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Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade

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Abstract

Several red wines were elaborated in order to study the influence of the grape harvesting date (degree of maturity of the grape) on their chromatic characteristics and polyphenolic contents. Wines made from two grape varieties, at three different harvesting dates and from two consecutive vintages, were selected for this study. The results showed that the harvesting date of grapes (directly correlated with the degree of maturity of the grapes), influenced the chromatic characteristics of the wines, although their polyphenolic compositions were clearly different, especially in Cabernet Sauvignon wines. In general, higher intensities of blue or violet tones were detected in wines made from the grapes collected on the second harvesting date, in which the ratios anthocyanins/proanthocyanidins and anthocyanins/(proanthocyanidins + catechins) were the lowest. These ratios are proposed as probable indicators of the aptitude for wine ageing.

No significant differences were observed between the levels of the phenolic compounds or between the chromatic characteristics of the wines from both vintages.

Keywords: Colour; Grapes; Maturity; Phenolic compounds; Red wines

1. Introduction

The ripening of grapes includes a broad range of physical and biochemical processes, that begins in ''veraison'' and ends with berry maturity, which are responsible for the grapes reaching optimum characteristics for their transformation or consumption (González-San-josé, Barrón, Junquera, & Robredo, 1991; Palacios, Cea, Cancer, Martínez, & Avenoza, 1986; Winkler, Cook, Kliewer, & Lider, 1974). The changes that take place during ripening do not occur simultaneously. Each compound evolves differently, and, in addition, is influenced by genetic, climatic and geographical factors and by cultural practices (Andrades & González-Sanjosé, 1995; Esteban, Villanueva, & Lissarrague, 2001; Jones & Davis, 2000; Junquera, Robredo, & Díez, 1988; Pérez-Magariño & González-Sanjosé, 2002a). For these reasons, the process of ripening will determine the quality of the grape, and the moment for harvesting will be an important factor in the making of quality wines (Du-Plessis, 1984; González-Sanjosé & Díez, 1992; Hamilton & Coombe, 1992).

In general, various types of maturity states can be distinguished that do not usually coincide in time, which are: physiological (germination), industrial (greater yield from the grape both in weight and in sugar content) and technological (optimum characteristics of the grape for

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their final destination, having in mind the type of wine that will be made) (Robredo, Junquera, González-San-josé, & Barrón, 1991).

Traditionally, the indicators of the date for harvesting have been determined using parameters such as the weight of the berry and the density of the must (Coombe, 1987), and according to the content and relationship between sugars and acidity (Junquera et al., 1988). This is due to (during grape ripening) the accumulation of sugars, water and other reserve substances (Peynaud, 1989), and the decrease of acidity (Iland & Coombe, 1988; King, Sims, Moore, & Bates, 1988; Robredo et al., 1991). In other words, until, recently only the ''industrial'' maturity was considered.

However, for the elaboration of quality wines, oenologists seek grapes with special characteristics, which include, not only an adequate amount of sugars and acids, but also appropriate levels of other important grape components, such as phenolic compounds and terpenoids (in aromatic varieties). During the past two decades, classic indices of maturity have been abandoned, and new parameters, such as ''phenolic maturity'' or the content of different phenolic compounds in the grape, considered. This change of thinking is due to the belief that only grapes with high and balanced levels of phenols could produce quality red wines, suitable for ageing (González-Sanjosé et al., 1991; Peynaud, 1989).

The phenolic compounds of the grape are responsible for the colour, flavour, body and structure of red wines. These compounds do not evolve during ripening of grapes in the same way as the sugars; that is to say, their maximum concentration does not normally coincide with the maximum accumulation of sugars (Maujean et al., 1983). In general, the phenolic compound content increases throughout ripening of the grape. However, there are different evolutions among different phenolic families (González-Sanjosé, Barrón, & Díez, 1990a; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999), and this is strongly influenced by intrinsic grape factors, such as the variety, as well as extrinsic factors, such as climatology, region and soil (Andrades & González-Sanjosé, 1995; González-Sanjosé & Díez, 1993; Reynolds, Pool, & Mattick, 1986).

It is also necessary to keep in mind that the phenolic content of wines will depend, not only on the composition of the grape, but also on enological practices (González-Sanjosé, Santa-María, & Díez, 1990b; Pérez-Magariño & González-Sanjosé, 1999a; Peña, Hernández, García-Vallejo, Estrella, & Suarez, 2000), since they can give wines with very different final phenolic contents. The ageing of the wine will also modify the phenolic composition because the phenolic compounds undergo different transformations, such as oxidation processes (Martínez & Whitaker, 1995), condensation and polymerisation reactions (Bakker et al., 1997; Dallas, Ricardo Da Silva, & Laureano, 1996; Es-Safi, LeGuernevé, Fulcrand, Cheynier, & Moutounet, 2000; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Liao, Cai, & Haslam, 1992; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2003; Revilla, Pérez-Magariño, González-Sanjosé, & Beltrán, 1999), and extraction from wood (Chatonnet & Dubourdieu, 1998). All these reactions are related to the colour, but also with other sensorial characteristics such as the flavour, bitterness and astringency of the final wine.

Winery experience shows that wines with more stable colour and with higher or more intense purple hues are obtained using more mature grapes. This fact should be correlated with the changes occurring in the phenolic composition of grapes during the last stages of the ripening process, and which are nowadays well-known (Andrades & González-Sanjosé, 1995; Esteban et al., 2001; Jones & Davis, 2000; Robredo et al., 1991). However, no papers have been found that examine the correlation of harvesting date (grape maturity states) and phenolic composition of the wines with the chromatic characteristics of the wines during the ageing period.

