destemming and crushing (DC)(n=44). *Total acetates x10; Total esters x10; Total acids x10. Different letters indicate significant differences between the samples ($p \leq 0.05$).

Figure 2. Content of microorganisms in wines elaborated by carbonic maceration (n=40) and by destemming and crushing (n=44).