“IMPACT OF PREFERMENTATIVE MACERATION ON THE PHENOLIC AND VOLATILE COMPOUNDS IN THE MONASTRELL RED WINES”.

Álvarez, I.*; J.L. Aleixandre, J.L.; García, M.J.; Lizama, V.

Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia. C/Camino de Vera s/n 46022. Valencia. Spain.
inmalva@tal.upv.es

ABSTRACT

The effects of two prefermentation treatments (cold soak at 6-8 ºC and cold soak at 0-2 ºC with dry ice) and two different maceration times (4 and 8 days) in must and wine composition have been studied. Grapes from Vitis vinifera var. Monastrell were harvested at two maturation levels. Total phenolic, colour density, anthocyanins and fractioned anthocyanins, ionized anthocyanins, polymeric pigments colour, condensed tannins and aromatic compounds were estimated. The effects of temperature in the grape juice composition were determined with constant monitoring during the prefermentation step. Significant differences were found among the measured parameters in the grape juice and wine. Prefermentative maceration produces wines with higher phenolics compounds, anthocyanins, especially malvidin-3-glucoside, ionized and polymeric anthocyanins and aromatic compounds values compared to control wines. This increase is more significant when the prefermentation treatments are done with the use it dry ice and the must is produced from less mature grapes. The maceration time showed not to be significant during the process.

Keywords: cryomaceration, dry ice, red wine, phenolic compounds, volatile composition.

1. INTRODUCTION

The organoleptic properties of red wines depend on the phenolic compounds and on the volatile composition. Also the colour in young red wines is related with the anthocyanin concentration and the ionization index, according to the pH, sulfur dioxide concentration and copigmentation [1]. Previous studies [2, 3] have shown that colour stability depends on the formation of polymeric pigment with anthocyanins and condensed tannins. The condensation of tannins with other compounds of the wine contributes to change the astringencie and increases the sensation of mouth volume and roundness [4]. The type of wine produced is influenced by the temperature, time and intensity of fermenting must recycling during maceration. The vinification process may extract high quantities of colour and tannins to give structure to wine and stability to the colour without becomes so tough and astringent. To obtain this type of wine from Monastrell grapes in which poliphenolic maturity is reached after pulp maturity [5] it's necessary use a specific method of winemaking.

Several studies regarding phenolics and aromatics extraction have investigated what happens during wine making in the others varieties [6-10]. To make wines from the Monastrell variety with a good quantity of phenoplic and aromatic compounds and along with balanced alcoholic degree can be interesting to increase the prefermentative maceration phase. The purpose is to increase the extraction and stabilization of the poliphenolic compounds in the liquid phase (antocyanins and tannins with low molecular weight) and decrease the extraction intensity during the fermentative process to avoid the extraction of the tough and bitter tannins from the seed [11-15].

If the prefermentation skin contact is carried out with dry ice in addition with retarding the start of the fermentation process breaking and disorganizing the skin cells through freezing. Freezing increase the volume of the intracellular liquids thus disrupting the membranes and providing an easy exit for the aromatic and phenolic compounds [16, 17].

The spectrophotometric calculations have been used by many authors to describe the evolution of colour, anthocyanins, polymeric and copigmented pigments, condensed tannins, astingency perception [18-22]. The introduction of chromatography to quantify flavonoids and fractions of anthocyanins has also provide useful information about the effects of the winemaking practices [23- 28]. Volatile compounds were determined by gas chromatography [29-31].The phenolic and aromatic compounds studied were selected on the basis of their significance in red wines [32, 33].

2. MATERIALS AND METHODS

2.1. Wine samples and protocols

The experimental work is carry out with the Monastrell grapes from Denomination of Origin Valencia. Two harvests were made at different concentration of sugars: 12 and 13 ºBé. Two prefermentation treatments were applied in the must: cold soak at 6-8 ºC and cold soak at 0-2 ºC with dry ice and two different maceration times 4 and 8 days. The control wines were vinified quickly without any further treatment. All the experiments were done in triplicate using 50 L stainless steels tanks. During the alcoholic fermentation temperature and density were measured daily and punching was carried out twice a day. All the tanks were emptied at same density after completed of the alcoholic and malolactic fermentation. Finally the wines were bottled and stored at 14 ºC. All analyses were done 3 months later.

2.2. Common and spectrophotometric parameters

The common parameters were performed according to the Regulation Official Methods established by UE [34]. These parameters are density, ethanol, pH, sugars, total and volatile acidity, total and free sulphurous content.

