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Influence of prefermentative maceration temperature on the colour and the phenolic and volatile composition of rosé wines

M Rosario Salinas,* José Garijo, Francisco Pardo, Amaya Zalacain and Gonzalo L Alonso

Cátedra Química Agrícola, ETSIA, Universidad de Castilla-La Mancha, Campus Universitario, E-02071 Albacete, Spain

Abstract: Prefermentative maceration for 8 h at 5, 10 and 15 °C was used to make rosé wines, and changes in their colour (colour intensity (CI), tone and CIELAB parameters), phenolic compounds (classic indices and individual compounds) and volatile compounds (major and minor) were monitored from the must stage to wines until 6 months after bottling. The 15 °C maceration temperature provided wines with the highest CI, a^* and C^* values, the greatest malvidin-3-glucoside content and the lowest alcohol and ethyl acetate levels. Only in these wines were terpenols released after 6 months in the bottle. The wines produced at 5 °C had the highest ester levels, which also remained more stable during storage. When using maceration temperature as the differentiating variable in a discriminant analysis, volatile compounds were important contributors. However, colour and phenolic compound parameters were important when sampling time was used as the differentiating variable. The best scoring wines in an informal sensory evaluation test were those subjected to 15 °C maceration, while the least appreciated were those macerated at 5 °C.

Keywords: rosé wine; prefermentative maceration; colour; phenolic compounds; volatile compounds

INTRODUCTION

Rosé wines present certain similarities to red wines, since they are both made from red grapes whose pigments confer on them the characteristic colour. They also present similarities to white wines as regards fruitiness and freshness, for which reason rosé and white wines usually use a similar technology consisting of separating by pressure the juice from the grapes, although a certain degree of maceration is necessary to obtain colour. The most important criterion for the qualification of rosé wines is colour, since their legal definition is not precise.[1] The actual elaboration of rosé wines is difficult, since a minimum of colour has to be extracted along with a maximum level of aromas in a short time. Their storage stage is also problematic, because both colour and aromas are fragile and frequently fleeting.

Colour and aroma are the most important quality characteristics of wines. The colour of rosé wines and its stability are strongly influenced by grape variety and directly dependent on parameters such as the anthocyanins extracted from the grape skins during the short maceration, and the reactions involving these compounds and other phenolic compounds during subsequent vinification and storage.[2–4] The aroma of rosé wines is principally due to the compounds formed during fermentation, and ensuring that the characteristic aromas of each grape variety (varietal aromas) are present is a worthy challenge, since it is these that give a wine personality and distinguish it from others.[5,6]

Low-temperature prefermentative maceration is frequently used in elaborating white wines to encourage contact between grape skins and juices in order to extract the greatest amount of aromas and their precursors, both mainly located in the skin of *vinifera* varieties.[7,8] Increased contact time between juice, seed and skin also increases the content of phenolic compounds and, consequently, the properties associated with them, such as colour and astringency. Du Pleissis[9] observed that polyphenol extraction increased 300-fold for the same contact time when the maceration temperature was raised from 15 to 35 °C. However, an adequate level of phenolic compounds and an improved aroma can be achieved by controlling both maceration time and temperature.[10,11] For example, Long and Lindblom[12] obtained better-quality Chardonnay wines when maceration was carried out at 10 °C for 8 h. For the same maceration time a low temperature diminishes

* Correspondence to: M Rosario Salinas, Cátedra Química Agrícola, ETSIA, Universidad de Castilla-La Mancha, Campus Universitario, E-02071 Albacete, Spain
E-mail: Rosario.Salinas@uclm.es
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the level of proteins in wine, making must clarification unnecessary, while higher temperatures may produce musts with a more intense colour and greater propensity to oxidation. In general, it can be affirmed that the most suitable maceration temperatures and times for white wines are between 5 and 20 °C and between 4 and 24 h respectively.[13] Low-temperature prefermentative maceration with longer maceration times than those used for white wines has also been used to make red wines in order to increase aroma and colour stability. Heatherbell *et al*,[14] for example, observed better sensorial characteristics in Pinot Noir wines after maceration at 4 than at 10 °C, whereas Cuénat *et al*,[15] also in Pinot Noir wines and working with temperatures of 4, 5 and 15 °C, obtained the best sensorial characteristics at the last temperature. For their part, Mahon *et al*[16] studied the effect of prefermentative maceration at 10 and 20 °C on the content of the aroma glycosidic precursors in Cabernet Sauvignon wines and found no significant effect of temperature.

We have not found scientific references concerning low-temperature prefermentative maceration processes applied to the elaboration of rosé wines. As the characteristics of rosé wines, especially colour, are intermediate between those of red and white wines, such a technique might be suitable for improving quality both before and after bottling. The aim of this study, then, was to ascertain the effect of prefermentative maceration at 5, 10 and 15 °C for 8 h on the colour and the phenolic and volatile composition of rosé wines. The evolution of these wines was monitored during their first 6 months in the bottle.

EXPERIMENTAL

Healthy Monastrell grapes from the same vineyard in the Jumilla area (Murcia, Spain) were picked once the maturation controls indicated the optimum conditions (21.6 Brix). Grapes were collected in 25 kg boxes and 15 mg kg^{-1} of SO_2 was added. In the cellar these grapes were mixed together to guarantee uniform sampling and then separated into four batches of 300 kg each. Wines were elaborated in duplicate with 150 kg of grapes each. A control wine (C) was elaborated following the standard winemaking procedure for rosé wine in the area (maceration time of 2 h at 16 °C; addition of 10 g hl^{-1} of dry active yeasts; must fermentation temperature between 18 and 21 °C; correction of total SO_2 up to 50 mg l^{-1}; stabilisation at 10 °C prior to bottling). Three other wines were also elaborated in the same way but with a prefermentative maceration step at 5, 10 and 15 °C for 8 h followed by grape pomace pressing with 65% yield.

