

Research Note

Location Effects on the Aromatic Composition of Monovarietal cv. Carignan Wines

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Abstract: The aromatic profile of monovarietal Carignan wine has been characterized, and the influence of the origin on the volatile composition wines from six different zones of Chile and from two different seasons has been studied. The solid phase microextraction conditions employed were previously studied. An extraction temperature of 45°C, 40 minutes of incubation, 1.5 g of salt and 180 seconds of fiber desorption were employed for the analysis of the 28 wine samples studied. Sixty-three volatile compounds were determined in Carignan cv. wines, and they were characterized by substantial amounts of ethyl esters and small amounts of volatile acid compounds among others. Analysis of variance results showed significant effects on the “location” factor ($p < 0.05$), which was corroborated by principal component analysis and linear discriminant analysis, where clear groups were observed regarding production area. The geographical origin affected the volatile composition of the studied wines and production areas

31 closer to the Andes Mountains showed significant lower contents of esters and acids than the
32 ones closer to the ocean.

33 **Keywords:** aroma, Carignan, characterization, chromatography, extraction, location

34 **Introduction**

35 The Carignan cultivar is originally from Spain, specifically from Cariñena, a small town located
36 in the region of Aragón, which provides the name of the wines with Origin Denomination (O.
37 D.) produced in this zone. This variety was introduced to France in the XIIth century, becoming
38 the most widely red grape cultivar in French vineyards (Galet 1990).

39 Historically, the grapes of this cultivar have been used to produce blend wines with other grapes
40 from Rioja O.D. Its intense acidity, deep violet-red color and fruity/floral aroma, which are now
41 highly valued organoleptic properties, have encouraged the production of monovarietal Carignan
42 wines.

43 While this variety is extensively cultivated in many European countries such as Spain, France
44 and Italy, it is also cultivated in the Americas, being Mexico, United States, and Chile the main
45 countries, respectively (OIV 2011). Among the 722 hectares approximately cultivated in Chile,
46 82% are located in the Maule Region, a valley that is one of the most productive wine regions in
47 this country (SAG, Servicio Agrícola y Ganadero, <http://www.sag.gob.cl/>).

48 Chilean orography is very heterogeneous, resulting in a wide range of climatic zones, with some
49 of them influenced more by the Pacific Ocean and others influenced more by the Andes
50 Mountains. Thus, the climate of the Maule Valley can be divided into five climatic zones with

51 three climate indexes (Montes 2006) (Figure 1). Climate indexes are used for the estimation of
52 the potential climate of a particular place to ensure the maturation of different varieties (Huglin
53 and Schneider 1998). The *Huglin Index* (HI) relates the average and maximum daily
54 temperatures during the active growing season and day length coefficient (Huglin 1978).
55 According to Huglin and Schneider (1998), the Carignan variety has an HI requirement of 2,200
56 units to obtain a sugar content of approximately 18 to 20 ° Brix. The *Cold Night Index* (CI) can
57 be used to determine the average minimum nightly temperatures during the maturation period. It
58 is very important to take into account this index because night temperatures influence the
59 formation of aromas in the grape and subsequently in wines (Tonietto 1999). Finally, the
60 *Drought Index* (DI) allows a characterization of the water component of the climate, indicating
61 the presence or absence of drought in a wine region, which accounts for the climatic demand of a
62 standard vineyard, evaporation from bare ground and rain without surface runoff or without
63 internal drainage (Tonietto 1999). The three indexes described above can be integrated, creating
64 a climate classification system for wine regions (Tonietto and Carbonneau 2004) which allows
65 for the classification and grouping together of regions according to their similarities (Montes
66 2006).

67 Climatic conditions in conjunction with soil characteristics, cultivar, biodiversity and human
68 practices configure *terroir* (Van Leeuwen et al. 2004), which is clearly related to the typicality
69 and quality that can be found in a wine produced in a specific region (Roullier-Gall et al. 2014).
70 Many studies have focused on the effects of climate because it is considered to be the main
71 influence (De Andrés-De Prado et al. 2007). One of the characteristics that may be affected by

72 *terroir* is the volatile profile of grapes (Jiang et al. 2013), and the aroma is one of the most
73 important factors defining the character and quality of wine (Vilanova et al. 2007).

74 The differences between wines produced from grapes grown in different areas separated by a few
75 kilometers are significant and these differences are even more significant between wines of the
76 same variety grown in different countries; this applies to wines produced from Carignan grapes.
77 Nevertheless, the volatile composition of Carignan wines has been scarcely studied. As far as we
78 known, there are only few works which involve the study of the volatile composition of wines
79 made from Carignan grape (Baumes et al. 1986, Arozarena et al. 2000, Hernández-Orte et al.
80 2002, Geffroy et al. 2015).

81 In addition, although solid-phase micro extraction (SPME) has been widely employed for the
82 analysis of volatiles in oenological samples, only Hernández-Orte et al. (2002) have applied
83 previously this extraction technique to Carignan wine samples, using it for the analysis of
84 volatile sulfur compounds.

85 Therefore, the objectives of this study were on the one hand, to optimize solid-phase
86 microextraction conditions to characterize the aromatic profile of pure Chilean Carignan wines
87 and on the other hand, to evaluate the influence of the location of production on the volatile
88 composition of these wines, using samples from six different zones of the Maule Valley and
89 from two different harvests, 2012 and 2014.

90

91 **Materials and Methods**

92 **Samples**

93 For this study, 28 commercial Carignan cv. wines were analyzed in triplicate and included 15
94 samples of 2012 harvest wines and 13 samples of 2014 harvest wines. The samples were
95 collected from wine cellars from six different zones of the Maule Region of Chile: Caliboro (8)
96 [35°49'27"S; 71°54'14"W]; Melozal (8) [35°42'0" S; 71°48'0" W]; Cauquenes (2)
97 [35°58'00"S; 72°21'00"W], Huerta del Maule (2) [35° 40' 0"S; 71° 57' 0" W]; Loncomilla (4)
98 [35°34'31"S; 71°45'24"W], Sauzal (4) [35°45'00"S; 72°07'00"W].

99 Sauzal and Cauquenes locations have a mean Huglin index (HI) of 2223 units, the average value
100 of Drought index (DI) of -240 mm, and a Cold Night Index (CI) of 9.6 °C. Caliboro and
101 Melozal, have a HI of 2088 units, DI of -145 mm of average and a CI de 8.8 °C. Huerta del
102 Maule and Loncomilla are characterized by a HI of 2400 units, DI of -222 mm and mean CI of
103 9.7 °C.

104 **Reagents and standards**

105 All the chemicals used were analytical-reagent grade and provided from the following sources:
106 ethyl isobutyrate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl isovalerate, ethyl crotonate, ethyl
107 hexanoate, ethyl octanoate, ethyl undecanoate, ethyl decanoate, ethyl 2-furoate, ethyl succinate,
108 ethyl phenylacetate, isobutyl acetate, isoamyl acetate, isoamyl butanoate, isoamyl hexanoate,
109 isoamyl lactate, isoamyl octanoate, methyl octanoate, methyl decanoate, isobutanol, butanol, 3-

110 methyl-1-butanol, 2 ethyl-1-hexanol, 3-methyl-1-pentanol, hexanol, 3-hexen-1-ol, 2,3-
111 butanediol, 1-nonanol, 1-decanol, 2-phenylethanol, 4-ethylphenol, limonene, o-cymene, trans-2
112 pinalol, linalool, alpha-terpineol, hexanoic acid, octanoic acid, decanoic acid, furfural,
113 benzaldehyde, 5-methyl-furfural, beta-damascenone, and oaklactone were purchased from
114 Sigma-Aldrich (Steinheim, Germany).