The phenolic composition was determined by spectrophotometric methods: Total Polyphenols [20]; Proanthocyanidins, Total Anthocyanins and Polymeric Index [2]; Copigmented Anthocyanin, Free Anthocyanin and Polymeric Anthocyanin [1]; Colour Density, Colour Shade, Ethanol Index and HCl Index [35]; Ionization Index [22], and PVP Index [36].

2.3. Liquid chromatographic determination

Separation and quantification of antocyanins were done by high performance liquid chromatography (HPLC) using a method modified from that one described [37]. Concentrations were determined using the external standard method and their factor response were deduced from their respective calibration curves. A Merck-Hitachi LaChrom system equipped with a D-7100 quaternary pump, D-7455 diode-array detector, D-7485 fluorescence detector, and L-7650 column oven was used. An analytical LiChrospher 100 RP-18 colum (250 x 4 mm, 5 μm) from Merck was utilized and protected by a guard column of the same material. Separation was performed at a flow rate of 1 mL/min with a mobile phase composed of (A) phosphoric acid/water, 1.5:98.5, v/v) and (B) phosphoric acid/acetic acid/acetonitrile/water, 1.5:25:20:54, v/v/v/v). Samples (25μL) of each wine were injected into the HPLC system after filtration through 0.2 μm Whatman inorganic Anodisc 13 membranes filters from Whatman International LTD (Maidstone, England). The multigradient solvent system was as follows: 0-30 min, from 100% A to 30 % A; 30-40 min, from 30% A to 35% A. The column was equilibrated whit starting conditions for 10 min after each analysis. The HPLC elutes were monitored by absorbance at 530 nm. The rest of the compounds whose standards are not available were quantified with Mv-3-gls as standard, because this is the most common anthocyanin in grapes.

2.4. Gas chromatographic conditions

Volatile components were quantified with chromatography with a HP-5890 (Hewlett Packard Corp., USA) chromatograph equipped with a flame ionization detector (FID) using nitrogen as a carrier gas.

Isobuthyl and isoamyl alcohols, ethyl and methyl acetates, methanol and 1-propanol were determined by the direct injection of 1μL of wine containing 4-methyl-2-penthanol as an internal standard, in a Carbowax 1500 capillary column (length 4 meter, internal diameter 0,32 cm) over Cromosorb to a 15%, with 80-100 meshes [39].

Minor wine components were determined by making a prior extraction. 2-phenylethanol, isoamyl acetate, isobuthyl acetate, ethyl butyrate, ethyl lactate, ethyl octanoate, diethylglutarate, diethylsuccinate, n-amylalcohol and γ-butyrelactone were extracted using organic solvents (diethyleter and n-pentane 2:1). As an internal standard 1 ml of 2-octanol was added to 500 ml of wine. The extraction procedure was optimized by means of ultrasound [29]. The combined extracts were dried on anhydrous sodium sulphate, reduced in volume to 20 μL in a vacuum rotatory evaporator, and then by a gentle stream of nitrogen. 1 μL to extracts were injected in HP-INNOWax (Crosslinked Polyethylene Glycol) capillary column of 60 m long and 0.25 mm internal diameter.

Compounds quantification was based on the internal standard method. The efficacy of the method was verified from the analysis performed on standard solutions of the components, and with the aid of an HP-5979 mass spectrophotometer linked to the chromatograph. The variance of the method was determined by the analysis of three replicates of each sample.

2.5. Statistical analysis

All data were statistically analyzed using Statgraphic Plus 5.1. Statistical methods employed were ANOVA. The statistical significance of each factor under consideration was calculated at the $\alpha=0.05$ level using the Student´s t-test.

3. RESULTS AND DISCUSSION

The composition of the musts from grapes having 12 and 13 ºBé and the composition of the wines with prefermentative maceration were studied using a complete factorial model, considering days and temperatures of maceration. The results obtained from the statistical analysis from the macerated wines and the control wine was compared to see the influence of the prefermentative maceration in the characteristics of the wines. All analyzes were conducted in triplicate and the average values calculated. Different letters within the some column for each treatment mean significant differences at 1% ($P<0.01$).

3.1. Common parameters of must and wines

Table 1 shows the mean values of common parameters in the musts from two harvests. The must with 13 ºBé has the highest value in probable alcohol degree and pH and lower value in total acidity.

It was found that the wines obtained from the two maturity degrees had significant differences in ethanol concentration (table 2 and 3). On the other hand no significant differences were found in pH and total acidity among the produced wines. The residual sugars, volatile acidity and sulphurous values are according with the values of normal dry young wines.