The classic analyses of the musts and wines were performed according to the official methods established by ECC[17] regulation. For each vinification, analyses of the chromatic parameters, phenolic compounds and volatile compounds were carried out on the must after centrifugation at 4500 rpm. The same analyses were also carried out on each wine after fermentation (F), after the postfermentation stabilisation process (3 months), immediately before bottling (B) and after 3 and 6 months in the bottle (B3 and B6). All analyses were carried out in triplicate.

Chromatic parameters

These measurements correspond to the CIELAB colour space values,[18] colour intensity (CI)[19] and tone.[3] A Perkin-Elmer Lambda 3B spectrophotometer (Norwalk, CT, USA) was used, scanning between 380 and 780 nm at 5 nm intervals, with a quartz cell of 10 mm thickness. The parameters were provided by the program 'Color of Wines—2001' from Perkin-Elmer Hispania (Madrid, Spain).

Phenolic compounds

Total and individual phenolic compounds were analysed: the total polyphenol index (TPI) and Folin–Ciocalteu index (FI) by the official methods,[17] total anthocyanin content following the method of Ribéreau-Gayon *et al*[20] and tannins according to Montedoro and Fantozzi.[21] For all analyses the same Perkin-Elmer Lambda 3B spectrophotometer was used.

The individual anthocyanins were isolated by Sep-pack C-18 (Waters, Milford, MA USA), previously conditioned with 2 ml of methanol and 5 ml of water. A 4 ml aliquot of wine was eluted with 8 ml of acetonitrile at 16% at pH 2 and concentrated. The resulting fractions were analysed following the high-performance liquid chromatography (HPLC) method of Johnston and Morris.[22] The HPLC analyses were carried out with a chromatograph composed of an LC 410 pump and LC ISS 200 410 autosampler from Perkin-Elmer, fitted to an HP 1100 diode array detector from Hewlett-Packard (Palo Alto, CA, USA). The column used was a Hewlett-Packard C18 Nucleosil 5 µm (200 mm × 4 mm id). The solvents employed for elution were: A, 10% formic acid; B, acetonitrile. The percentage profile was as follows: 98% A (1 min); 94% A (4 min) to 86% A (20 min). The flow rate was set at 1.5 ml min^{-1}, the oven temperature at 25 °C and the injection volume at 50 µl.

Compounds were identified by comparing their spectra with those in Ref 23 and quantified using malvidin-3-glucoside chloride as an external standard (Extrasynthése, Genay, France). All solvents were of chromatographic grade.

The low-molecular-weight compounds (gallic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, tyrosol, (+)-catechin, tryptofol and *cis*-resveratrol) were analysed by HPLC.[24] Compounds were identified by comparing their spectra at 280 and 320 nm with those of corresponding standards (Fluka Chemica, Buchs, Switzerland). A calibration curve of five points for each compound was used, with a correlation coefficient $R^2 > 0.98$.

Volatile compounds

Major and minor volatile compounds were analysed. The major compounds were analysed following the gas chromatography (GC) method based on direct injection of the sample into a Perkin-Elmer 8310 gas chromatograph with a PTV injector and a flame ionisation detector.[25] The compounds analysed were ethyl acetate, methanol, 1-propanol, isopentyl acetate, isobutanol, 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol. 3-Pentanol was used as an internal standard. Identification was carried out by comparison of the retention times with those of corresponding chromatographic standards (Chem-Service Inc, West Chester, PA, USA), and quantification was based on the calibration curves of respective standards (range concentration) in a 12% (v/v) ethanol/water solution; their correlation coefficient was $R^2 > 0.96$.

For minor volatile compound analysis the method followed was that described by Salinas and Alonso[26] based on dynamic headspace. The compounds analysed were ethyl hexanoate, hexyl acetate, ethyl lactate, 1-hexanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, ethyl octanoate, benzaldehyde, linalool, isobutyric acid, ethyl decanoate, isovaleric acid, geraniol, 2-phenylethyl acetate, ethyl dodecanoate, hexanoic acid, benzyl alcohol, 2-phenylethanol, nerolidol and octanoic acid (Sigma-Aldrich, Madrid, Spain; Chem-Service Inc). Methyl caprilate was used as an internal standard. Compounds were analysed by thermal desorption, gas chromatography and mass spectrometry. Isolation was carried out in triplicate using a Dynamic Thermal Stripper 1000 (Dynatherm Analytical Instruments Inc, Kelton, PA, USA). The purge phase used 50 ml of wine, with helium being bubbled through the sample for 20 min at a flow rate of 84.3 ml min^{-1} and at 30 °C; the dry phase lasted 5 min under the same conditions. Volatiles were adsorbed on 0.17 g of Tenax-TA (60 mesh; Alltech, Deerfield, IL, USA) contained in a metallic tube at 25 °C and introduced into the desorption thermal equipment (Perkin-Elmer Desorption ATD 400) under the following conditions: oven, 300 °C; desorption time, 4 min; cold trap, −30 °C with 0.02 g of Tenax adsorbent. The inlet, outlet and desorption flows were 45, 9 and 53 ml min^{-1} respectively. The compounds passed through a transfer line at 200 °C into an HP 6890 gas chromatograph coupled to an LC 3D mass detector with a fused silica capillary column (BP21 stationary phase, 50 m length, 0.22 mm id, 0.25 μm film thickness; SGE, Ringwood, Victoria, Australia). The chromatographic programme was: 50 °C (2.5 °C min^{-1}); 180 °C (2 min) and up to 200 °C (1 °C min^{-1}). For mass spectrometry the EI mode was used (ionisation energy 70 eV, source temperature 250 °C). The acquisition was made in scanning mode (mass range 35–500 u). Identification was carried out using the NIST library, and quantification was based on the calibration curves of respective standards ($R^2 > 0.88$) in a 12% (v/v) ethanol/water solution at pH 3.6.