Therefore, the main objective of this paper was to study the influence of the harvesting date, directly correlated with the degree of grape maturity, on the chromatic characteristics and the phenolic composition of red wines. This study is of practical interest because if it were possible to know why more mature grapes produce more stable wine colour, there would be objective criteria for establishing the optimum technological maturity moment, that is the best date for harvesting the red grapes. Wines made from two varieties harvested at three different dates were studied. Wines were aged for one year in American new oak barrels, followed by six months of storage in the bottle. The study was carried out over two consecutive vintages.

2. Materials and methods

2.1. Samples

Two varieties of red grape were selected, Tinto Fino (TF) and Cabernet Sauvignon (CS), which were cultivated on two adjoining vineyards. These varieties were selected because Tinto Fino is the main and more extensively cultivated variety in the Spanish Denomination of Origin (DO) ''Ribera del Duero'', the area in which the grapes were cultivated and the wines made, and Cabernet Sauvignon is a French variety used as complementary variety in the winemaking of ''Ribera del Duero'' DO.

Three different harvesting dates were considered. The first was the usual moment for harvesting which was decided by the usual criteria of the winemaker, according to °Brix, titratable acidity, visual colour of grapes and astringency of the skins. Wines made from these

grapes were labelled as TF-A and CS-A, respectively. The second and third studied harvesting dates were one week later (wines labelled as TF-B and CS-B), and two weeks later (wines labelled as TF-C and CS-C). During the additional two weeks, there was no significant rainfall, and temperature and light levels were suitable for continued ripening, so the grapes remained turgid, senescence did not set in, and no putrefaction was observed, in either of the years studied.

All the grapes used in this study were grown in the same vineyards, which were divided in to nine parts (Fig. 1). Grapes from a group of three parts were harvested at each date to make a respective wine. The three parts of TF or CS vineyards harvested at each date were chosen randomly, such as shown in Fig. 1.

Every studied red wine was made in duplicate in stainless steel tanks from approximately 1200 kg of grapes, following the traditional method of red wine-making. Thus, after manual harvesting and selection to eliminate the damaged raisins, the grapes were destemmed with minimum physical damage, and the mass lightly sulphited (0.04 g/l), then filled into tanks to develop fermentation at a controlled temperature (25–28 °C). The wines were racked off into new tanks when the total polyphenol index (TPI, measurement of wine absorbance at 280 nm) showed the same value during two consecutive days, which coincided, in the majority of cases, with nearly complete consumption of the reducing sugars (<3 g/l). Operating in this way, maceration time was around 14 days, with slight differences in time for each wine (variety, maturity degree and vintage). At this moment, considered as the end of the alcoholic fermentation, an initial sample was taken (''no aged wines'' or ''0 months of ageing''). After slight spontaneous clarification process, occurring in the tank, wines were transferred to new American oak barrels with a medium–high degree of toasting, in which malolactic fermentation was carried out. Furthermore, the wines under study were kept in this type of barrel, three for each type of wine, and the pertinent racking (cask to cask) allowed a spontaneous clarification process during the ageing period, which had a duration of 12 months, under normal cellar conditions (80% of relative humidity and 14 °C). During this time, samples, at 2, 8 and 12 months of storage in barrels, were taken, which were proportional mixtures of the three barrels of each wine. After oak ageing, the wines were filtered and bottled and final samples were taken after 6 months of storage in the bottle.

Aβ	Bχ	Cα
Cα	Bχ	Aβ
Cβ	Aα	Bχ

Tinto Fino vineyard

Aα	Bβ	Aχ
Bχ	Cα	Cβ
Cα	Aχ	Bβ

Cabernet Sauvignon vineyard

Fig. 1. Harvesting scheme: A, B, and C show, respectively, the parcels harvested at the first, second and third date, in the first studied vintage. α,β and χ show the order in which the parcels were harvested in the second studied vintage.

2.2. Analyses of levels of phenolic compounds in wines

Several phenolic families were measured: total polyphenols (TP) were measured by reaction with Folin–Ciocalteu reagent, and were expressed as gallic acid equivalents (GAE); total anthocyanins (TA) were quantified according to the variation of colour in function of pH and were expressed as mg/l of malvidin-3-glucoside; low-polymeric polyphenols (LPP) were evaluated and expressed as GAE after salt precipitation of highly polymeric phenols; proanthocyanidins (PRO) were measured after their acid hydrolysis with heat, and were expressed as mg/l of cyanidin chloride; and catechins (CAT) were determined by their reaction with vanillin, and were expressed as mg/l of D-catechin. All these methods are conventional ones, used traditionally in wineries and oenological stations, and they are set out in García-Barceló (1990).

2.3. Analyses of grapes and musts

Representative samples of grapes (about 2 kg) were collected at random from several vines and from different parts of various clusters. One hundred berries were used to evaluate their total weight and the juice/solids ratio, which was calculated after manual pressing of the grapes and weight of the solid residue (skins and seeds) and measurement of the volume obtained.

Skin extracts were obtained from 50 berries (in duplicate), following the method proposed by Izcara and González-Sanjosé (2001). Grape skins were separated manually and macerated with methanol–formic acid. Total polyphenols, total anthocyanins, catechins and proanthocyanidins were analysed in these extracts, following the methods previously cited in wines.