3.2. Phenolic composition of wines

Figures 1 and 2 show the effect of the different treatments in the anthocyanin concentration, colour shades, proanthocyanidines concentration and total polyphenols in wines, which have been bottled for a period of 6 moths. The prefermentative maceration has a positive effect on the total concentration of the polyphenolic compounds of wines elaborated with the less matures grapes. The anthocyanins concentration is slightly higher in wines obtained by cryomaceration, but a significant increase of the procyanidine concentration is observed in these wines. The significant decrease of colour shade with prefermentative maceration wines indicates an increase protection of anthocyans against oxidation. The prefermentative maceration treatments at low temperatures seem to have a positive effect on the phenolic parameters.

The cryomacerated wines have a significantly higher value in colour density because the anthocyanins concentration is higher due to increased ionisation index. The increase of the colour is related with the increase of copigmented anthocyans as well as the values of anthocyans polimerized and measured by the polymeric index, polymeric antocyanins and PVPP index [35]. The significant increase of the polymeric polyphenols due to the prefermentative maceration have a positive effect not only on the colour density but also on the colour stability. The prefermentative maceration increases the polymerised tannins too as shows of the ClH index increase but the polymerised polysacarides and tannins do not increase. This is because of the non-significant difference in the ethanol index between control and cryomacerated wines.

The cold soak treatments increase the anthocyanin extraction, mainly the extraction of malvidine-3-glucoside which contributes to the stability during storage (tables 4 and 5). The increase is higher when the dry ice treatment is for 4 days. Prefermentative maceration produce wines with higher phenolic compounds, proanthocyanidines, anthocyanins especially malvidin-3-glucoside, ionised and polymeric anthocyanins compounds values compared with control wines values.

The prefermentative maceration has a positive effect on the total concentration of the polyphenolic compounds of wines elaborated with the less matures grapes. These result is in agreement with those publisher previously for wines of other grape varieties [11, 12, 17]. The polyphenolic concentration is not directly related with the duration of the treatments because the results are not improved if the prefermentative maceration time increases, just as it were observed in a previous work [16]. The wines obtained from prefermentative maceration with dry ice have high polyphenolic concentration it is important to point out that the addition of the dry ice in the cold soak allows us to obtain some results but reducing the time of the treatment to 4 days. When the maceration time is extended to 8 days with the addition of dry ice it does not show to have a significant effect in the increase of the polyphenolic concentration.

3.3. Aromatic compounds in wines

The concentration of volatile compounds identified and quantified in the Monastrell wines produced with different treatments are shown in tables 6 and 7. The prefermentative maceration with dry ice showed a significant increase in the concentration of most part of the acetates and esters analysed as well as a significant increase of the diethylglutarate, isobutanol, 2-phenylethanol and isoamylic alcohols in all the wines obtained by cryomacetarion independent of grape maturity. The ethylacetate and methylacetate concentrations are higher in the wines obtained from 12 ºBé grapes but not in the wines obtained from 13 ºBé grapes. However the isoamylacetate, isobutylacetate, ethylbutirate and gammabutirolactone concentrations are significantly increased in the wines obtained from the grapes having 13 ºBé. The diethylsuccinate concentration has a significant decrease in the wines with cryomaceration according to several authors [30, 32, 33].

The wines obtained with dry ice (0-2 ºC) have slightly higher volatile compounds concentration than the obtained with prefermentative maceration at 5-8 ºC but these differences are only significant in a few compounds. The increase in the duration of cryomaceration does not give an increase of the aromatic compounds concentration.

Esters and higher alcohols are very important in wine aroma giving a fruity odour [31, 39]. The formation of higher alcohols and esters is linked to the matabolism of the aminoacids thus their concentration depends the grape composition, the yeast density and its condition [30, 32, 40]. The decomposition of the skins produced with the use of dry ice increases the aromatic precursors [22] and consequently a more significant increase in the aromatic compounds.

The wines obtained from 13 ºBé grapes have a lower concentration in aromatic compounds than the wines obtained from less mature grapes. However the prefermentative maceration effect is evident in the wines obtained from grapes more mature having the most part of compounds analysed similar concentrations to the wines obtained from 12 ºBé grapes.

4. CONCLUSIONS

The results obtained show that the technique of cold soak with or without dry ice increases polyphenolic compounds concentration and his stabilization in Monastrell red wine. This increase is most important when prefermentative treatments are made with dry ice. The better results are in wines from 12 ºBé grapes. The effect of maceration time on phenolic compounds is not so significant.