115 The internal standard employed, 4-methyl-2-pentanol, and also acetic acid, ethyl acetate and
116 sodium chloride were purchased from Merck (Darmstadt, Germany).

117 **Headspace solid-phase micro-extraction (HS-SPME) and GC/MS conditions**

118 The HS-SPME method was optimized to give the most suitable extraction and desorption of
119 wine volatiles, with the optimized parameters including incubation temperature (35, 45, 55 and
120 65°C), extraction time (20, 30, 40 and 50 minutes), desorption time (180, 300 and 420 seconds)
121 and concentration of sodium chloride (1, 1.5 and 2 g). While one variable was studied, the rest
122 were fixed to an average value (35°C, 30 minutes, 300 seconds and 1.5 g).

123 In all cases, 7 mL of wine was placed into a 20-mL glass vial with sodium chloride and 15 μ L of
124 4-methyl-2-pentanol (IS) at 0.75 g/L, and the vial was then positioned in the autosampler tray for
125 HS-SPME sampling.

126 Static headspace sampling was employed after the fiber was cleaned and conditioned (1 hour at
127 270 °C before the first use, and 5 minutes before each extraction). After incubating with
128 temperature and agitation speed of 500 rpm, the volatiles in the headspace of the vial were
129 extracted with a 2-cm 50/30- μ m Carboxen/DVB/PDMS SPME fiber (Supelco, Bellefonte, PA,

130 USA). The fiber penetration in the vial during extraction was 30 mm, and after extraction, the
131 fiber was desorbed. The sample was injected using the splitless (3 min) mode with an injector
132 temperature of 250 °C and a transfer line temperature of 280 °C.

133 Gas chromatographic analysis was carried out using a 7890B Agilent GC system coupled to an
134 Agilent 5977 inert quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA).

135 A DB Wax capillary column (60 m x 0.25 mm, 0.25 µm film thickness, J&W Scientific, Folsom,
136 CA, USA) was used, with helium carrier gas at a flow rate of 1 mL/min. The oven temperature
137 program was as follows: the temperature was 35 °C for 10 min and was subsequently raised to
138 100 °C at 5 °C/min and then to 210 °C at 3 °C/min (holding 40 minutes). The electron ionization
139 mass spectra (40 – 300 amu) were acquired in scan mode at 70 eV.

140 All data were recorded using MS ChemStation software (Agilent Technologies, Palo Alto, CA,
141 USA). The samples were analyzed in triplicate, and blank runs using empty glass tubes were
142 performed before and after each analysis.

143 **Identification and quantitation**

144 Compound identification was based on mass spectra matching using version 2.0 of the standard
145 NIST library and the retention index (LRI) of authentic reference standards or the data in the
146 literature. Quantitation has been carried out employing calibration curves of five levels of
147 concentration in triplicate for each volatile compound (relative area versus concentration). The
148 relative area was calculated by dividing the peak area of the target ion of each compound by the

149 peak area of the target ion of the internal standard. Those identified compounds whose
150 commercial standards were unavailable were quantified either with the calibration curve of an
151 equivalent compound (Table 1) or by means of relative area (Table 2).

152 **Statistical analysis**

153 Statistical analysis was carried out using the Statistica 8.0 software package (StatSoft, Inc. Tulsa,
154 OK, USA). Analysis of variance (ANOVA) followed by a post hoc comparison test (Tukey's
155 test), principal component analysis (PCA) and linear discriminant analysis (LDA) were
156 performed.

157 **Results and Discussion**

158 **Optimization of the extraction conditions**

159 For the optimization of the extraction parameters, a Carignan wine from the 2015 harvest was
160 employed. Among the different conditions assayed, the temperature and extraction time showed
161 the highest influence on the results obtained. Thus, the higher the temperature employed, the
162 larger the recovery obtained of the less volatile compounds, especially the acids (Figure 2).
163 However, some highly volatile compounds, such as some esters and alcohols, were lost during
164 extraction and exhibited poorly resolved chromatographic peaks. Incubation times also affected
165 recoveries, and therefore, intermediate conditions were selected, i.e., 45 °C and 40 minutes of
166 incubation. Varying the salt concentration generally affected recoveries, with the addition of 1.5

167 g being optimum for most compounds. Finally, 180 seconds of fiber desorption showed the
168 highest recoveries of the studied volatile compounds and the best peak resolutions.

169 **Characterization of the aromatic profile**

170 A total of 62 volatile compounds were determined in monovarietal Carignan cv. wine samples
171 (Table 1, Figure 3). The most abundant chemical groups were esters (32), followed by alcohols
172 (15) and terpenes (5). Compounds from other families such as acids (4), aldehydes (3), lactones
173 (2) and C13 norisoprenoid (1) were also identified.

174 The volatile profile of these wines was very similar, despite growing zone differences, with
175 respect to major volatile compounds. Table 2 shows mean concentrations of the volatile
176 compounds in the wines from the different studied locations, grouping 2012 and 2014 vintages
177 together.

178 The results showed that the Chilean wines of Carignan cv. were characterized by important
179 amounts of ethyl esters such as ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl succinate,
180 ethyl isobutyrate, ethyl butyrate and ethyl 2-methylbutyrate. Geffroy et al. (2015) found in
181 Carignan wines from Aragón (Spain), ethyl 2-methylbutyrate as the major ester volatile
182 compound followed by isobutyl acetate, ethyl furoate and ethyl acetate. Baumes et al. (1986)
183 identified as the major esters ethyl 2-hydroxy propionate and ethyl butanoate, followed by ethyl
184 succinate in Carignan wines from Montpellier (France).

185 These previous reports, however, are in accordance with our results regarding the major volatile
186 alcohols found in Chilean Carignan cv. wines, such as 3-methyl-1-butanol, isobutanol, 2-
187 phenylethanol and hexanol.

188 Carignan Chilean wines contained low amounts of volatile acid compounds (Table 2). The major
189 acid volatile compound found was acetic acid, followed by hexanoic, octanoic and decanoic
190 acids, being in agreement with the order found by Geffroy et al. (2015).

191 Among the volatile terpenes identified, trans-2-pinalol and o-cymene were the terpenes with the
192 highest concentration depending on the location, followed by alpha-terpineol. However, in
193 Spanish Carignan wines from the above mentioned studies, the major terpene compound was
194 linalool followed by alpha-terpineol, and citronellol was the most abundant terpene found in the
195 studied French wines (Baumes et al. 1986, Geffroy et al. 2015).

196 As seen, some differences in composition are found upon comparing Chilean and European
197 wines, so volatile composition seems to be dependent on the origin.

198 **Effect of location and production year**

199 Differences observed in volatile composition of wines are usually produced not only by the
200 location but also by the production conditions. To minimize that factor, all wines analyzed in this
201 research come from a group of Chilean winemakers that employ similar practices of production
202 and harvesting, so we can suppose that the differences observed could be mainly derived from
203 production area and harvest year. To identify significant differences these variables, the

204 quantitative compound data were subjected to analysis of variance (ANOVA). Table 3 shows the
205 results for the two factors studied.