Sugar content, pH and titratable acidity were measured in the must obtained after pressing the grapes. They were determined using the OIV methods (1990).

2.4. Analytical standards and reagents

Gallic acid and D-catechin were purchased from Sigma–Aldrich (St. Louis, MO), and malvidin-3-glucoside and cyanidin chloride from Extrasynthèse (Lyon, France).

The rest of reagents were purchased from Panreac (Madrid, Spain).

2.5. Colour evaluation of wines

The measurement of the colour of the wines was carried out using the Glories methods (1986) and CIELab space (1986). The chromatic Glories parameters are colour intensity (CI), tonality (To), percentage of yellow (%Ye), percentage of red (%Red) and percentage of blue (%Blue). They are widely known and more and more frequently used by oenologists. The CIELab parameters are less well known although both the "Commission Internacionale of L'Eclairage" (CIE), as well as several authors, consider that they better define the colour of wines, and permit better differentiation (Almela, Javaloy, Fernández-López, & López-Roca, 1996; Bakker, Bridle, & Timberlake, 1986; Heredia, Troncoso, & Guzmán-Chozas, 1997; Pérez-Magariño & González-Sanjosé, 1999b).

The parameters that define the space of colour, CIELab, that represent the different chromatic characteristics are: a^* (red/green values), b^* (yellow/blue values) and L^* (lightness), from which the psychophysical parameters correlated with the perception of colour by human observers are obtained. These are: L^*, C^* (chroma or saturation) and h^* (hue angle) (Iñiguez, Ortega, Rosales, Ayala, & Puras, 1995). All these CIELab parameters were calculated automatically by a suitable programme installed in a Beckman DU-650 spectrophotometer with diode-array of UV–Vis (Analytical Development Center, Beckman Instruments Inc., 1995), using the illuminant D_{65} (daylight source) and a 10° standard observer (perception of a human observer), following the CIE recommendations (1986) and the latest suggestions of the OIV (1994).

All the spectrophotometric measurements were carried out using quartz cells of 1 mm path length.

2.6. Statistical analyses

All the compounds and parameters reported below were evaluated in duplicate, in each of the samples.

The statistical analysis of the data was carried out by analysis of the variance (ANOVA) and the LSD test (least significant difference) to show measurements which can be considered statistically different. A significance level of $\alpha = 0.05$ was used.

All statistical analyses were carried out using the statistics package Statgraphics Plus 4.0 (1999).

3. Results and discussion

Table 1 shows the results of analyses carried out on the grapes. In both years, it was observed that the weight of berries increased gradually in the last two weeks, especially during the first additional week. At the same time, an accumulation of skin phenols was observed, also especially during the first additional week, with no significant differences between the second and third harvesting dates.

During the additional weeks, acidity decreased, mainly during the second one, falling, in some cases,

Table 1
Values of the cited parameters for Tinto Fino (TF) and Cabernet Sauvignon (CS) grapes, at the three chosen harvesting date (A, B and C) and for the two studied vintages

	TF-A	TF-B	TF-C	CS-A	CS-B	CS-C
First vintage						
Weight of 100 berries (g)	130	147	153	88	100	105
Juice/solids ratio	2.08	2.70	2.13	1.67	1.85	1.35
TP (mg/100 berries)[a]	234	245	246	190	205	206
TA (mg/100 berries)[b]	235	241	218	186	201	189
PRO (mg/100 berries)[c]	160	173	150	105	163	125
CAT (mg/100 berries)[d]	26	61	36	13	32	28
Sugar content (g/l)	225	240	242	225	238	240
TAc (g/l tartaric acid)[e]	6.2	5.6	4.6	7.8	7.6	6.4
pH	3.55	3.77	3.79	3.36	3.36	3.47
Second vintage						
Weight of 100 berries (g)	135	145	151	91	97	100
Juice/solids ratio	2.00	2.63	2.22	1.59	1.89	1.32
TP (mg/100 berries)[a]	231	252	253	202	212	210
TA (mg/100 berries)[b]	231	244	224	192	204	196
PRO (mg/100 berries)[c]	158	176	154	109	160	128
CAT (mg/100 berries)[d]	30	66	41	16	34	31
Sugar content (g/l)	233	245	249	231	240	244
TAc (g/l tartaric acid)[e]	5.7	5.9	4.4	7.8	7.4	6.6
pH	3.62	3.73	3.74	3.47	3.50	3.51

[a, b, c, d] TP, total polyphenols; TA, total anthocyanins; PRO, total proanthocyanidins; CAT, total catechins levels on the skin of the berries.
[e] TAc, titratable acidity of the must.

to levels below the recommendation values for wine quality (TF-C grapes, both years).

In general, even if wines from the first vintage showed apparently higher levels of phenolic compounds than the wines from the second one, no statistical differences were found between wines of the two years, and no vintage factors were detected between these wines. Tables 2 and 3 show the α values for the variability induced by the vintage factor. All the α values obtained, with the exception of one case (the percentage of red in TF-A wines), were above 0.05. Therefore, a vintage factor was not detected in both in young wines (not aged ones, Table 2) and aged wines (Table 3). These results, together with the fact that a similar evolution of the wines was observed in both vintages (data not showed in this paper), suggested that it was possible to consider all the results together, independent of the vintages in which the wines were made. Furthermore, in this way, the presentation and discussion of the data are simplified.

Tables 2 and 3 also summarise the results relative to the LSD test, showing the statistically significant differences induced by the harvesting data factor, for each variety and studied parameter.