Sensory evaluation

At the same time that wine samples were taken for analysis, an informal sensory evaluation, with a structured scoring scale, was carried out by a team of seven panellists belonging to the Tasting Committee of Jumilla Regulatory Council. The panellists used an official penalisation profile in which the lowest points were given to the best-considered attributes: visual appreciation, olfactory appreciation (intensity and quality), taste appreciation (intensity and quality) and harmony.

Statistical analyses

Significant differences among musts, wines and sampling times for each of the parameters were assessed by one-way analysis of variance as well as using the SPSS Version 10.0 statistical package for Windows (SPSS Inc, Chicago, IL, USA). Statistical differences among means were evaluated using Duncan's test at 0.05% level in order to evaluate the significance of the analysis. To establish the relationship between the analysed parameters, the maceration temperatures (5, 10 and 15 °C), the sampling times (F, B, B3 and B6) and the panellist profile, three discriminant analyses were carried out using the SPSS program. An unbalanced two-way (maceration temperature and sampling time) discriminant analysis was also carried out taking into account the existence of interactions between the factors.

Different correlations (linear and exponential) between parameters were studied using Microsoft Excel 2000.

RESULTS AND DISCUSSION

Musts

The results of classic analyses of the musts after the maceration step at 5, 10 and 15 °C for 8 h were very similar (mean values: 210 g l^{-1} of sugar, pH 3.5, total acidity of 6.3 g l^{-1} as tartaric acid). After this an exhaustive control of the fermentation process was carried out. Parameters such as density 15/15, Baumé grades and temperature of the fermentation tanks were checked daily (data not shown). Therefore in this paper the differences observed in colour, polyphenolic compound and volatile compound parameters of the different samples were assumed to be mainly due to the maceration temperature variable. Table 1 shows the analytical results for the musts after the pressing step. The CIELAB co-ordinates a^* (red/green), b^* (yellow/blue) and L^* (clarity) define a sample's colour. C^* and H^* are psychophysical magnitudes which express the colour obtained from a^* and b^* and provide information on the 'chromaticness' of a coloured object (C^*) and on the appearance by which a colour is identified (H^* or hue).[18] Since the co-ordinate b^* stands out over a^*, musts are more yellowish than reddish. The highest maceration temperature showed greater values of CI, a^*, b^*, C^* and H^* and lower L^*.

Table 1. Must composition before alcoholic fermentation

Parameter	5 °C	10 °C	15 °C	Control
Chromatic parameters				
Colour intensity	0.47[a]	0.64[b]	0.77[c]	0.67[d]
Tone	1.01[a]	0.98[a]	0.95[a]	0.96[a]
*a**	3.29[a]	8.89[b]	9.17[b]	9.06[b]
*b**	8.03[a]	11.55[b]	24.55[c]	11.76[b]
*L**	92.37[b]	101.06[c]	67.22[a]	102.90[c]
Phenolic parameters				
TPI	5.85[a]	6.05[a]	6.35[a]	6.10[a]
Folin index	6.85[a]	7.10[a]	7.30[a]	6.95[a]
Total anthocyanins (mg l^{-1})	69.50[a]	76.00[b]	91.01[c]	87.50[c]
Tannin (mg tannic acid l^{-1})	108.25[a]	105.35[a]	102.45[a]	102.60[a]
Malvidin-3-glucoside (mg l^{-1})	44.31[a]	46.57[a]	66.74[c]	61.65[b]
Volatile compounds				
Methanol (mg l^{-1})	23.48[a]	37.11[b]	43.13[c]	34.25[a]
2-Phenylethanol (mg l^{-1})	0.20[c]	0.19[c]	0.12[a]	0.17[b]
Benzyl alcohol (mg l^{-1})	0.11[c]	0.09[b]	0.06[a]	0.08[b]
C6 compounds (mg l^{-1})	7.48[c]	7.12[b]	6.30[a]	6.19[a]
Terpenols (μg l^{-1})	2.67[b]	2.09[a]	2.47[b]	2.06[a]

TPI: total polyphenol index. C6 compounds: sum of 1-hexanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol. Terpenols: sum of linalool, geraniol and nerolidol. Different superscript letters within a row indicate significant differences at 0.05% level.

Although it is not accepted for CIELAB parameters, L^* was higher than 100 in some must conditions (Table 1), probably because must matrices are more difficult to determine it in than in wines. The phenolic compounds with greater contribution to must colour are anthocyanins, which increased significantly with increasing maceration temperature. It is necessary to point out the important tannin concentration in all musts before the fermentation process. Such content may came from the skin, as alcoholic fermentation has not taken place and the amount of ethanol is not sufficient to extract these compounds from the seeds.[27] A tannin/anthocyanin ratio between 1.6 and 1.8 was observed, decreasing with increasing maceration temperature. The most abundant anthocyanin was malvidin-3-glucoside, representing more than 57% of the total group, but it only increased significantly when the 15 °C maceration temperature was used. Methanol was the most abundant volatile component in the musts and increased significantly with increasing maceration temperature. This may be due to the greater pectinmethylesterase enzymatic activity which releases methanol from pectins.[28] The other volatile compounds found in the musts, from higher to lower concentration, were C6 compounds, 2-phenylethanol, benzyl alcohol and terpenols. The higher concentrations were always found in the must macerated at 5 °C.