206 Although almost half of the compounds exhibit significant differences when the “year” factor
207 was taken into account, grouping similar compounds together showed that the wines from the
208 same area had generally similar total volatile profiles between 2012 and 2014 (Figure 4). This
209 indicates that production year is not a very relevant parameter that affects volatile composition of
210 the studied wines.

211 Twenty-five compounds showed significant differences when production zones ($p < 0.05$, Table
212 3) was taken into account. Therefore, a post hoc comparison Tukey’s test was applied to the data
213 according to the variable “location”, with the results presented in Table 2. Production area does
214 affect compound family profiles, such as esters and acids. As seen, significantly higher contents
215 of these types of compounds were found in wines from Sauzal and Cauquenes, both of which are
216 the closest regions studied to the Pacific Ocean. Conversely, significantly lower amounts of
217 esters were found in wines from Loncomilla and Caliboro, and lower contents of terpenes were
218 found in wines from Melozal and Huerta of Maule, the closest regions to the Andes Mountains
219 (Figure 1). Production region does appear to affect the content of these compounds in wines and
220 therefore could result in decreased floral and fruity characteristics of these samples. In addition,
221 significantly lower concentrations of acids were detected in wines from Huerta of Maule and
222 Loncomilla.

223

224 **Multivariate statistical analysis**

225 On the basis of the results obtained from ANOVA, the data were analyzed by multivariate
226 statistical analysis. Non-supervised pattern recognition statistical analysis, such as principal
227 component analysis (PCA), was employed to identify possible patterns related to the
228 classification factors. From PCA, 14 significant principal components (PCs) were obtained,
229 according to Kaiser's criterion (eigenvalues>1), and explained 89.10% of the total variability (7
230 PCs explained the 73.10% of the variability). Figure 5 shows a plot of the first two PCs, which
231 explained 38.06% of the total variability. The majority of samples from Melozal and Caliboro
232 are located in the first and second sectors of the plot (positive values of first PC), so this PC
233 could be related to distance to the Andes Mountains. Compounds with a higher value on this PC
234 were esters such as isoamyloctanoate, isoamyldcanoate, ethyl tetradecanoate, ethyl methyl
235 succinate, ethyl dodecanoate, ethyl isovalerate, ethyl isobutyrate, ethyl phenyl acetate and ethyl
236 decanoate and acids such as hexanoic acid, octanoic acid and decanoic acid. As seen, this is in
237 concordance with the ANOVA results, which showed a relation between ester and acid content
238 and proximity to the Andes Mountains.

239 To obtain suitable classification rules for the samples, supervised pattern recognition statistical
240 analysis such as forward stepwise linear discriminant analysis (LDA), according to Wilks'
241 lambda and employing the leave-one-out method, was applied to the set of data. "Location" was
242 employed as a classification criterion using six categories: Caliboro, Melozal, Cauquenes, Huerta
243 of Maule, Loncomilla and Sauzal. All of the samples were correctly classified using the leave-

244 one-out method. Figure 6 shows a scatterplot of the samples on the plane defined by the first two
245 discriminant functions for a “location” classification criterion. As seen, six clear groups were
246 formed, and all samples were correctly classified. Twenty-eight variables were obtained
247 ($p < 0.05$), with esters being the majority of the compounds that contributed to the classification
248 function, with the coefficients shown in Table 4. Again, this chemical family contributes to the
249 differentiation of the samples and therefore to their classification.

250 Conclusions

251 In this work, 28 monovarietal Carignan cv. wines from six different Chilean production zones
252 and two harvest seasons have been studied. This cultivar had been scarcely studied previously,
253 and this is the first time that Chilean wines have been chemically characterized. Some
254 differences have been found in comparison to other Carignan wines from different European
255 regions, so location may be affecting volatile composition. The effect of Chilean production zone
256 and year has been studied to corroborate this fact. Wines from locations closer to the Andes
257 Mountains showed significantly lower contents of esters and acids. After multivariate statistical
258 analysis, 100% of the studied wines were correctly classified regarding production zone, with
259 esters being the chemical family that contributed most to the correct classification of the
260 samples. Future researches could include different cultivars to be compared with in order to
261 enhance the specificity of Carignan wines.

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- 314

315 **Table 1** Identified compounds in Chilean Carignan cv. wines.

Compounds	LRI ^a	Qion ^b	ID ^c
Ethyl esters			
Ethyl isobutyrate	1010	43	A, C
Ethyl butyrate	1055	71	A, B, C
Ethyl 2-methylbutyrate	1084	57	A, B, C
Ethyl isovalerate	1089	88	A, B, C
Ethyl crotonate	1214	69	A, B, C
Ethyl hexanoate	1245	88	A, B, C
Ethyl heptanoate ^{*1}	1348	88	B, C
Ethyl octanoate	1437	88	A, B, C
Ethyl nonanoate ^{*1}	1534	88	C
Ethyl decanoate	1647	88	A, B, C
Ethyl 2-furoate	1661	95	A, C
Ethyl succinate	1679	101	A, B, C
Ethyl 9-decenoate ^{*1}	1659	88	C
Diethyl glutarate	1746	143	C
Ethyl phenylacetate	1799	91	A, B, C
Ethyl dodecanoate ^{*1}	1854	88	B, C
Ethyl methyl succinate	1892	115	C
Ethyl 3-methylbutyl succinate	1942	101	C
Ethyl tetradecanoate ^{*1}	2041	88	B, C
Diethyl 2-hydroxy-3-methylsuccinate	2154	131	C
Ethyl hexadecanoate	2233	88	C
Ethyl hydrogen succinate ^{*2}	2292	101	C
Acetate esters			
Ethyl acetate	879	43	A, B, C
Isobutyl acetate	1015	43	A, B, C
Isoamyl acetate	1122	43	A, B, C
Isoamyl esters			
Isoamyl butanoate	1306	71	A, C
Isoamyl hexanoate	1479	70	A, B, C
Isoamyl lactate	1524	45	A, C
Isoamyl octanoate	1680	70	A, B, C
Isoamyl decanoate ^{*3}	1888	70	B, C
Methyl esters			
Methyl octanoate	1342	74	A, C
Methyl decanoate	1558	74	A, C
Alcohols			
Isobutanol	1074	43	A, B, C
Butanol	1172	56	A, B, C
1-Penten-3-ol ^{*4}	1200	57	C
3-methyl-1-butanol	1200	55	A, B, C
4-methyl-1-pentanol ^{*5}	1315	56	B, C
3-methyl-1-pentanol	1332	56	A, B, C

Hexanol	1375	56	A, B, C
3-Hexen-1-ol	1410	41	A, B, C
1-Octanol ^{*6}	1577	56	B, C
2,3-Butanediol	1534	45	A, B, C
1-Nonanol	1681	56	A, B, C
1-Decanol	1793	70	A, B, C
2-phenylethanol	1940	91	A, B, C
Benzyl alcohol	1883	79	B, C
4-ethylphenol	2212	107	A, B, C
Terpenes			
Limonene	1179	68	A, B, C
o-cymene	1242	119	A, B, C
Trans-2 pinalol	1320	43	A, C
Linalool	1555	71	A, B, C
Alpha-terpineol	1693	59	A, B, C
Acids			
Acetic acid	1484	43	A, C
Hexanoic acid	1880	60	A, B, C
Octanoic acid	2076	60	A, B, C
Decanoic acid	2329	60	A, B, C
Aldehydes			
Furfural	1438	96	A, B, C
Benzaldehyde	1565	77	A, C
5-methyl-furfural	1565	110	A, C
C₁₃ Norisoprenoid			
Beta-Damascenone	1849	69	A, B, C
Lactones			
Oaklactone	1947	99	A, B, C
γ -Carboethoxy- γ -butyrolactone	2315	85	C

316 ^aLRI: Linear Retention Index.