Prefermentative maceration increases aromatic compounds concentration in wines except diethylglutarate concentration, which show a significant decrease in its values. No differences exist between wines that were cryomacerated with dry ice and wines that were macerated at 4-8 ºC. With wines from 13 ºBé grapes the maceration effect is higher. The increase of the maceration time does not increase the concentration of aromatic compounds.

On the other hand using dry ice increases the cost of the process and it only justified when is necessary to do an extraction and stabilization of phenolic compounds in wines from 12 ºBé grapes. In this case the extraction of the aromatic compounds is not important. However in Monastrell wines from 13 ºBé grapes prefermentative maceration is more effective to increase the aromatic compounds concentration and less for the extraction and stabilization of polyphenolic compounds.

5. REFERENCEES

[1] R. Boulton, Am. J Enol Vitic. 52 (2001) 2.

[2] T.C. Somers, M.E. Evans, J. Sci. Food Agric. 28 (1977) 279.

[3] J. Bakker, C.F. Timberlake, J. Agric. Food Chem. 45 (1997) 35.

[4] A.C. Noble, Physiol. Behav. 56 (6) (1994) 1251.

[5] H. McMahon, B.W. Zoecklein, Y.W. Jasinsky, Am. J. Vitic. Enol. 50 (4) (1999) 385.

[6] A. Zimman, W.S. Joslin, M.L. Lyon, J. Meier, A.L. Waterhouse, Am. J. Enol. Vitic. 53(2) (2002) 93

[7] M,Gómez-Míguez, F.J. Heredia, J. Agric. Food Chem. 52 (2004) 5117.

[8] J. Burns, W. Mullen, N. Landrault, P.L. Teissedre, M. E. J. Lean, A. Crozier. J. Agric. Food Chem. 50 (2002) 4096.

[9] Y. Kotseridis, R. Baumes, J. Agric. Food Chem. 48 (2000) 400.

[10] J.A. Fernández-López, L. Almela, J.A. Muñoz, V. Hidalgo, 1998. Food Research International. 31(9) (1998) 667.

[11] D. Delteil. Rev. Oenol., 112 (2004) 112.

[12] P. Cuenta, F.Lorenzini, Rev. Suiss. Viti. Hortic. Arboric, 28 (4) (1996) 259.

[13] M. Feuillat. Rev des Oenologues, 82 (1997) 29.

[14] R.Gómez-Plaza, R. Gil-Muñoz, J.M. López-Roca, A. Martínez-Cutillas, J.I. Fernández-Fernández, Lebensm.-Wiss. u.-Technol., 35 (2002) 46.

[15] D. Healherber, M. Dicey, S. Goldsworth, L. Vanhanen, New Zeland Society for Viticulture and Enology Symposium. Chistghurch. (1997)

[16] I. Álvarez, M.A. García, R. González, P. Martín, In Avances en ciencias y técnicas enológicas, Ed. ITACyL y GIENOL (2005) 121.

[17] M. Couasnon, Rev. Oenol. Tech. Vitic. Oenol, 92 (1999) 26.

[18] N. Vivas, Y. Glories, L. Lagune, C. Saucier, M. Augustin, Journal International des Sciences de la Vigne et du Vin, 4 (1994) 319.

[19] J.H. Thorngate, B. H. Gump (Ed.). Am. Chem. Soc, Symp. Series 536 A. C. S. Washington D. C. (1993) 57.

[20] V.L. Singleton, J.A. Rossi, Am. Journal of Vitic. and Enol., 16 (1965) 144.

[21] Y. Glories, Conn. Vigne Vin, 18(3) (1984a) 195.

[22] Y. Glories, Conn. Vigne Vin, 18(4) (1984b) 253.

[23] I. Revilla, S. Pérez-Magariño, M.L. González-SanJosé, S. Beltrán, Journal of Chromatography. A, 847 (1999) 83.

[24] J.P. Roggero, S. Coen, B. Ragonnet, Am. J. Enol. Vitic. 37 (1986) 77.

[25] E. García-Beneytez, F. Cabello, E. Revilla, J. Agric. Food Chem. 51 (2003) 5622.

[26] J.M. Ryan, E. Revilla, J. Agric. Food Chem. 51 (2003) 3372.

[27] E. Revilla, E. García-Beneytez, F. Cabello, G. Martín Ortega, J. M. Ryan, J. Chromatogr. A 915 (2001) 53.

[28] A. Bertrand, P. Ribereau-Gayon, *Bull. O.I.V.* 79/FV (1972) 409.