Wines

After the alcoholic fermentation stage the wines had the following characteristics: wine at 5 °C, alcoholic grade 12.38, total acidity 6.63 g l^{-1} tartaric acid, volatile acidity 0.30 g l^{-1} acetic acid, pH 3.6, 7 mg l^{-1} free SO_2 and 28 mg l^{-1} total SO_2; wine at 10 °C, alcoholic grade 11.66, total acidity 7.16 g l^{-1} tartaric acid, volatile acidity 0.33 g l^{-1} acetic acid, pH 3.5, 7 mg l^{-1} free SO_2 and 29 mg l^{-1} total SO_2; wine at 15 °C, alcoholic grade 12.00, total acidity 7.09 g l^{-1} tartaric acid, volatile acidity 0.25 g l^{-1} acetic acid, pH 3.52, 7 mg l^{-1} free SO_2 and 26 mg l^{-1} total SO_2; control wine, alcoholic grade 12.21, total acidity 6.29 g l^{-1} tartaric acid, volatile acidity 0.35 g l^{-1} acetic acid, pH 3.55, 9 mg l^{-1} free SO_2 and 37 mg l^{-1} total SO_2. After fermentation the alcoholic degree, total acidity and pH of the wines were seen to have been significantly affected by the maceration temperature. From 5 to 10 °C, for example, the alcoholic degree decreased, total acidity increased and, consequently, pH fell, while between 10 and 15 °C the alcoholic degree increased.

Colour

Figure 1 shows the evolution of the chromatic parameters with the maceration temperature and sampling time. Only in the wines after fermentation were the CI values lower than in the musts. The highest CI values were recorded in all wines at the time of bottling, after which they decreased. As the maceration temperature increased, so did the CI values.

The highest tone values were seen in the 5 °C wine. There were no significant differences in tone between the 10 and 15 °C wines after 3 months in the bottle, but after 6 months it was significantly lower in the 15 °C wine. Tone evolved similarly in all wines, increasing quickly up to 3 months and then remaining constant up to 6 months.

The wines after fermentation had higher values of a^* and b^* than the musts, but L^* varied randomly. The chromatic parameters (A^*, b^* and C^*) increased significantly with increasing maceration temperature; while a^* increased between 5 and 10 °C, b^* and C^*

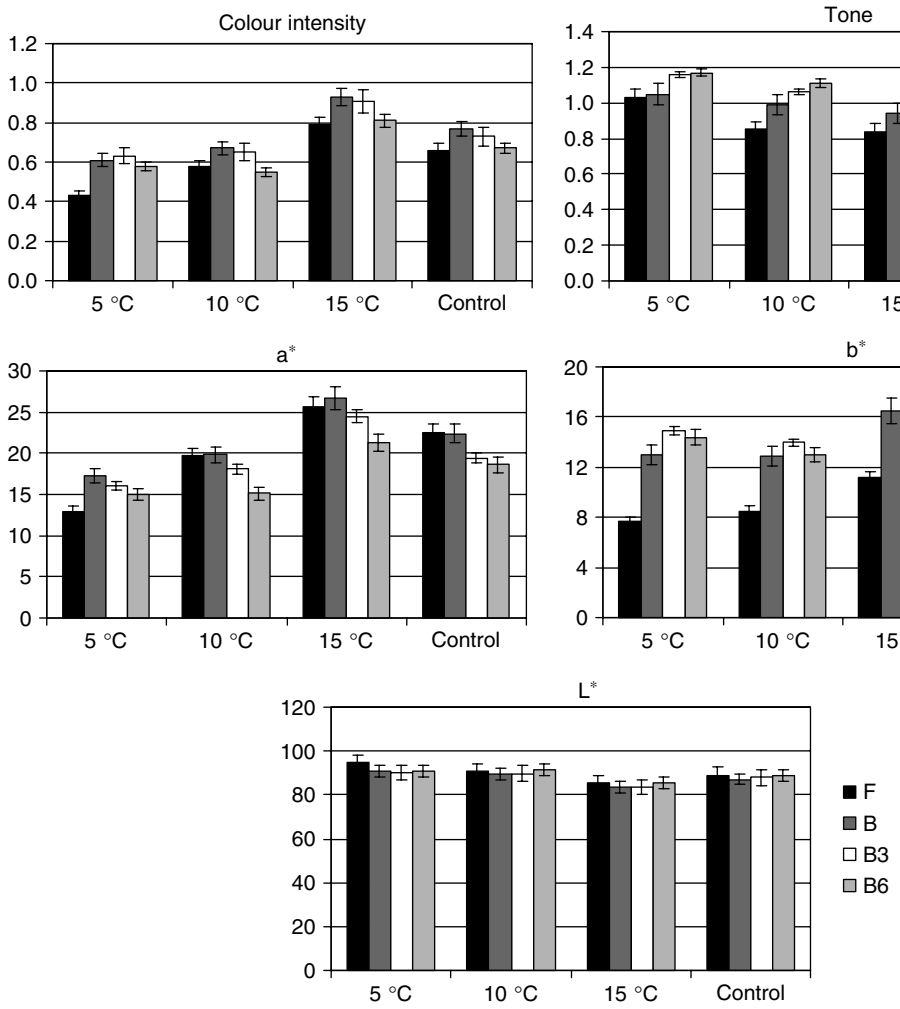

Figure 1. Evolution of wine chromatic parameters according to maceration temperature. Sampling times: F, after fermentation; B, before bottling; B3, 3 months after bottling; B6, 6 months after bottling.

did so between 10 and 15 °C. In all wines the red coordinate (a^*) predominated over the yellow (b^*). No significant differences were seen in H^*. This explains why the wine made with a maceration temperature of 15 °C showed more chromaticness before bottling (B). To ascertain colour stability, the colour differences (ΔE^*_{ab})[29] were calculated using as reference sample the 15 °C wine before bottling. All wines showed lower ΔE^*_{ab} values at sampling time B, which increased during the first 6 months in the bottle. The 15 °C wine presented the lowest ΔE^*_{ab} values at all sampling times and therefore the best and most stable colour.