317 ^bQion: Quantification ion.

318 ^cID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass
 319 spectrum agreed with mass spectral data base and LRI agreed with the literature data: Ferreira et al.
 320 2001, Gurbuz et al. 2006, Raposo et al. 2016, Tao et al. 2008; C, mass spectrum agreed with mass
 321 spectral data base.

322 ^{*1}Ethyl undecanoate equivalents; ^{*2}Ethyl succinate equivalents; ^{*3}Isoamyl octanoate equivalents; ^{*4}
 323 2-Ethyl-1-hexanol equivalents; ^{*5}3-Methyl-1-pentanol equivalents ^{*6}Nonanol equivalents.

324

325

Table 2 Mean concentrations ($\mu\text{g/L}$) of the studied compounds determined in the wine samples from the different production zones. Results from Tukey's test according to the variable "location."

Compounds	Caliboro	Melozal	Cauquenes	Huerta Maule	Loncomilla	Sauzal
Ethyl esters						
Ethyl isobutyrate	115 ^{ab}	141 ^b	42.3 ^a	121 ^{ab}	90.6 ^{ab}	77.8 ^{ab}
Ethyl butyrate	109 ^a	133 ^a	149 ^a	134 ^a	119 ^a	152 ^a
Ethyl 2-methylbutyrate	72.6 ^{ab}	109 ^b	27.1 ^a	87.3 ^{ab}	69.9 ^{ab}	63.9 ^{ab}
Ethyl isovalerate	15.7 ^a	30.0 ^b	5.24 ^a	22.4 ^{ab}	15.9 ^{ab}	20.0 ^{ab}
Ethyl crotonate	16.0 ^a	15.7 ^a	7.50 ^a	4.03 ^a	6.51 ^a	3.95 ^a
Ethyl hexanoate	1094 ^a	1231 ^a	1182 ^a	1094 ^a	918 ^a	1418 ^a
Ethyl heptanoate	6.03 ^a	5.92 ^a	4.84 ^a	4.61 ^a	3.69 ^a	5.19 ^a
Ethyl octanoate	152 ^{ab}	147 ^{ab}	174 ^{ab}	134 ^a	144 ^{ab}	226 ^b
Ethyl nonanoate	5.86 ^a	5.93 ^a	23.1 ^b	5.84 ^a	5.42 ^a	5.71 ^a
Ethyl decanoate	41 ^a	40.3 ^a	62.8 ^a	44.2 ^a	41.7 ^a	53.8 ^a
Ethyl 2-furoate	0.16 ^a	0.62 ^a	0.09 ^a	0.21 ^a	0.15 ^a	0.22 ^a
Ethyl succinate	149 ^{ab}	141 ^{ab}	112 ^a	168 ^b	140 ^{ab}	151 ^{ab}
Ethyl 9-decenoate	9.4 ^a	6.39 ^a	5.95 ^a	5.75 ^a	6.08 ^a	6.35 ^a
Diethyl glutarate*	0.01 ^{ab}	0.02 ^b	0.01 ^a	0.02 ^b	0.02 ^{ab}	0.02 ^{ab}
Ethyl phenylacetate	4.01 ^{ab}	5.61 ^b	2.28 ^a	4.54 ^{ab}	3.89 ^{ab}	5.11 ^{ab}
Ethyl dodecanoate	41 ^a	28.9 ^a	82.2 ^a	62.3 ^a	63.3 ^a	56.5 ^a
Ethyl methyl succinate*	0.17 ^a	0.03 ^a	0.51 ^a	0.05 ^a	0.26 ^a	0.37 ^a
Ethyl 3-methylbutyl* succinate	0.54 ^a	0.40 ^a	0.49 ^a	0.61 ^a	0.57 ^a	0.62 ^a
Ethyl tetradecanoate	4.35 ^a	2.71 ^a	14.0 ^b	5.46 ^a	6.52 ^{ab}	6.10 ^{ab}
Diethyl 2-hydroxy-3- methylsuccinate*	0.03 ^a	0.04 ^a	0.03 ^a	0.04 ^a	0.03 ^a	0.04 ^a
Ethyl hexadecanoate	13.5 ^a	13.5 ^a	41.2 ^b	18.4 ^a	18.8 ^a	18.2 ^a
Ethyl hydrogen succinate	55.1 ^a	49.6 ^a	59.7 ^a	61.0 ^a	49.5 ^a	66.5 ^a
Total	1903^a	2108^a	1997^a	1979^a	1703^a	2337^a

Acetate esters						
Ethyl acetate	59279 ^{ab}	70279 ^b	53279 ^{ab}	68279 ^{ab}	50779 ^a	63279 ^{ab}
Isobutyl acetate	65.6 ^a	62.2 ^a	39.8 ^a	64.2 ^a	45.2 ^a	69.6 ^a
Isoamyl acetate	394 ^a	380 ^a	376 ^a	405 ^a	376 ^a	376 ^a
Total	59738^{ab}	70721^b	53695^{ab}	68748^{ab}	51200^a	63725^{ab}
Isoamyl esters						
Isoamyl butanoate	0.51 ^a	0.59 ^{ab}	0.41 ^a	0.59 ^{ab}	0.54 ^{ab}	0.72 ^b
Isoamyl hexanoate	0.68 ^a	0.67 ^a	0.73 ^a	0.65 ^a	0.55 ^a	0.93 ^a
Isoamyl lactate	66.0 ^a	40.8 ^a	50.3 ^a	48.5 ^a	51.8 ^a	78.7 ^a
Isoamyl octanoate	127 ^a	76.1 ^a	291 ^a	126 ^a	121 ^a	189 ^a
Isoamyl decanoate	24.6 ^a	16.3 ^a	170 ^b	70.9 ^{ab}	73.8 ^{ab}	77.4 ^{ab}
Total	219^b	134^a	512^c	247^b	248^b	347^b
Methyl esters						
Methyl octanoate	1.06 ^{ab}	0.96 ^a	1.41 ^b	1.18 ^{ab}	0.84 ^a	1.38 ^b
Methyl decanoate	0.10 ^a	0.07 ^a	0.51 ^c	0.20 ^{ab}	0.05 ^a	0.33 ^{bc}
Total	1.16^a	1.02^a	1.92^b	1.37^{ab}	0.86^a	1.71^b
Total esters	61861^{ab}	72947^b	56205^{ab}	70973^{ab}	53151^a	66408^{ab}
Alcohols						
Isobutanol	43243 ^a	39910 ^a	42577 ^a	39243 ^a	43577 ^a	40910 ^a
Butanol	1083 ^a	823 ^a	1243 ^a	1280 ^a	1167 ^a	957 ^a
1-Penten-3-ol	0.31 ^a	0.15 ^a	0.14 ^a	0.15 ^a	1.22 ^a	1.92 ^a
3-methyl-1-butanol	99317 ^a	103460 ^a	113317 ^a	104317 ^a	107603 ^a	109031 ^a
4-methyl-1-pentanol	40.8 ^a	39.3 ^a	48.5 ^a	46.3 ^a	50.0 ^a	52.0 ^a
3-methyl-1-pentanol	300 ^a	333 ^a	388 ^a	340 ^a	355 ^a	345 ^a
Hexanol	4427 ^b	2252 ^a	3702 ^{ab}	3452 ^{ab}	3377 ^{ab}	4327 ^b
3-Hexen-1-ol	23.6 ^a	27.6 ^{ab}	27.6 ^{ab}	36.1 ^b	21.1 ^a	24.1 ^{ab}
1-Octanol	2.40 ^a	2.25 ^a	3.55 ^a	2.23 ^a	2.36 ^a	2.83 ^a
2,3-Butanediol	82.9 ^a	88.2 ^a	87.1 ^a	92.2 ^a	80.2 ^a	78.2 ^a
1-Nonanol	0.29 ^a	0.39 ^a	5.08 ^b	0.50 ^a	0.87 ^a	0.27 ^a
1-Decanol	1.15 ^a	1.31 ^a	1.40 ^a	1.11 ^a	1.66 ^a	1.52 ^a