In general, the young wines from the second harvesting date of both varieties (TF-B and CS-B) had higher contents of the different phenolic families analysed. Only the initial total anthocyanin content did not show this and, even in CS wines, no statistically significant differences were found among them. This fact could be due to the intensive maceration applied, to the high levels of anthocyanins in the grapes and to the easy extractability

Table 2
Phenolic compound levels and colour parameter values of un-aged wines made from Tinto Fino (TF) and Cabernet Sauvignon (CS) grapes harvested at the three chosen dates (A, B and C)

Phenolic compounds	TF-A	TF-B	TF-C	CS-A	CS-B	CS-C
Total anthocyanins (mg/l)	875 ± 23 b* 0.4475[a]	908 ± 16 b 0.3087	834 ± 14 a 0.2677	1217 ± 31 a 0.0831	1202 ± 9 a 0.1920	1259 ± 13 a 0.2296
Total polyphenols (mg/l)	1498 ± 17 a 0.2887	1604 ± 12 b 0.3711	1457 ± 17 a 0.5602	1951 ± 15 a 0.1221	2232 ± 12 c 0.1732	2112 ± 12 b 0.2160
Low-polymeric polyphenols (mg/l)	1269 ± 13 a 0.4523	1405 ± 11 c 0.5708	1338 ± 9 b 0.3257	1769 ± 8 a 0.2016	1987 ± 17 b 0.3056	1976 ± 16 b 0.2714
Catechins (mg/l)	447 ± 25 a 0.3044	542 ± 11 b 0.3018	441 ± 12 a 0.4665	887 ± 27 a 0.2023	1026 ± 12 c 0.2703	960 ± 13 b 0.2864
Proanthocyanidins (mg/l)	847 ± 15 a 0.2106	1011 ± 10 b 0.2356	851 ± 9 a 0.1928	1219 ± 1 a 0.1856	1666 ± 7 c 0.1222	1485 ± 9 b 0.1701
L^*	61.0 ± 0.01 b 0.1469	59.4 ± 0.10 a 0.2502	62.6 ± 0.20 c 0.2446	49.6 ± 0.14 c 0.6616	46.2 ± 0.09 a 0.2816	47.1 ± 0.29 b 0.3591
a^*	50.4 ± 0.01 c 0.0925	49.9 ± 0.08 b 0.3450	48.1 ± 0.12 a 0.3934	63.6 ± 0.02 b 0.1961	63.1 ± 0.11 a 0.2740	64.8 ± 0.20 c 0.2802
b^*	−6.32 ± 0.03 b 0.2784	−4.67 ± 0.04 c 0.2877	−6.53 ± 0.04 a 0.2468	1.49 ± 0.06 a 0.3903	4.41 ± 0.07 b 0.3187	1.22 ± 0.11 a 0.6735
C^*	50.8 ± 0.01 c 0.1075	50.1 ± 0.08 b 0.3478	48.6 ± 0.13 a 0.3873	63.6 ± 0.01 a 0.1728	63.3 ± 0.12 a 0.2623	64.8 ± 0.21 b 0.2866
h^*	−7.15 ± 0.04 b 0.2489	−5.35 ± 0.04 c 0.2793	−7.73 ± 0.03 a 0.2742	1.33 ± 0.06 b 0.3611	4.00 ± 0.06 c 0.3041	1.08 ± 0.10 a 0.6680
Colour intensity	1.73 ± 0.028 c 0.1552	1.62 ± 0.008 b 0.2649	1.42 ± 0.005 a 0.2965	2.59 ± 0.023 a 0.2712	2.75 ± 0.001 c 0.7935	2.66 ± 0.003 b 0.2929
Tonality	0.422 ± 0.001 a 0.5528	0.446 ± 0.001 c 0.5528	0.428 ± 0.001 b 0.6985	0.358 ± 0.004 a 0.3932	0.392 ± 0.003 b 0.2965	0.359 ± 0.001 a 0.5528
%Yellow	26.3 ± 0.02 a 0.9990	27.7 ± 0.03 c 0.1548	27.0 ± 0.01 b 0.5528	24.2 ± 0.13 a 0.1755	25.7 ± 0.10 b 0.1948	24.3 ± 0.04 a 0.2999
%Red	62.3 ± 0.13 b **0.0342**	62.0 ± 0.06 a 0.4053	63.1 ± 0.04 c 0.4477	67.5 ± 0.40 b 0.1256	65.7 ± 0.14 a 0.2588	67.6 ± 0.05 b 0.2965
%Blue	11.4 ± 0.13 c 0.0565	10.4 ± 0.02 b 0.7905	9.85 ± 0.03 a 0.5337	8.26 ± 0.27 a 0.2837	8.59 ± 0.03 a 0.7644	8.12 ± 0.01 a 0.8378

Mean values ± standard deviation, and α values from ANOVA analyses by vintages.
[a] α values > 0.05 indicates not vintage effect for each parameter and harvesting date.
* Values with the same letter, for each variety and parameter, were not significantly different according to LSD test.