Phenolic composition

Figure 2 shows the evolution of the Folin index (FI), total polyphenol index (TPI), total anthocyanin content (TA) and tannins with the maceration temperature and sampling time. The lower anthocyanin contents in the wines in comparison with the musts may be attributed to mud losses and to complex compound formation with other molecules, while the tannin increment effect may be due to the solubilising effect of ethanol on the skin and seed wastage. As can be seen, all the above parameters showed higher values in the 15 °C wine. TPI diminished slowly with time. The tannin content and FI increased in the 3 months between the end of fermentation and the moment of bottling and then fell sharply in the bottle (tannins by 80% and FI by 60%). The total anthocyanin content fell by more than 60% in all wines after fermentation. The tannin/anthocyanin (T/A) ratio has been used previously to ascertain whether rosé wines have been made by direct pressing of the fresh grapes or by a drawing-off method, which implies a short maceration.[26] In our case the highest mean value of the T/A ratio was 4.6, which was reached in all wines before bottling (B); 3 months later, however, the value had fallen to 1.2 and then remained constant up to 6 months. This value was slightly higher in the 5 °C wine. As already stated, the highest T/A value was reached before bottling, when CI was also at its highest and CIELAB colour the best. This may be due to the greater copigmentation between anthocyanins and phenolic compounds at this time, this factor being considered responsible for up to 50% of the colour of young wines, while the breakdown of copigmented forms is considered to be mainly responsible for colour loss with time.[2,30]

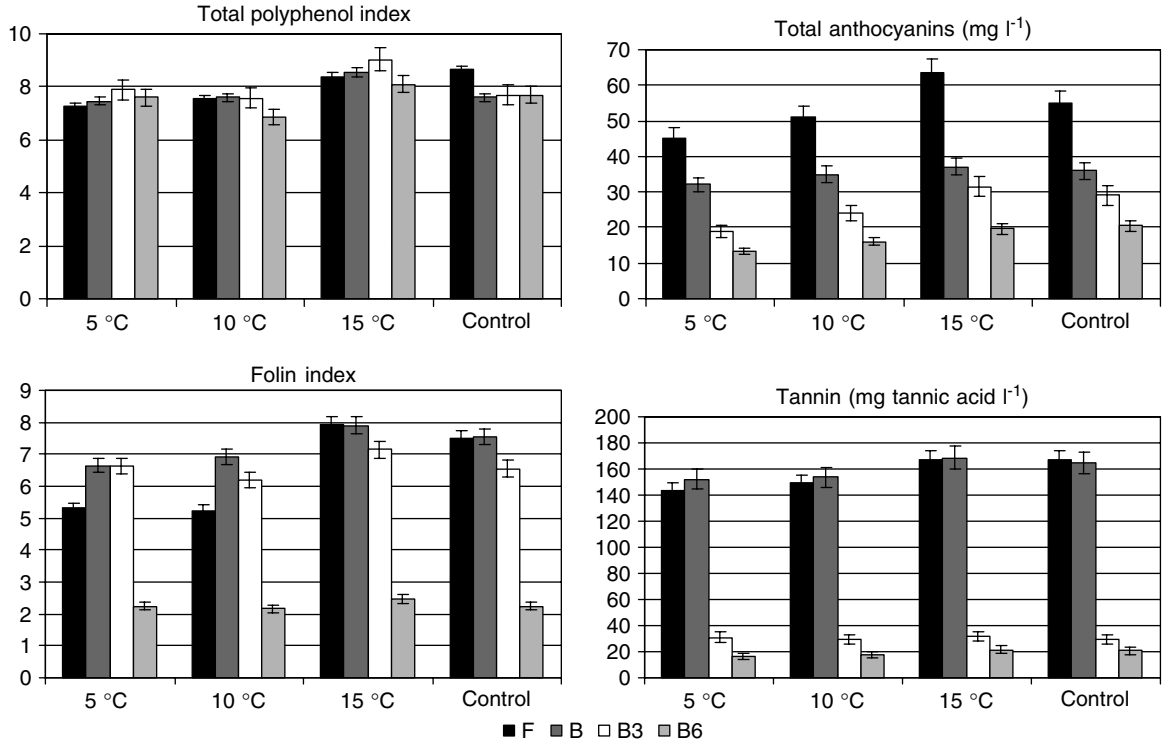

Figure 2. Evolution of wine phenolic compounds according to maceration temperature. Sampling times: F, after fermentation; B, before bottling; B3, 3 months after bottling; B6, 6 months after bottling.

Regardless of the temperature used during maceration, a linear relation was seen between tannin content and TPI after fermentation (tannin = 18.21TPI + 11.8, $R^2 = 0.98$). Such a relation was not seen in later samples, an effect observed previously in enzymatically treated rosé wines.[31]

Table 2 shows the levels of some individual phenolic compounds, with malvidin-3-glucoside (Mv3G) representing more than 80% of total anthocyanins. This is followed, in decreasing order of abundance, by the 3-glucoside derivatives of petunidine, peonidine, delphinidine and cyanidine. The content of all these increased with increasing maceration temperature, although for Mv3G this effect was only significant after fermentation had finished and after 6 months in the bottle. A linear decrement was observed in the anthocyanin/Mv3G ratio in relation to the maceration temperature once fermentation had finished, although this effect was not seen in the bottle. All anthocyanin levels fell as time in the bottle increased, although the exact behaviour depended on the anthocyanin concerned and the maceration temperature. Hence the hydroxylated anthocyanins delphiinidin-3G and cyanidin-3G decreased more slowly than their methoxylated counterparts petunidin-3G and peonidin-3G regardless of the maceration temperature, while the behaviour of Mv3G depended on the temperature, since it showed the highest level 6 months after bottling at 15 °C.