2-phenylethanol	18909 ^a	18509 ^a	17409 ^a	18709 ^a	20109 ^a	18709 ^a
Benzyl alcohol*	0.03 ^a	0.03 ^a	0.07 ^a	0.03 ^a	0.05 ^a	0.07 ^a
4-ethylphenol	14.7 ^a	32.7 ^{ab}	32.0 ^{ab}	24.9 ^a	31.6 ^a	107 ^b
Total	167444^a	165477^a	178839^a	167543^a	176374^a	174544^a
Terpenes						
Limonene	0.05 ^b	0.03 ^a	0.04 ^{ab}	0.04 ^{ab}	0.05 ^b	0.05 ^b
o-cymene	2.42 ^{ab}	2.72 ^{ab}	1.11 ^a	3.43 ^b	3.27 ^b	2.73 ^{ab}
Trans-2 pinalol	4.22 ^{ab}	2.66 ^{ab}	5.01 ^{ab}	1.61 ^a	6.59 ^b	7.29 ^b
Linalool	0.86 ^a	0.03 ^a	1.24 ^a	0.03 ^a	0.52 ^a	1.13 ^a
Alpha-terpineol	2.09 ^{bc}	1.54 ^{ab}	0.91 ^a	1.80 ^{abc}	2.43 ^c	2.80 ^c
Total	9.64^{ab}	6.98^a	8.31^{ab}	6.91^a	12.8^b	14.1^b
Acids						
Acetic acid	1147 ^a	1267 ^a	1207 ^a	1207 ^a	1001 ^a	1259 ^a
Hexanoic acid	857 ^a	847 ^a	990 ^a	770 ^a	722 ^a	1045 ^a
Octanoic acid	376 ^a	304 ^a	492 ^a	249 ^a	323 ^a	510 ^a
Decanoic acid	104 ^a	94.2 ^a	105 ^a	82.9 ^a	95.8 ^a	142 ^a
Total	2484^{ab}	2512^{ab}	2793^{ab}	2309^a	2142^a	2955^b
Aldehydes						
Furfural	82.4 ^a	82.0 ^a	122 ^a	67.3 ^a	58.2 ^a	63.0 ^a
Benzaldehyde	1.81 ^a	10.7 ^b	4.36 ^{ab}	2.30 ^{ab}	7.48 ^{ab}	3.41 ^{ab}
5-methyl-furfural	5.25 ^a	4.83 ^a	0.75 ^a	7.25 ^a	2.00 ^a	1.67 ^a
Total	89.5^a	97.6^a	127^a	76.9^a	67.7^a	68^a
C₁₃ Norisoprenoid						
Beta-Damascenone	2.28 ^{ab}	2.27 ^{ab}	2.18 ^{ab}	1.07 ^a	2.39 ^{ab}	6.44 ^b
Lactones						
Oaklactone	34.8 ^a	68.1 ^a	60.8 ^a	54.5 ^a	6.85 ^a	36.7 ^a
γ-Carboethoxy-γ-butyrolactone	0.04 ^a	0.04 ^a	0.05 ^a	0.05 ^a	0.05 ^a	0.05 ^a
Total	34.8^b	68.1^b	60.9^b	54.6^b	6.89^a	36.7^b

Different letters in different columns indicate significant differences according to Tukey's test ($P < 0.05$). From letter 'a' to letter 'c' indicates increasing concentrations. *: Result expressed in relative area with respect to the internal standard 4-methyl-2-pentanol.

1 **Table 3** Analysis of variance results for the factors studied.
2

Compound	Location		Year	
	F-value	p-value	F-value	p-value
Ethyl esters				
Ethyl isobutyrate	2.78	0.0274 ^a	10.68	0.0019 ^a
Ethyl butyrate	2.43	0.0479 ^a	0.47	0.4977
Ethyl 2-methylbutyrate	2.95	0.0207 ^a	29.03	0.0000 ^a
Ethyl isovalerate	3.87	0.0048 ^a	33.49	0.0000 ^a
Ethyl crotonate	3.84	0.0051 ^a	0.22	0.6423
Ethyl hexanoate	1.32	0.2706	0.31	0.5801
Ethyl heptanoate	1.00	0.4253	0.73	0.3954
Ethyl octanoate	1.52	0.2000	9.82	0.0028 ^a
Ethyl nonanoate	60.28	0.0000 ^a	0.52	0.4744
Ethyl decanoate	1.38	0.2485	14.73	0.0003 ^a
Ethyl 2-furoate	2.37	0.0525	0.00	0.9582
Ethyl succinate	2.24	0.0652	21.37	0.0000 ^a
Ethyl 9-decenoate	1.25	0.2996	0.04	0.8388
Diethyl glutarate	5.20	0.0006 ^a	26.23	0.0000 ^a
Ethyl phenylacetate	4.46	0.0019 ^a	17.44	0.0001 ^a
Ethyl dodecanoate	1.44	0.2276	15.36	0.0003 ^a
Ethyl methyl succinate	1.76	0.1388	11.28	0.0014 ^a
Ethyl 3-methylbutyl succinate	3.35	0.0110 ^a	1.18	0.2822
Ethyl tetradecanoate	5.62	0.0003 ^a	12.35	0.0009 ^a
Diethyl 2-hydroxy-3-methylsuccinate	1.86	0.1183	0.82	0.3684
Ethyl hexadecanoate	4.41	0.0021	35.36	0.0000 ^a
Ethyl hydrogen succinate	0.97	0.9746	0.52	0.4725
Acetate esters				
Ethyl acetate	3.30	0.0119 ^a	37.48	0.0000 ^a
Isobutyl acetate	1.98	0.0976	1.39	0.2434
Isoamyl acetate	0.18	0.9671	3.69	0.0601
Isoamyl esters				
Isoamyl butanoate	2.90	0.0225 ^a	1.73	0.1937
Isoamyl hexanoate	1.08	0.3816	5.22	0.0263 ^a
Isoamyl lactate	1.47	0.2164	3.98	0.0510
Isoamyl octanoate	2.09	0.0817	14.64	0.0003 ^a
Isoamyl decanoate	3.42	0.0099 ^a	17.22	0.0001 ^a
Methyl esters				
Methyl octanoate	4.54	0.0017 ^a	0.09	0.7695
Methyl decanoate	8.88	0.0000 ^a	4.59	0.0367 ^a
Alcohols				