Table 3
Phenolic compound levels and colour parameter values of 18 month-aged wines made from Tinto Fino (TF) and Cabernet Sauvignon (CS) grapes harvested at the three chosen dates (A, B and C)

Phenolic compounds	TF-A	TF-B	TF-C	CS-A	CS-B	CS-C
Total anthocyanins (mg/l)	341 ± 4 a 0.5485[a]	377 ± 7 b 0.5162	365 ± 6 b 0.4803	387 ± 3 a 0.3933	417 ± 6 b 0.3172	423 ± 5 b 0.4005
Total polyphenols (mg/l)	1482 ± 5 a 0.4908	1578 ± 5 b 0.3998	1489 ± 7 a 0.4651	1843 ± 4 a 0.2447	2137 ± 5 c 0.2587	2046 ± 7 b 0.3024
Low-polymeric polyphenols (mg/l)	950 ± 11 a 0.4807	1133 ± 7 b 0.5113	1225 ± 10 c 0.4518	1313 ± 6 a 0.3172	1409 ± 13 b 0.3331	1573 ± 8 c 0.2994
Catechins (mg/l)	496 ± 10 a,b 0.4301	510 ± 13 b 0.3898	472 ± 11 a 0.4739	774 ± 11 a 0.3507	886 ± 7 b 0.3681	782 ± 11 a 0.3434
Proanthocyanidins (mg/l)	962 ± 9 a 0.3624	1139 ± 4 b 0.3981	962 ± 5 a 0.3823	1134 ± 7 a 0.3303	1680 ± 4 c 0.3078	1438 ± 5 b 0.2998
L^*	71.64 ± 0.08 a 0.1971	72.51 ± 0.01 b 0.2247	74.48 ± 0.11 c 0.2338	60.86 ± 0.07 c 0.4722	54.12 ± 0.04 a 0.3470	59.23 ± 0.09 b 0.3726
a^*	26.50 ± 0.03 c 0.1822	23.85 ± 0.02 b 0.3440	23.09 ± 0.07 a 0.4175	41.63 ± 0.09 a 0.2381	43.66 ± 0.07 c 0.3407	42.41 ± 0.06 b 0.3099
b^*	0.01 ± 0.01 a 0.3118	1.57 ± 0.03 c 0.3193	0.56 ± 0.03 b 0.3050	2.76 ± 0.02 a 0.4072	6.03 ± 0.08 b 0.3891	2.82 ± 0.04 a 0.4627
C^*	26.50 ± 0.03 c 0.2230	23.90 ± 0.02 b 0.3857	23.10 ± 0.07 a 0.3777	41.72 ± 0.09 a 0.2187	44.07 ± 0.08 c 0.3061	42.50 ± 0.06 b 0.3552
h^*	0.02 ± 0.01 a 0.3427	3.77 ± 0.06 c 0.4002	1.39 ± 0.07 b 0.3778	3.80 ± 0.01 a 0.4621	7.86 ± 0.09 b 0.4273	3.81 ± 0.05 a 0.5937
Colour intensity	0.917 ± 0.002 c 0.2793	0.887 ± 0.003 b 0.3043	0.783 ± 0.003 a 0.2899	1.558 ± 0.004 a 0.3101	1.912 ± 0.002 c 0.6061	1.613 ± 0.003 b 0.3779
Tonality	0.684 ± 0.001 a 0.6371	0.739 ± 0.001 c 0.6647	0.722 ± 0.001 b 0.6999	0.582 ± 0.002 a 0.5308	0.609 ± 0.001 c 0.5070	0.600 ± 0.001 b 0.6363
%Yellow	35.38 ± 0.03 a 0.7502	36.06 ± 0.02 b 0.4276	36.18 ± 0.03 b 0.5481	33.09 ± 0.05 a 0.2832	33.56 ± 0.04 b 0.2771	33.46 ± 0.04 b 0.3304
%Red	51.76 ± 0.07 c 0.2235	48.80 ± 0.07 a 0.4145	50.12 ± 0.07 b 0.4769	56.79 ± 0.12 c 0.2536	55.05 ± 0.06 a 0.2706	55.78 ± 0.07 b 0.2998
%Blue	12.86 ± 0.04 a 0.2387	15.14 ± 0.05 c 0.6081	13.70 ± 0.04 b 0.6119	10.17 ± 0.06 a 0.4227	11.39 ± 0.01 c 0.6621	10.77 ± 0.03 b 0.6973

Mean values ± standard deviation, and α values from ANOVA analyses by vintages.
[a] α Values > 0.05 indicate no vintage effect for each parameter and harvesting date.
[*] Values with the same letter, for each variety and parameter, were not significantly different according to LSD test.

of the anthocyanins. The lower content of total anthocyanins of the TF-C wines accords with the fact, previously published that, after complete maturity, the content of anthocyanins falls, which could be an indicator of the beginning of the post-maturity and senescence process (Andrades & González-Sanjosé, 1995).

Regarding chromaticity, it was observed that the wines from the second harvesting date were the darkest (lower L^*), which agrees with their higher global phenolic content. These results are in agreement with those found in a previous paper (Pérez-Magariño & González-Sanjosé, 2001). According to Bakker et al. (1986) and Almela, Javaloy, Fernández-López, and López-Roca (1995), the darkest wines should also show the greatest chromatic intensity; however, this was only observed for the wines of CS. However, since previous works (Revilla & González-Sanjosé, 2001) indicated that the blue component of wines was directly and closely related to their chromatic intensity, then possibly the high value of the blue component of the wine TF-A could be the responsible for its high chromatic intensity.

The wines from the second harvesting date (B) had slightly higher values of tonality, and also, slightly higher percentages of yellow and lower red tones. However, though all the wines showed very low values of tonality, the differences were not appreciable in a visual test.