Among the phenolic acids the most abundant were the hydrobenzoics, which in turn were significantly higher in the 15 °C wine at sampling stages F and B, while also increasing in the bottle. Hydroxycinnamic acids reached their highest levels in the 5 °C wine, although after 6 months in the bottle the levels were similar in all wines.

The (+)-catechin content was higher in the 5 °C wine after bottling and remained constant in the bottle (control, 5 and 10 °C wines), although it decreased in the 15 °C wine.

Tyrosol was more abundant than tryptofol in all wines. After 3 and 6 months in the bottle the level of both compounds was significantly lower in the 15 °C wine than in the others. The *cis*-resveratrol content was very low and not significantly different from the level in the control wine. It remained constant in the bottle and then fell only in the 15 °C wine after 6 months.

In summary, the samples showing the highest CI, a^* and C^* scores also showed the highest Mv3G content and best colour, the 15 °C wine being the most representative of this phenomenon. This finding seems to confirm the observations of Zimman *et al*[32] for some wines in which copigmentation increased with temperature. On the other hand, the 5 °C wine showed the highest (+)-catechin content and ΔE^*_{ab}, while 6 months after bottling, the lowest (+)-catechin content was observed in the 15 °C wine, which had the lowest ΔE^*_{ab} values. Several authors have observed that (+)-catechin acts as a copigment with various anthocyanins, increasing the colour in model solutions.[33,34]

Volatile composition

Table 3 shows the volatile compounds determined in the wines. The most abundant compounds were higher alcohols, whose total content is shown in the

Table 2. Evolution of individual phenolic compounds in wines ($n = 6$)

Compound (mg l^{-1})	5 °C	10 °C	15 °C	Control
After fermentation (F)				
Malvidin-3-glucoside	5.31^{a,3}	6.92^{b,3}	8.33^{c,3}	8.11^{c,3}
Hydroxybenzoic acids	0.09^{a,1}	0.09^{a,1}	0.13^{b,1}	0.11^{b,1}
Hydroxycinnamic acids	0.02^{c,1}	0.01^{b,1}	0.008^{a,1}	0.01^{b,1}
(+)-Catechin	0.12^{b,1}	0.11^{a,3}	0.13^{bc,4}	0.14^{c,3}
Tyrosol	1.11^{b,1}	1.44^{c,2}	1.01^{a,2}	1.00^{a,1}
Tryptofol	0.16^{b,2}	0.21^{c,4}	0.16^{b,3}	0.07^{a,1}
cis-Resveratrol	0.007^{a,1}	0.01^{c,1}	0.009^{b,2}	0.02^{d,1}
Before bottling (B)				
Malvidin-3-glucoside	8.63^{a,4}	7.21^{a,4}	12.86^{b,4}	13.04^{b,4}
Hydroxybenzoic acids	0.12^{a,2}	0.14^{ab,2}	0.20^{c,3}	0.15^{b,2}
Hydroxycinnamic acids	0.03^{b,2}	0.017^{a,1}	0.03^{ab,2}	0.02^{a,1}
(+)-Catechin	0.14^{b,3}	0.08^{a,1}	0.09^{a,2}	0.08^{a,2}
Tyrosol	1.52^{b,3}	1.29^{a,1}	1.27^{a,3}	1.34^{a,2}
Tryptofol	0.19^{a,3}	0.09^{a,1}	0.12^{b,3}	0.16^{c,3}
cis-Resveratrol	0.007^{b,1}	0.005^{a,1}	0.008^{c,2}	0.007^{b,1}
3 months in bottle (B3)				
Malvidin-3-glucoside	5.10^{b,2}	5.23^{c,2}	5.23^{c,1}	4.93^{a,2}
Hydroxybenzoic acids	0.17^{a,3}	0.15^{a,2}	0.17^{a,2}	0.17^{a,2}
Hydroxycinnamic acids	0.04^{b,3}	0.02^{a,1}	0.02^{a,2}	0.02^{a,1}
(+)-Catechin	0.14^{c,2}	0.10^{b,2}	0.11^{b,3}	0.04^{a,1}
Tyrosol	1.35^{b,2}	1.44^{b,2}	1.01^{a,2}	1.10^{a,1}
Tryptofol	0.18^{d,2}	0.16^{c,3}	0.09^{b,1}	0.07^{a,1}
cis-Resveratrol	0.009^{c,1}	0.008^{b,1}	0.010^{c,2}	0.006^{a,1}
6 months in bottle (B6)				
Malvidin-3-glucoside	2.48^{a,1}	2.92^{b,1}	5.94^{c,2}	3.45^{b,1}
Hydroxybenzoic acids	0.20^{a,4}	0.23^{a,3}	0.21^{a,3}	0.19^{a,3}
Hydroxycinnamic acids	0.02^{b,1}	0.02^{b,1}	0.02^{b,2}	0,02^{b,1}
(+)-Catechin	0.13^{d,2}	0.10^{c,3,2}	0.06^{b,1}	0.02^{a,1}
Tyrosol	1.57^{c,3}	1.39^{b,1}	0.98^{a,1}	0.96^{a,1}
Tryptofol	0.14^{d,1}	0.11^{c,2}	0.08^{a,1}	0.10^{b,2}
cis-Resveratrol	0.007^{c,1}	0.003^{b,1}	0.002^{a,1}	0.004^{c,1}

Hydroxybenzoic acids: sum of gallic acid, vanillic acid and syringic acid. Hydroxycinnamic acids: sum of *p*-coumaric acid and ferulic acid. Different superscript letters within a row indicate significant differences at 0.05% level between different temperatures of maceration. For each compound, different superscript numbers within a column indicate significant differences at 0.05% level between different sampling times.