[29] M.D. Herranz, Talanta 50 (1999) 413.

[30] T. Cabaroglu, A. Canbas, R. Baumes, C.L. Bayonove, J.P. Lepoutre, Z. Günata, Journal of Food Science, 62 (1997) 680.

[31] S. Selli, A. Canbas, T. Cabaroglu, H. Erten, Z. Günata, Food Chemistry (2005) in press.

[32] M.I. Spranger, M.C. Climaco, B. Sun, N. Eiriz, C. Fortunato, A. Nunes, C. Leandro, M.L. Avelar, A.P. Belchior. Differentiation of red winemaking technologies by phenolic and volatile composition. Analytica Chimica Acta, 513 (2004) 151.

[33] R.A. Peinado, J. Moreno, J.E. Bueno, J.A. Moreno, J.C. Mauricio, Food Chemistry 84 (2004) 585.

[34] UE. Official Methods to Wine Analyses. Reglamento 440/2003.

[35] Y. Glories, Thèse a L'Université de Bordeaux II. (1978).

[36] J. Blouin, Manuel pratique d'analyse des moûts et des vins. Chambre d'Agriculture de la Gironde. (1977).

[37] Ch. Honda, N. Kotoda, M. Wada, S. Kondo, S. Kobayashi, J. Soejima, Z. Zhang, T. Tsuda, T. Moriguchi, Plant Physiol. Biochem. 40 (2002) 955.

[38] P.X. Etiévant, WineIn H. Maarse (Ed), Volatile compounds in Food and Beverages, (1991) 483 New York: Marce Dekker.

[39] O.I.V. Recopilación de los métodos internacionales de análisis de vinos (1990) 16.

[40] E. Sanchez, M.C.Diaz Maroto, M.A. González, M.S. Perez, Food Chemistry 92 (2005) 627.

Acknowledgements

The authors wish to acknowledge gratefully the financial support received from the “Consellería de Cultura, Educación i Esport to Generalitat Valenciana (GV04B-423)”.

Table 1. Means values of conventional parameters in must

	Vintage 12 º Bé	Vintage 13 º Bé
Probable Ethanol Degree	12,5 ± 0,2 a	13,8 ± 0,15 b
Density	1091 ± 16 a	1100 ± 8 b
pH	3,38 ± 0,12 a	3,57 ± 0,45 b
Total Acidity (g/L)	6,6 ± 0,12 a	5,8 ± 0,25 b
Total sulphurous (mg/L)	48,50 ± 5 a	45,7 ± 3,5 a

Different letters within the same column for each effect mean significant differences ($P < 0,01$)

Table 2. Means values of conventional parameters in the wines coming with vintage to 12 º Bé

Effects	**Ethanol**	**pH**	**Total Acidity**
Control wine	12,75 ± 0,353 a	3,989 ± 0,061 a	4, 183 ± 0,37 a
Cold soak5-8ºC (4)	12,85 ± 0,636 a	3,935 ± 0,103 a	4,315 ± 0,155 a
Cold soak 5-8ºC (8)	13,22 ± 0,141 b	3,962 ± 0,012 a	4,385 ± 0,049 a
Cold soak dry ice (4)	12,95 ± 0,141 a	3,969 ± 0,012 a	4,235 ± 0,162 a
Cold soak dry ice (8)	13,02 ± 0,282 a	3,925 ± 0,002 a	4,275 ± 0,106 a

Different letters within the same column for each effect mean significant differences ($P < 0,01$)

Table 3. Means values of conventional parameters in the wine winemaking with vintage to 13 º Bé

Effects	Ethanol	pH	Total Acidity
Control wine	13,85 ± 0,361 a	4,018 ± 0,072 a	4,421 ± 0,006 a
Cold soak 5-8ºC (4)	14,25 ± 0,141 a	3,958 ± 0,029 a	4,495 ± 0,106 a
Cold soak 5-8ºC (8)	14,17 ± 0,282 a	4,062 ± 0,026 a	4,465 ± 0,162 a
Cold soak dry ice (4)	14,05 ± 0,061 a	4,022 ± 0,057 a	4,435 ± 0,162 a
Cold soak dry ice (8)	14,22 ± 0,282 a	3,972 ± 0,002 a	4,435 ± 0,162 a

Different letters within the same column for each effect mean significant differences (P<0,01)