Isobutanol	0.64	0.6687	0.84	0.3626
Butanol	3.53	0.0082 ^a	3.06	0.0857
1-Penten-3-ol	1.14	0.3515	10.81	0.0018 ^a
3-methyl-1-butanol	1.21	0.3199	2.29	0.1358
4-methyl-1-pentanol	2.18	0.0710	0.25	0.6185
3-methyl-1-pentanol	0.58	0.7145	2.84	0.0978
Hexanol	4.23	0.0028 ^a	0.14	0.7127
3-Hexen-1-ol	7.10	0.0000 ^a	1.77	0.1888
1-Octanol	1.50	0.2081	0.05	0.8325
2,3-Butanediol	0.98	0.4401	4.67	0.0351 ^a
1-Nonanol	12.43	0.0000 ^a	0.00	0.9965
1-Decanol	0.78	0.5721	15.45	0.0002 ^a
2-phenylethanol	0.60	0.7023	6.77	0.0119 ^a
Benzyl alcohol	2.92	0.0218 ^a	0.42	0.5203
4-ethylphenol	2.78	0.0270 ^a	1.72	0.1955
Terpenes				
Limonene	5.99	0.0002 ^a	3.14	0.0820
o-cymene	2.42	0.0488 ^a	17.43	0.0001 ^a
Trans-2 pinalol	1.66	0.1606	4.82	0.0325 ^a
Linalool	1.40	0.2399	12.09	0.0010 ^a
Alpha-terpineol	4.90	0.0010 ^a	0.17	0.6810
Acids				
Acetic acid	1.59	0.1808	19.74	0.0000 ^a
Hexanoic acid	1.05	0.4013	0.79	0.3784
Octanoic acid	1.80	0.1297	6.75	0.0121 ^a
Decanoic acid	1.35	0.2600	19.04	0.0001 ^a
Aldehydes				
Furfural	0.94	0.4630	48.05	0.0000 ^a
Benzaldehyde	2.39	0.0507	8.63	0.0049 ^a
5-methyl-furfural	0.91	0.4809	34.94	0.0000 ^a
C₁₃ Norisoprenoid				
Beta-Damascenone	0.87	0.5052	0.41	0.5243
Lactones				
Oaklactone	2.79	0.0268 ^a	10.87	0.0017 ^a
γ -Carboethoxy- γ -butyrolactone	2.20	0.0693	2.65	0.1091

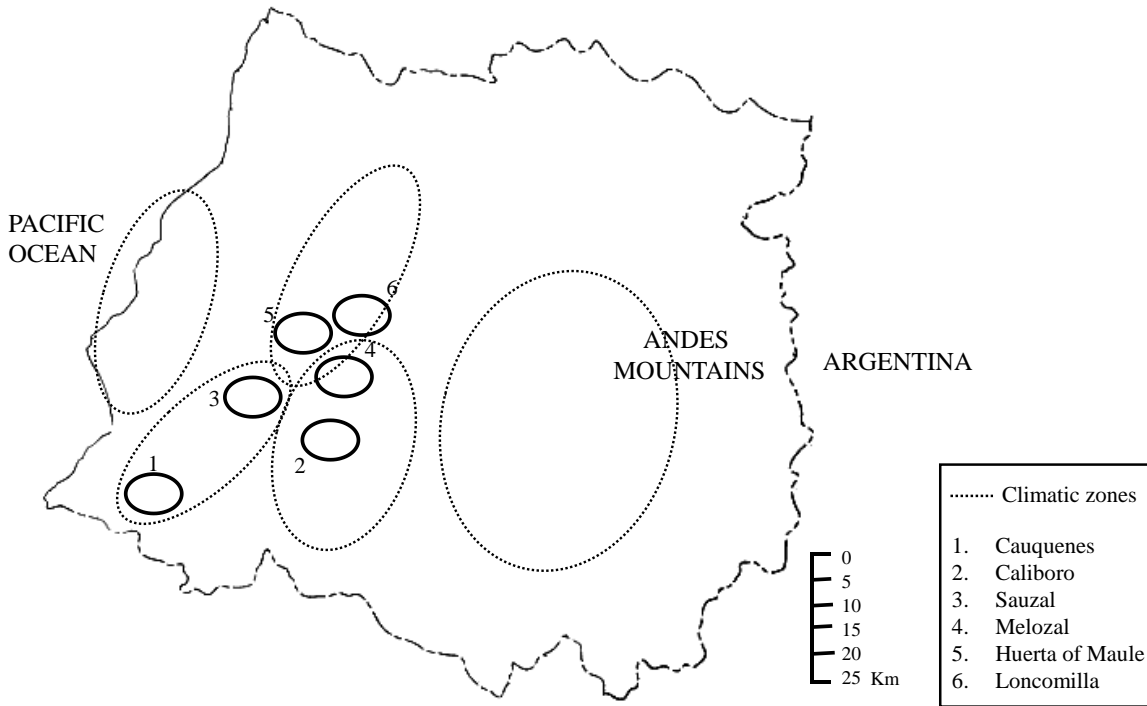
^aValues are significant at $p < 0.05$.

5 **Table 4** Linear Discriminant Analysis (LDA). Coefficients of the equation of classification.

Variable	Caliboro $p^a = 0.2857$	Melozal $p^a = 0.2857$	Cauquenes $p^a = 0.0714$	Huerta Maule $p^a = 0.0714$	Loncomilla $p^a = 0.1429$	Sauzal $p^a = 0.1429$
Ethyl acetate	45.0	55.0	48	92.9	45.7	47.0
Ethyl isobutyrate	421.7	-56.8	-10601	-2806.6	-2724.2	885.8
Isobutyl acetate	-8065.3	-7406.0	-53516	-20222.1	-16938.4	-3260.8
Ethyl-2-methylbutyrate	2583.7	2163.2	16294	4109.9	4429.4	-145.0
Ethyl isovalerate	-642.9	1167.2	7917	1511.5	496.4	433.5
Butanol	3482.9	-2454.3	-12105	-5340.8	400.7	3597.6
Ethyl crotonate	848.5	859.2	781	281.3	91.5	357.3
3-methyl-1-butanol	157.2	161.7	914	395.6	329.7	112.0
o-Cymene	2886.4	865.3	3078	8623.4	7783.6	3705.0
Ethyl heptanoate	-4140.1	-3081.7	-36841	-10669.7	-10267.7	-582.5
3-hexen-1-ol	13796.1	44286.6	256114	122831.3	87668.8	10499.9
Methyl octanoate	13062.1	11845.8	57717	22041.2	17891.7	6718.1
Ethyl octanoate	-87.2	-128.9	-577	-265.3	-159.5	-80.0
Isoamyl hexanoate	-2360.6	-383.4	-12858	-3967.1	-5818.3	-122.5
Ethyl nonanoate	11427.7	9720.3	83832	22490.5	21426.0	2075.7
Methyl decanoate	-18320.0	-9238.5	-129479	-31860.6	-37362.0	2668.9
5-methyl-furfural	46578.0	36839.5	457279	125977.2	118957.1	-3740.0
Alpha-terpineol	-5225.2	-11747.9	15902	8330.2	20305.7	1522.3
Decanol	13838.4	22311.9	178671	50598.4	44965.0	-207.1
Ethyl phenylacetate	-6864.7	-6238.9	-36506	-11267.7	-9727.5	-2821.8
Ethyl dodecanoate	34.8	-75.3	-138	-19.0	36.0	15.8
Beta-Damascenone	3189.7	2478.6	14161	6034.4	5441.8	1967.6
Hexanoic acid	2229.1	1909.9	8400	2923.3	2335.7	1105.7
Benzyl alcohol	1663.1	1149.0	18519	4884.1	4782.5	-17.9
Oaklactone	-1439.2	-1668.4	-15521	-5131.1	-3754.1	537.3
Diethyl 2-hydroxy-3-methylsuccinate	-43896.3	-45029.4	-229274	-88589.1	-70965.7	-27789.3
γ -Carboethoxy- γ -butyrolactone	32715.0	34554.9	159667	60648.2	46679.4	19006.4
Ethyl hydrogen succinate	44.0	32.5	195	312.3	159.4	132.4
Intercept	-1130.0	-1141.7	-14982	-2345.2	-1666.9	-830.1

7 ^ap: A priori probability.