table (ethanol is not included). These alcohols have unpleasant aromas and all exceeded the perception threshold, although in concentrations considered acceptable for quality wines.[35] The total alcohol content was significantly lower in the 15 °C wine than in the 5 °C wine. Among the alcohols, of note for their particularly high levels were isopentyl alcohol (from 100 to 168 mg l^{-1}), followed by methanol (from 58 to 90 mg l^{-1}), isobutanol (from 17 to 37 mg l^{-1}), 2-methyl-1-butanol (from 12 to 27 mg l^{-1}) and 1-propanol (from 8 to 14 mg l^{-1}). The concentration of all these alcohols fell during their time in the bottle, as mentioned in other works.[31,36] From a sensorial point of view the most interesting alcohol analysed was 2-phenylethanol, which has a floral, rose-like aroma and which exceeded its olfactory threshold (estimated at 7.5 mg l^{-1})[37] in all wines after fermentation. Although this compound was found in the musts, it increased considerably in the wines after fermentation owing to release of its precursors and mainly to its formation by yeast action.[38–40] The 15 °C wine had the lowest 2-phenylethanol content, which fell during bottling, reflecting the findings of Marais *et al*.[39]

After alcohols, esters were the most abundant compounds in the wines. Some authors consider that acetates and esters of short-chain fatty acids are the compounds mainly responsible for the aromas of young wines.[40,41] The most abundant was ethyl acetate, for which reason it will be discussed separately. It exceeded its perception threshold by a considerable margin but remained below the level considered by Piggott and Findlay[42] to suppress the aroma of other esters, and therefore must exert a positive effect on the overall sensorial quality of the wines. During its time in the bottle the control wine showed the highest ethyl acetate content, while among the wines subjected to prefermentative maceration the 5 °C wine had the highest content. Levels increased with time in the bottle, as previously observed by Marais and Pool.[43]

Ethyl esters predominated over acetates, and both were highest in the control wine. Ethylic esters were more abundant in the 5 °C wine than in the 15 °C wine and diminished with time in the bottle. During this stage, too, there was a significant decrease in acetate concentrations in the wines macerated at higher

Table 3. Evolution of volatile compounds in wines ($n = 6$)

Compound	5 °C	10 °C	15 °C	Control
After fermentation (F)				
Total alcohols (mg l^{-1}) (1)	$285.88^{b,3}$	$287.77^{b,3}$	$249.61^{a,3}$	$251.41^{a,3}$
2-Phenylethanol (mg l^{-1})	$8.09^{a,1}$	$15.18^{b,2}$	$13.54^{b,3}$	$14.68^{b,2}$
Ethyl acetate (mg l^{-1})	$32.46^{b,2}$	$27.03^{a,2}$	$41.35^{c,4}$	$41.19^{c,2}$
Ethylic esters (mg l^{-1}) (2)	$3.11^{b,3}$	$1.99^{a,2}$	$2.65^{b,3}$	$3.31^{b,2}$
Acetates (mg l^{-1}) (3)	$2.79^{a,2}$	$2.34^{a,3}$	$2.96^{a,4}$	$2.68^{a,2}$
C6 compounds (mg l^{-1}) (4)	$3.17^{b,2,3}$	$2.36^{a,2}$	$2.51^{a,2}$	$2.74^{a,2,3}$
Terpenols (mg l^{-1}) (5)	$7.32^{a,1}$	$9.12^{b,1}$	$7.65^{a,1}$	$8.76^{ab,1}$
Acids (mg l^{-1}) (6)	$6.13^{ab,2}$	$3.33^{a,1}$	$4.66^{a,1}$	$8.66^{b,2}$
Before bottling (B)				
Total alcohols (mg l^{-1}) (1)	$282.03^{a,3}$	$316.75^{b,4}$	$241.57^{a,3}$	$251.41^{a,3}$
2-Phenylethanol (mg l^{-1})	$13.54^{a,4}$	$16.42^{b,2}$	$11.99^{a,3}$	$13.47^{a,2}$
Ethyl acetate (mg l^{-1})	$30.67^{c,1}$	$21.20^{a,1}$	$26.00^{b,2}$	$51.35^{d,3}$
Ethylic esters (mg l^{-1}) (2)	$2.52^{b,2}$	$2.00^{a,2}$	$2.17^{a,2}$	$3.41^{c,2}$
Acetates (mg l^{-1}) (3)	$1.97^{c,2}$	$0.82^{b,2}$	$0.63^{a,3}$	$2.44^{d,1,2}$
C6 compounds (mg l^{-1}) (4)	$2.59^{a,1}$	$2.25^{a,2}$	$2.39^{a,1}$	$2.35^{a,1}$
Terpenols (μg l^{-1}) (5)	$12.92^{a,2}$	$13.23^{ab,2}$	$13.66^{b,2}$	$12.11^{a,2}$
Acids (mg l^{-1}) (6)	$7.93^{b,3}$	$4.22^{a,1}$	$8.56^{b,3}$	$10.19^{c,3}$
3 months in bottle (B3)				
Total alcohols (mg l^{-1}) (1)	$270.73^{b,1}$	$248.88^{b,1}$	$216.42^{a,1}$	$210.38^{a,1}$
2-Phenylethanol (mg l^{-1})	$11.82^{b,3}$	$14.34^{d,2}$	$9.09^{a,2}$	$13.66^{c,2}$
Ethyl acetate (mg l^{-1})	$36.35^{c,3}$	$24.38^{a,2}$	$28.25^{b,1}$	$38.20^{c,1}$
Ethylic esters (mg l^{-1}) (2)	$2.17^{ab,1}$	$1.79^{a,1}$	$1.89^{a,1}$	$2.43^{b,1}$
Acetates (mg l^{-1}) (3)	$1.11^{c,1}$	$0.48^{b,1}$	$0.31^{a,1}$	$1.16^{c,1}$
C6 compounds (mg l^{-1}) (4)	$2.43^{ab,1}$	$2.06^{a,1}$	$2.22^{a,1}$	$2.66^{b,1,2}$
Terpenols (μg l^{-1}) (5)	$12.77^{a,2}$	$15.87^{d,3}$	$14.33^{c,3}$	$13.36^{b,3}$
Acids (mg l^{-1}) (6)	$4.84^{a,1}$	$5.82^{b,3}$	$5.45^{b,2}$	$6.64^{c,1}$
6 months in bottle (B6)				
Total alcohols (mg l^{-1}) (1)	$260.02^{b,2}$	$250.33^{b,2}$	$219.89^{a,2}$	$211.19^{a,2}$
2-Phenylethanol (mg l^{-1})	$9.17^{b,2}$	$9.12^{b,1}$	$6.71^{a,1}$	$8.01^{ab,1}$
Ethyl acetate (mg l^{-1})	$35.73^{a,3}$	$33.92^{a,3}$	$32.49^{a,3}$	$42.75^{b,2}$
Ethylic esters (mg l^{-1}) (2)	$2.68^{c,2}$	$1.84^{a,1}$	$2.07^{b,2}$	$3.12^{d,2}$
Acetates (mg l^{-1}) (3)	$0.96^{b,1}$	$0.47^{a,1}$	$0.46^{a,2}$	$1.40^{c,1}$
C6 compounds (mg l^{-1}) (4)	$2.85^{b,2}$	$2.13^{a,1}$	$2.47^{ab,1,2}$	$2.91^{b,3}$
Terpenols (μg l^{-1}) (5)	$12.52^{b,2}$	$11.97^{a,2}$	$16.36^{d,4}$	$13.63^{c,3}$
Acids (mg l^{-1}) (6)	$6.01b^{c,2}$	$4.75^{a,2}$	$5.84^{b,2}$	$6.84^{c,1}$