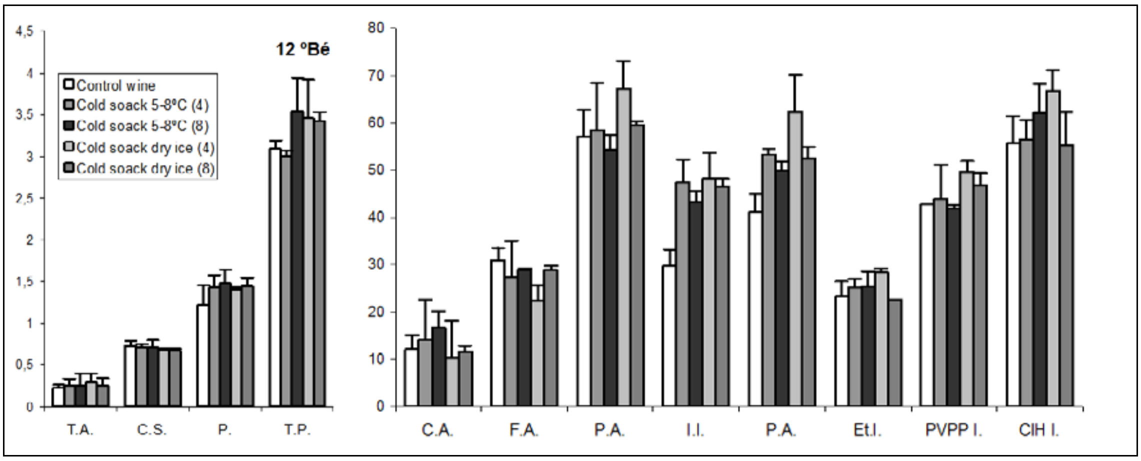

Fig. 1. Wine phenolic composition after 6 moths of being bottled in the wines winemaking with vintage to 12 ºBé

T.A. (Total Anthocyanins g/L)
C.S. (Color Shade g/L)
P. (Proanthocyanidins g/L)
T.P. (Total Polyphenols g/L)
C.A. (Copigmented Anthocyanin %)
F.A. (Free Anthocyanin %)
P.A. (Polymeric Anthocyanin %)
C.D. (Color Density %)
I.I. (Ionization Index %)
P.I. (Polymeric Index %)
Et. I. (Ethanol Index %)
PVPP I. (PVP Index %)
ClH I. (HCl Index %)

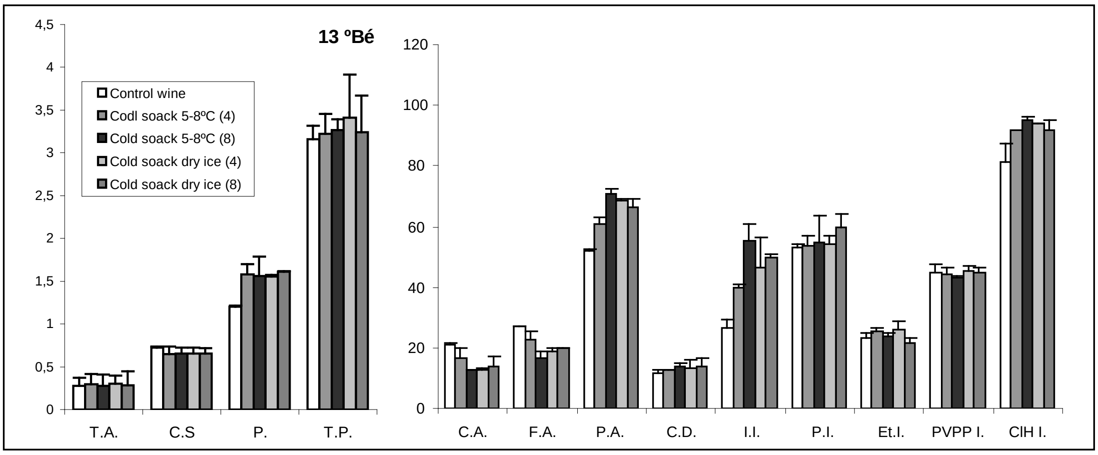

Fig. 2. Wine phenolic composition after 6 moths of being bottled in the wines winemaking with vintage to 13 ºBé

T.A. (Total Anthocyanins g/L)
C.S. (Color Shade g/L)
P. (Proanthocyanidins g/L)
T.P. (Total Polyphenols g/L)
C.A. (Copigmented Anthocyanin %)
F.A. (Free Anthocyanin %)
P.A. (Polymeric Anthocyanin %)
C.D. (Color Density %)
I.I. (Ionization Index %)
P.I. (Polymeric Index %)
Et. I. (Ethanol Index %)
PVPP I. (PVP Index %)
ClH I. (HCl Index %)