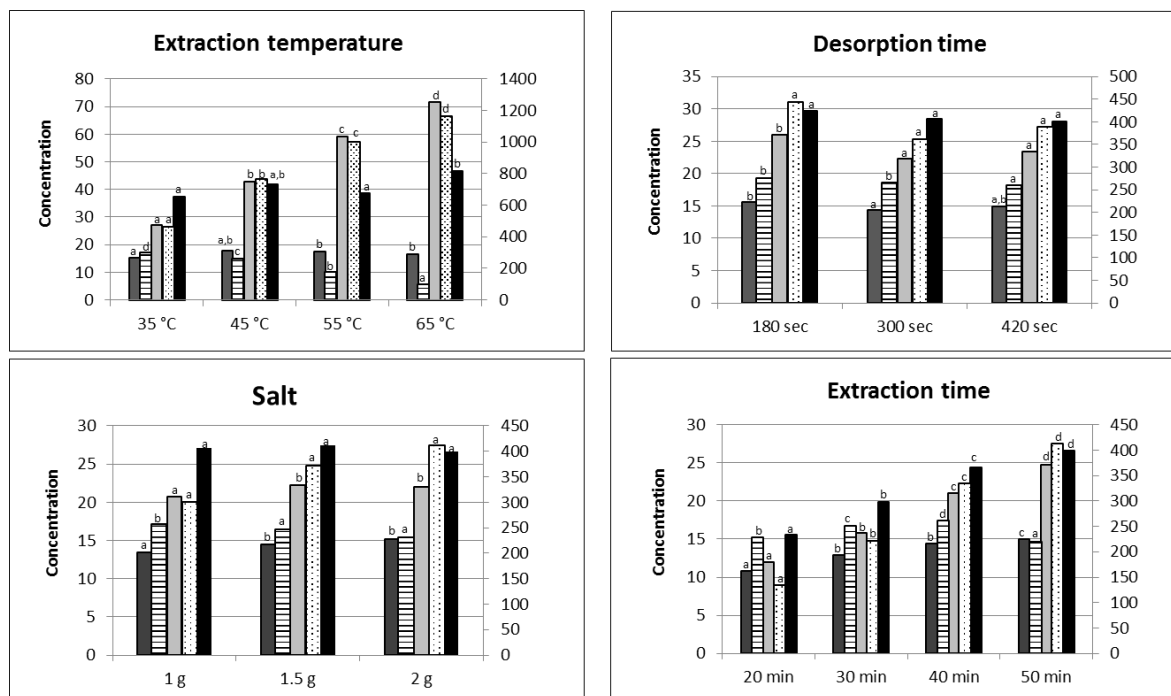
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10 **Figure 1** Climatic zones of Maule region. Locations studied.

11

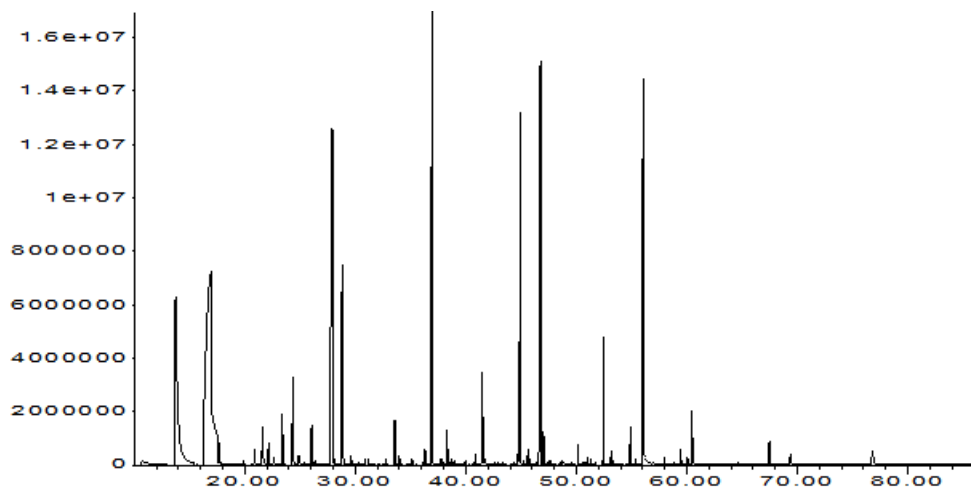


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13 **Figure 2** Mean concentrations of volatile compounds in the optimization of the extraction
 14 conditions: ■Alcohols; □Lactones; ■Aldehydes (Left axis). □Esters; ▣Acids (right axis).
 15 Concentration expressed in $\mu\text{g/L}$, except alcohols and esters in mg/L . The bars of the same
 16 chemical family with different letters show significant differences ($p < 0.05$).