(1) Sum of methanol, 1-propanol, isobutanol, 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol. (2) Sum of ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate. (3) Sum of isopentyl acetate, hexyl acetate and 2-phenylethyl acetate. (4) Sum of 1-hexanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol. (5) Sum of linalool, geraniol and nerolidol. (6) Sum of hexanoic acid and octanoic acid. Different superscript letters within a row indicate significant differences at 0.05% level between different temperatures of maceration. For each compound, different superscript numbers within a column indicate significant differences at 0.05% level between different sampling times.

temperatures up to 3 months. Numerous authors attribute the loss of the typical fruity aroma of young wines to the fall in ester levels, particularly acetates, during storage.[31,35,36,41,43–45] Esters are synthesised enzymatically during fermentation in higher quantities than would be expected from their equilibrium concentration, but during storage they hydrolyse to reach approximate chemical equilibrium with their acids and alcohols.[11] In the present study the maceration temperature influenced the synthesis of both ethylic and acetic esters, whose concentrations were higher at 5 than at 15 °C, except at sampling time F, suggesting that temperature has a selective effect on their precursors. When the isopentyl alcohol/isopentyl acetate ratio was plotted against sampling time, the 5 °C wine showed the lowest evolution in relation to the others.

In the 5 °C wine, C6 compounds showed significantly higher concentrations after fermentation, but lower concentrations than in the must. These compounds decreased slightly or remained constant in all bottled wines.

The 5 °C wine showed the lowest terpenol content. Up to 3 months after bottling, terpenols were gradually released from their precursors and increased in concentration without exceeding their olfactory perception threshold. It cannot be ruled out, however, that synergies between individual terpenols contributed to the aroma of the wines. Six months after bottling, only the 15 °C wine showed a significant increase in terpenols. Linalool was the most abundant terpenol.

Maceration temperature did not seem to have any clear effect on acid levels, which remained constant

Table 4. Standardised coefficients of canonic discriminant function 1 when maceration temperature and sampling time were used as differentiating variable

Maceration temperature		Sampling time	
Tone	−2.42	*b**	6.78
*L**	21.84	*C**	25.08
*b**	−7.69	Folin index	13.47
Folin index	7.13	TPI	−17.21
(+)-Catechin	1.75	Total anthocyanins	−11.49
Ethyl acetate	−4.96	Tannin	−19.23
Acetates	−25.31	(+)-Catechin	1.42
Ethylic esters	3.62	Ethylic esters	2.59
C6 compounds	−1.90	C6 compounds	1.81
Terpenols	13.73		

TPI: total polyphenol index.

between 3 and 6 months after bottling. The most abundant acid was octanoic acid.

Sensory evaluation

All wines were better assessed before bottling; as time progressed, their scores increased, meaning that the quality was falling. After 6 months in the bottle the best evaluated wines were those subjected to 15 °C maceration, while the worst evaluated were those macerated at 5 °C.

Sample discrimination

The interaction between the two differentiating variables (maceration temperature and sampling time) showed that the parameter tannin had a discriminating power close to 100%. The effects of both variables were also studied separately (Table 4). The wines were clearly separated by two canonic discriminating functions when maceration temperature was used as the differentiating variable, the first of which explained 95.7% of the variance. Volatile compounds and L^* were the variables that contributed most to the differentiation. When the differentiating variable used was sampling time (F, B, B3 and B6), again two canonic discriminating functions correctly separated all samples, the first of which explained 94.1% of the variance. The variables with greater contribution were phenolic compound and colour parameters.

CONCLUSIONS

The wines made with a prefermentative maceration temperature of 15 °C had the highest colour intensity, a^* and C^* values, together with the highest Mv3G content and the lowest ethyl acetate and alcohol contents. Only in these wines, furthermore, were terpenols still being released after 6 months in the bottle, although they never exceeded their olfactory threshold. The wines made with a prefermentative maceration temperature of 5 °C, on the other hand, had the highest ester content, which remained relatively stable during storage, but these wines received the lowest sensorial evaluation.

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