The ageing process induced clear modifications of the chromatic characteristics of the wines and also of their phenolic levels. These changes are summarised and presented in graph form (Figs. 2 and 3), in which highly correlated chromatic parameters (Pérez-Magariño & González-Sanjosé, 2002b) are omitted in order to simplify the presentation. So, three Glories and two

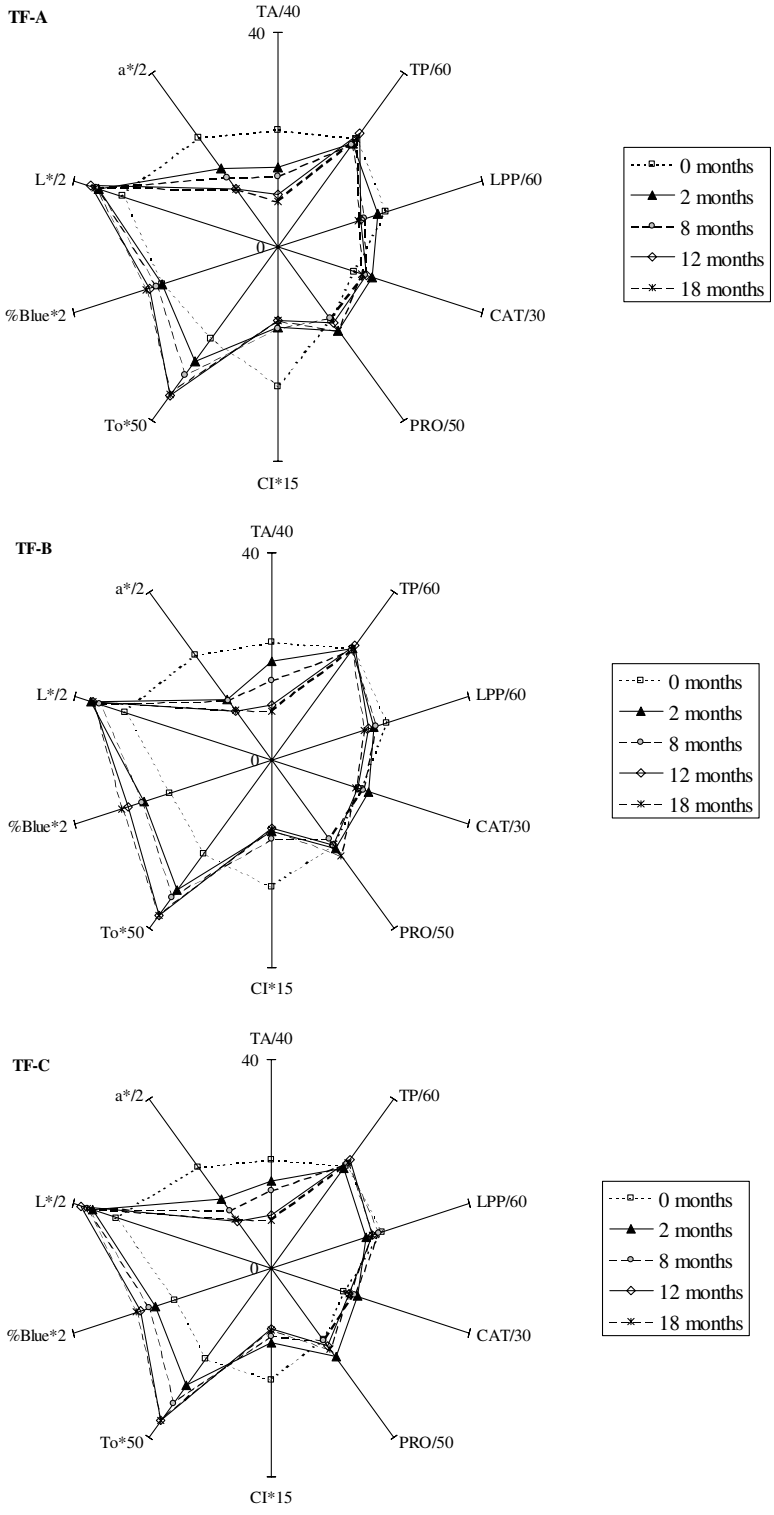

Fig. 2. Evolution of the selected chromatic parameters and phenolic family levels of the Tinto Fino wines (TF) made from the grapes collected at the first harvesting date (TF-A), at the second harvesting date (TF-B) and at the third harvesting date (TF-C). Values were transformed to fit on the scale.

CIELab parameters are shown: colour intensity (correlated with C^*), tonality (highly correlated with h^* and %Ye), percentage of blue (associated with the parameter b^*), a^* (correlated with the %Red), and L^*. Furthermore, in order to improve the quality of the graphs, equivalent scales were applied to all shown parameters, so values were transformed, and the conversion factor is shown in the legend of each parameter.