Table 4. Levels of anthocyanins (mg/L) after 6 moths of had been bottled in wines 12 ºBé winemaking with vintage to 13 ºBé

	Control wine	Cold soak 5-8ºC (4)	Cold soak 5-8ºC (8)	Cold soak dry ice (4)	Cold soak dry ice (8)
Df-3-Gl	11.64 ± 2.56	15.27 ± 1.65	14.37 ± 2.46	13.71 ± 2.50	13.12 ± 2.35
Cy-3-Gl	6.79 ± 0.95	5.45 ± 0.65	5.72 ± 0.36	8.25 ± 0.89	8.51 ± 0.55
Pt-3-Gl	20.90 ± 3.90	34.48 ± 3.80	31.84 ± 4.60	33.04 ± 5.60	31.69 ± 3.80
Pn-3-Gl	13.22 ± 1.36	13.09 ± 1.09	13.03 ± 1.02	11.11 ± 1.41	9.67 ± 2.01
Mv-3-Gl	213.08 ± 25.10	219.31 ± 23.11	206.38 ± 24.3	209.74 ± 28.30	217.49 ± 19.31
Mv-3-ac	1.91 ± 0.50	2.73 ± 0.29	1.14 ± 0.16	1.38 ± 0.18	1.17 ± 0.14
Mv-3-Cu	12.48 ± 2.60	5.45 ± 1.60	5.96 ± 1.20	5.08 ± 1.30	4.70 ± 1.0
Total Anthocyans	280.03 ± 12.0	295.9 ± 15.0	278.43 ± 12.0	304.31 ± 15.0	286.34 ± 12.0

Table 5. Levels of anthocyanins (mg/L)after 6 moths of had been bottled in wines 12 ºBé winemaking with vintage to 13 ºBé

	Control wine	Cold soak 5-8ºC (4)	Cold soak 5-8ºC (8)	Cold soak dry ice (4)	Cold soak dry ice (8)
Df-3-Gl	20.44 ± 3.34	19.87 ± 2.05	16.98 ± 2.94	12.82 ± 0.38	12.27 ± 0.52
Cy-3-Gl	4.35 ± 0.36	4.97 ± 0.45	3.01 ± 0.25	4.29 ± 1.10	6.51 ± 0.36
Pt-3-Gl	29.92 ± 3.51	22.35 ± 3.20	27.37 ± 5.32	29.61 ± 3.63	28.27 ± 4.91
Pn-3-Gl	9.76 ± 1.58	8.41 ± 2.51	11.05 ± 3.00	14.43 ± 3.16	9.58 ± 3.01
Mv-3-Gl	167.42 ± 10.10	181.82 ± 12.21	184.99 ± 17.10	209.18 ± 17.50	184.43 ± 15.00
Mv-3-ac	2.27 ± 0.50	1.56 ± 0.35	2.03 ± 0.36	2.18 ± 0.50	1.35 ± 0.30
Mv-3-Cu	14.03 ± 3.5	10.93 ± 2.5	11.95 ± 2.3	13.72 ± 3.6	9.93 ± 2.3
Total Anthocyans	229.17 ± 14.61	249.9 ± 17.21	257.78 ± 16.31	286.23 ± 20.22	252.33 ± 13.23

Table 6. Mean values (mg/L) of aromatic compounds in wines from 12 ºBé grapes 6 moths after bottling.