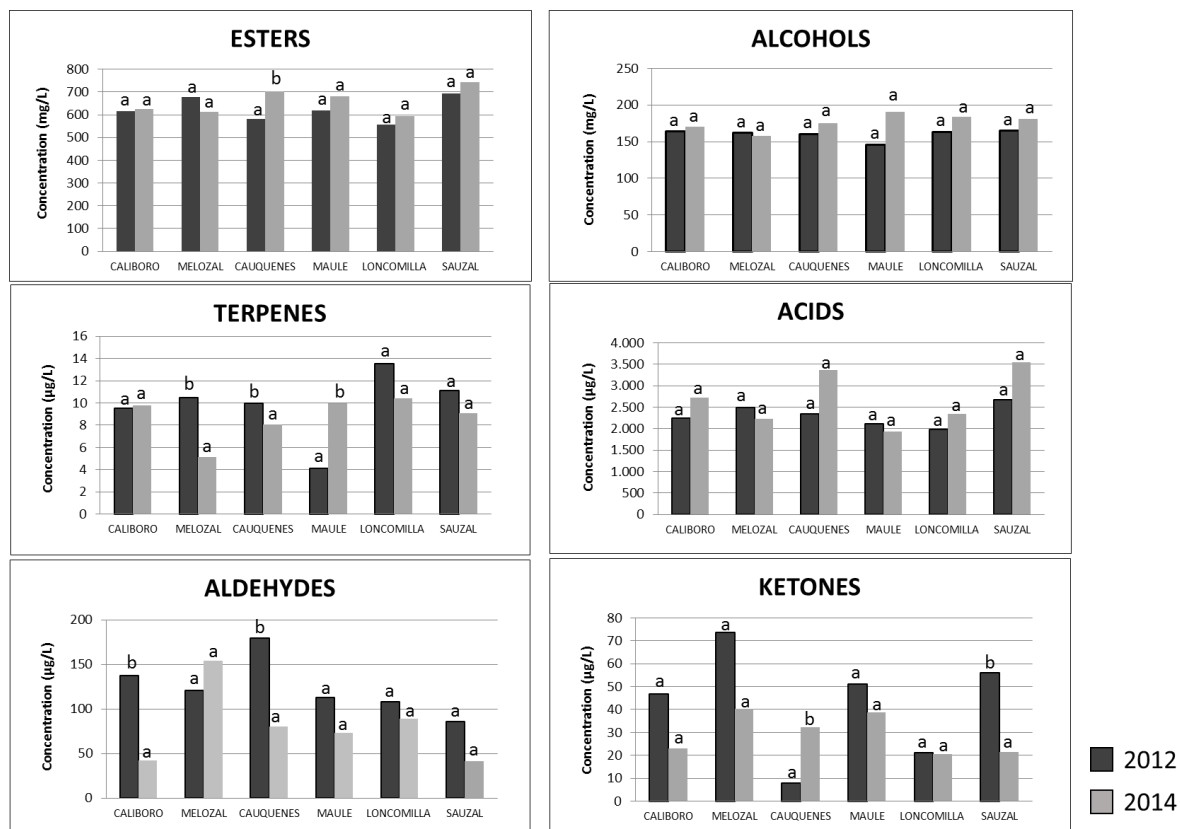
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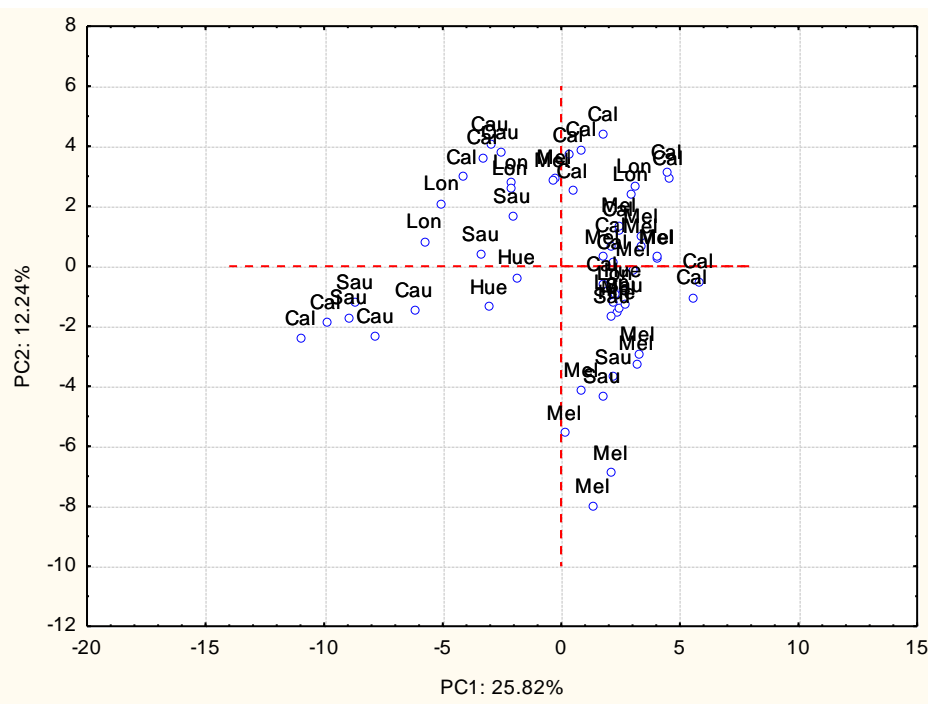


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20 **Figure 3** TIC of a monovarietal Carignan wine obtained by SPME–GC/MS.



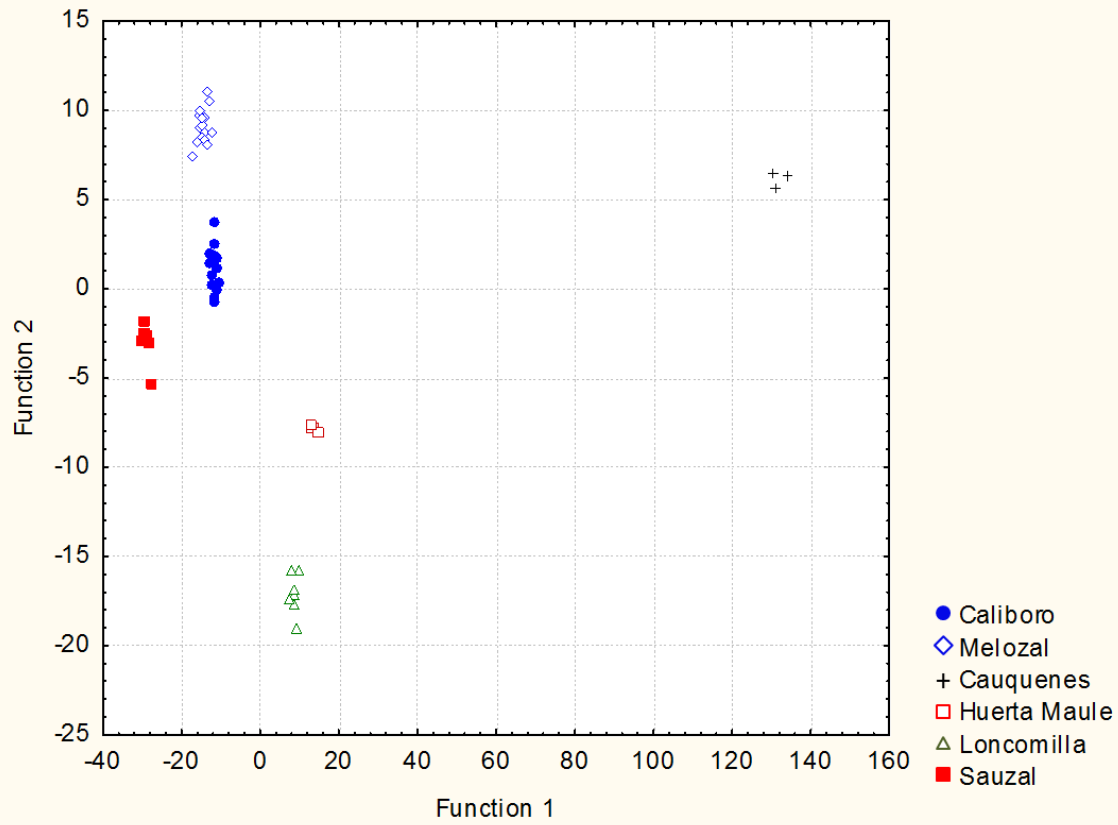
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 22 **Figure 4** Volatile compounds in the different locations and in the different harvests studied. The
 23 bars of the same year with different letters show significant differences ($p < 0.05$).
 24



25

26 **Figure 5** Principal component analysis (PCA). Plot of the samples in the plane defined by the
 27 first two principal components (PC). Abbreviations: Cal: Caliboro; Mel: Meloza; Cau:
 28 Cauquenes; Lon: Loncomilla; Hue: Huerta of Maule; Sau: Sauzal.

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30

31 **Figure 6** Linear discriminant analysis (LDA). Scatterplot of the samples on the plane defined by
 32 the discriminant functions.

33