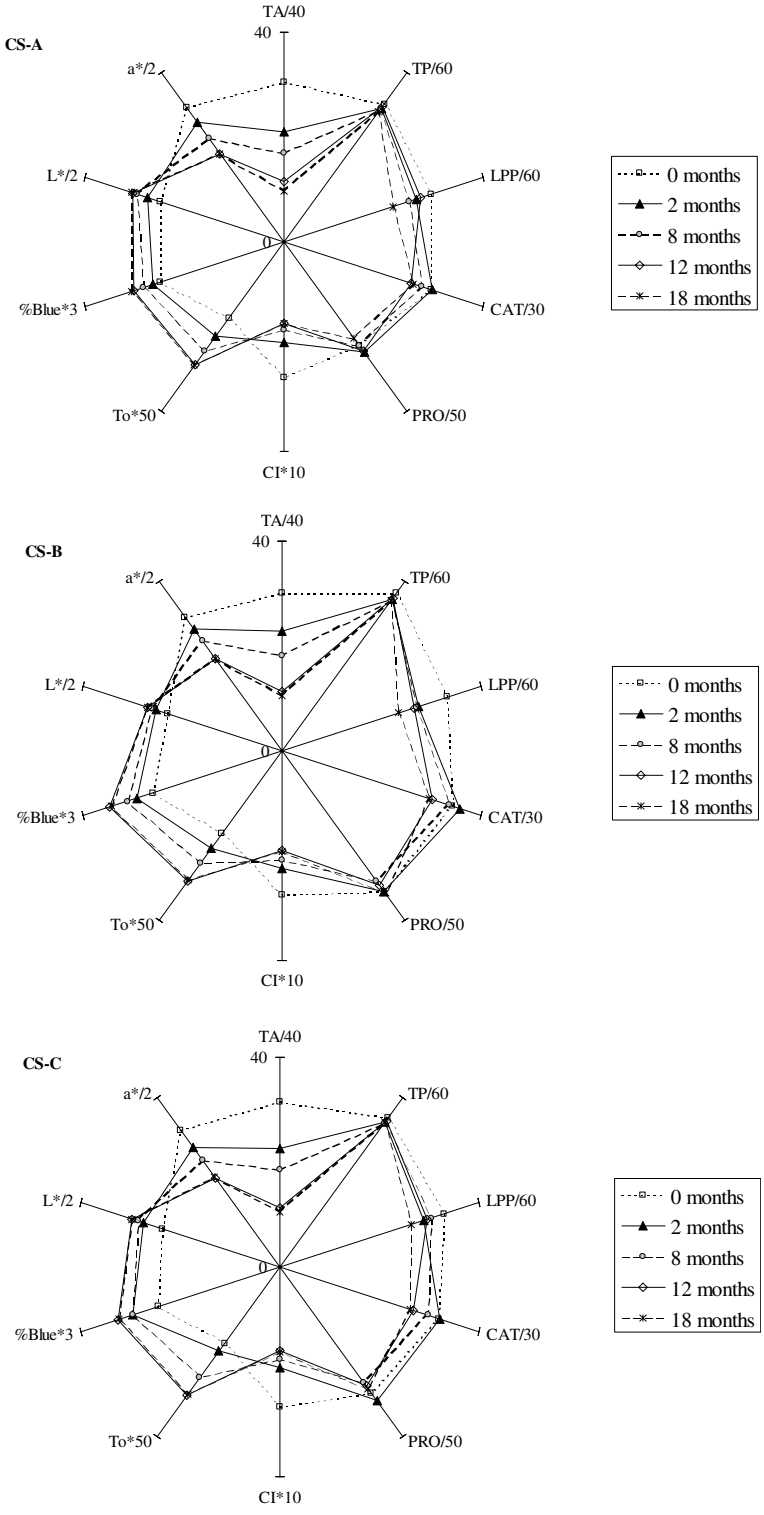

Fig. 3. Evolution of the selected chromatic parameters and phenolic family levels of the Cabernet Sauvignon wines (CS) made from the grapes collected at the first harvesting date (CS-A), at the second harvesting date (CS-B) and at the third harvesting date (CS-C). Values were transformed to fit on the scale.

During the ageing process, the total polyphenol content remained practically constant in all the wines studied. This could be due to the fact that losses of the wine's phenols, and the gains of the phenols extracted from the wood, balance one another out. It must not be forgotten that the barrels are new and with a medium–high degree of toasting, and therefore with a considerable capacity for transferring phenol compounds to the wine. In gen-

eral, the levels of low molecular weight phenols decreased in all the wines; however, while levels of catechins and proanthocyanidins increased in TF wines, they decreased in CS ones or remained constant. An important decrease in total anthocyanin content was observed, which is directly correlated with the reactions in which these pigments are involved during ageing (cycloaddition, or condensation with tannins) (Bakker et al., 1997; Fulcrand et al., 1998; Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002).

It is well known that changes in the phenolic fraction have a strong influence on the chromaticity of the wine. Thus, the colour intensity fell with ageing, with a simultaneous increase in lightness (L^*). At the same time, red tones fell (values of a^*) and tonality increased. This loss of red tones is mainly due to the loss of free anthocyanins already indicated. However, colour intensity did not decrease proportionally to anthocyanin decrease. This fact is due to the increase of violet tonalities (%Blue) during ageing, which make an important contribution to colour intensity (Revilla & González-Sanjosé, 2001). The increase of violet tonalities was especially detected in the wines from TF. The higher value of the blue component of these wines is probably due to a good co-pigmentation or condensation of the anthocyanins with tannins, whether they are ellagitannins, proanthocyanidins or catechins, giving rise to newly formed anthocyanins which stabilise the violet tonalities of the wine (Bakker et al., 1997; Fulcrand et al., 1998; Mateus et al., 2003; Pérez-Magariño, Izcara, González-Sanjosé, & González,