	Control wine	Cold soak 5-8ºC (4)	Cold soak 5-8ºC (8)	Cold soak dry ice(4)	Cold soak dry ice(8)
Methylacetate	10,383±0,705 a	12,841±1,170 ad	15,135±1,286 be	16,941±6,999 fe	13,048±1,492 ade
Ethylacetate	53,078±6,182 a	71,458±7,191 b	60,832±4,708 ac	58,010±6,906 ade	61,913±6,930 aec
Isobutylacetate	0,035±0,004 a	0,052±0,001 a	0,035±0,005 a	0,043±0,002 a	0,050±0,007 a
Ethylbutirate	0,065±0,002 a	0,059±0,0021 a	0,058±0,002 a	0,051±0,002 a	0,051±0,006 a
Isoamylacetate	0,366±0,098 ab	0,415±0,070 a	0,335±0,126 ab	0,376±0,012 ab	0,315±0,031 b
Ethyllactate	7,172±0,089 a	8,075±0,548 a	7,440±0,253 a	8,608±2,125 a	5,906±0,601 a
Ethyloctanoate	0,283±0,029 a	0,230±0,023 a	0,166±0,030 a	0,313±0,102 a	0,221±0,049 a
Diethylglutarate	0,171±0,038 a	0,282±0,011 ab	0,323±0,034 ab	0,370±0,109 b	0,27±0,070 ab
Diethylsuccinate	7,419±0,270 a	2,208±0,850 b	0,970±0,145 b	1,593±0,840 b	2,671±1,014 b
γ- Butyrelactone	0,941±0,116 a	0,868±0,066 a	0,976±0,429 a	0,786±0,030 a	0,579±0,132 a
Methanol	212,350±14,28a	227,252±3,20 a	228,712±7,66 a	230,249±18,53 a	215,898±3,78 a
1-propanol	33,022±4,436 a	38,024±12,478 a	32,426±0,909 a	29,887±1,360 a	38,120±0,843 a
Isobutanol	106,004±1,55 a	120,523±22,823 ab	123,473±6,629 ab	121,341±12,10 ab	219,205±5,605 b
n-amylalcohol	0,053±0,006 a	0,038±0,003 a	0,060±0,002 a	0,048±0,004 a	0,036±0,006 a
1 Butanol	3,185±1,413 a	3,049±0,294 a	2,070±0,247 a	2,364±0,233 a	2,496±0,843 a
2-Pheylethanol	4,831±0,014 ab	6,143±0,225 ab	6,373±1,197 ab	7,703±1,702 a	4,890±0,732 b
Isoamilic alcohols	369,73±24,15 ab	371,881±12,664 ab	372,824±26,984 ab	391,151±1,136 a	349,205±5,605 b

Different letters within the same column for each effect mean significant differences (P<0,01)

Table 7. Mean values (mg/L) of aromatic compounds in wines from 13 ºBé grapes 6 moths after bottling.

	Control wine		Cold soak 5-8ºC (4)		Cold soak 5-8ºC (8)		Cold soak dry ice (4)		Cold soak dry ice (8)	
Methylacetate	20,437±11,262	a	18,438±3,281	a	19,718±5,082	a	26,664±7,126	a	19,465±2,057	a
Ethylacetate	54,739±6,748	a	66,419±7,450	a	64,874±4,313	a	65,870±38,934	a	67,809±16,901	a
Isobutylacetate	0,0325±0,005	a	0,064±0,008	b	0,0875±0,01	c	0,0815±0,002	dc	0,0965±0,002	ec
Ethylbutirate	0,061±0,010	a	0,077±0,029	b	0,103±0,007	b	0,092±0,003	b	0,103±0,011	b
Isoamylacetate	0,360±0,099	a	0,511±0,010	ab	0,610±0,0	b	0,654±0,06	b	0,728±0,201	b
Ethyllactate	6,264±0,706	a	8,080±1,121	a	7,090±0,416	a	5,306±0,323	a	6,656±0,806	a
Ethyloctanoate	0,266±0,078	a	0,200±0,003	a	0,374±0,033	a	0,360±0,016	a	0,205±0,021	a
Diethylglutarate	0,185±0,105	a	0,285±0,021	ab	0,327±0,003	b	0,287±0,003	ab	0,302±0,01	ab
Diethylsuccinate	4,040±0,153	a	1,047±0,039	b	1,302±0,045	cb	0,996±0,009	db	0,917±0,008	eb
γ- Butyrelactone	0,759±0,068	a	1,141±0,100	ac	1,268±0,135	bc	1,009±0,258	ac	0,818±0,123	ac
Methanol	240,184±42,022	a	233,909±5,035	a	290,927±0,828	b	239,234±15,330	a	225,395±37,326	a
1-propanol	30,519±2,569	a	24,799±2,612	a	30,622±5,022	a	25,651±5,549	a	35,392±13,866	a
Isobutanol	83,000±3,850	a	75,209±3,443	ab	90,493±19,192	ab	89,941±0,311	a	97,865±6,772	b
n-amylalcohol	0,0805±0,014	a	0,119±0,012	b	0,0925±0,003	ac	0,078±0,026	ad	0,0675±0,003	ae
1 Butanol	2,990±1,575	a	2,056±0,650	a	2,120±0,604	a	3,171±0,000	a	4,168±0,665	a
2-Pheylethanol	4,26±0,276	a	7,753±1,177	b	7,042±1,196	b	5,658±0,915	c	6,276±0,111	b
Isoamilic alcohols	318,199±14,240	a	371,698±15,130	b	361,605±21,335	b	387,692±4,005	b	387,769±28,013	b

Different letters within the same column for each effect mean significant differences (P<0,01)