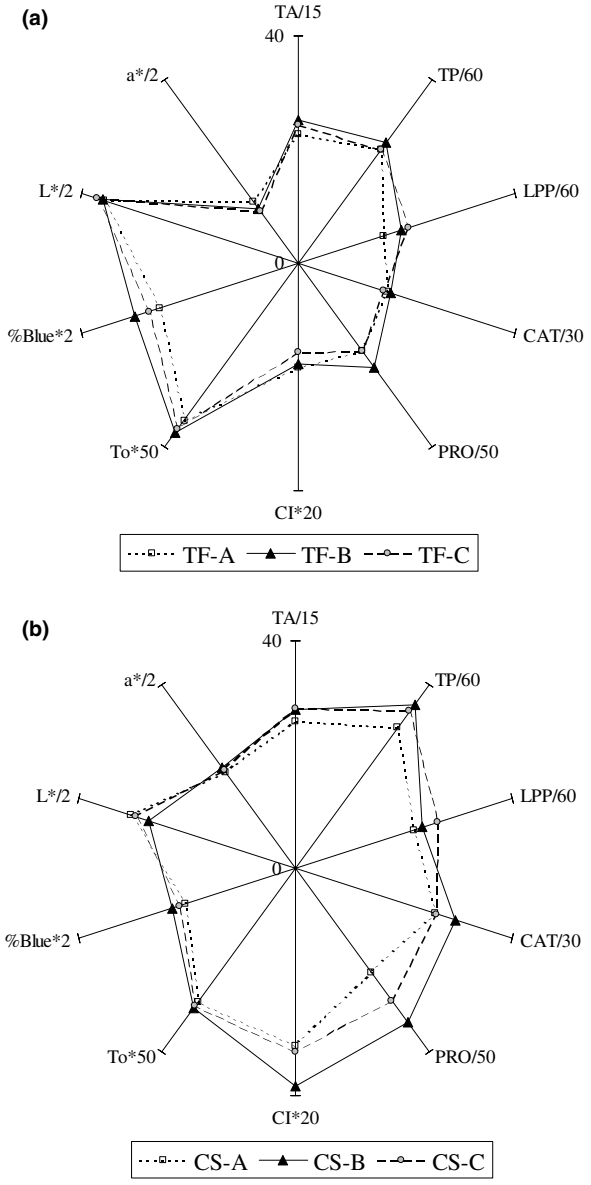

Fig. 4. Representation of the selected chromatic parameters and phenolic families, comparing the wines made from the grapes collected at the three studied harvesting dates (A, B and C) after 18 months of ageing. Values were transformed to fit on the scale.

2001 Revilla et al., 1999). Some of the quoted authors indicate that these pigments are formed in greater quantity in wines richer in phenols, particularly in anthocyanins and proanthocyanidins (Revilla & González-Sanjosé, 2001), conditions which occur, above all, in the wines from the second harvesting date, as previously described (Table 2).

According to Figs. 2 and 3, the wines from the third harvesting date are evidently evolved in a similar way during the ageing process. To support this hypothesis, Tables 3 and Fig. 4(a) and (b) show the different studied parameters of the wines after 18 months of ageing.

Thus, it is possible to affirm that wines from the second harvesting date (B) had higher levels of phenolic compounds, such as were found in the un-aged wines (Table 2). However, it is interesting to note that aged wines from the third harvesting date (C) showed finally better results than wines from the first harvesting date (A), having higher levels of anthocyanins and percentage of blue and, in CS wines, higher colour intensity. Furthermore, it was observed that the aged wines of CS made from the grapes collected on the second harvesting date (B) remained more intense and darker (lower L^*). This fact was not so clearly observed in the wines of TF.

In summary, the wines of each variety after 18 months of ageing (close to the date of being marketed) had different phenolic contents and chromatic profiles (Fig. 4), which could be due to the different states of maturity of the grapes employed to make them. It seems that the most important difference correlated with harvesting time was the evolution of violet tonality (%Blue), which increased significantly during ageing of all the studied wines, but showed the highest increase in wines from the second time of harvesting for the two studied grape varieties (TF-B and CS-B).

The greater violet tones of the wines from the second harvesting of CS, as well as their colour intensity and their higher proanthocyanidin and catechin levels, indicate their better aptitude for ageing. Something similar occurs with the wines of TF, although, in this case, the incidence of the degree of maturity of the grape is not so notable on the phenolic composition of wine but mainly notable on the chromatic characteristics. This fact could be due to the proper evolution of ripening process for each variety, which occurs quicker for TF.

The proportions found among the different phenolic compounds could explain these results. Thus, the best chromatic characteristics were observed in wines in which the ratios anthocyanins/proanthocyanidins and anthocyanins/(proanthocyanidins + catechins) were the lowest (Table 4), made from grapes with the lowest ratios of these phenolic compounds.

These observations seem to indicate that wine colour quality is improved using grapes with a major quantity of "co-pigments", which occurs in grapes harvested later than is traditional. However, from the point of view that wines from the third date of harvesting were not better than those of the second date, and that the grapes of these third dates showed higher values of the phenolic ratios, the harvesting should not be postponed unduly.

Table 4
Mean values of the ratios: total anthocyanins (TA)/proanthocyanidins (PRO) and TA/PRO + catechins (CAT) evaluated in berries extracts and wines of TF: Tinto Fino and CS: Cabernet Sauvignon, at the three chosen harvesting dates (A, B and C)

	TF-A	TF-B	TF-C	CS-A	CS-B	CS-C
Data from 100 berries						
TA/PRO	1.47	1.39	1.45	1.77	1.25	1.52
TA/(PRO + CAT)	1.25	1.02	1.16	1.56	1.04	1.24
Data in no aged wines						
TA/PRO	1.03	0.90	0.98	1.00	0.72	0.85
TA/(PRO + CAT)	0.68	0.58	0.65	0.58	0.45	0.51

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