Free Radical Scavenging Capacity in the Aging of Selected Red Spanish Wines

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Free radical scavenging capacity by the DPPH• method and main physicochemical properties, polyphenols content by HPLC, color by a tristimulus colorimeter, and UV−vis spectra in the aging of selected red Spanish wines, were studied. As the wines age, they become darker (lower lightness, L^*) and increase their hue angle (lower red color) as well as the ratio of absorbance at 420 nm to that at 520 nm. Main polyphenolics identified in the samples were tannic acid, oenin, and gallic acid. The antiradical efficiency of the samples increased during aging, which could be related to an increase in the tannic acid concentration shown by the following correlationship: $EC_{50} = 1/(0.18 + 0.0011[\text{tannic acid}]_{mg/L})$ with a correlation coefficient of 0.744.

Keywords: *Red wine; aging; free radical scavenging; polyphenols; color*

INTRODUCTION

Wine is a complex system capable of undergoing many different changes during storage. These alterations are known as aging. There are two successive phases in the aging of wines: first, maturation in barrels or tanks in which the oxygen is present; and second, when the wine has been bottled and protected from the air (Macheix et al., 1991; Gómez-Cordovés and González-San José, 1995).

Depending on the procedure followed during the maturation phase, some diffusion between wine and the compounds out of the barrel wall may occur, and by this, oak wood containers contribute to the formation and development of the sensorial characteristics of aged wines (Andrade et al., 1998). The amount of phenolics extracted into wine depends on aging time, oak type, as well as barrel size (Shahidi and Naczk, 1995). According to aging time, Spanish wines are classified as young or "joven" (best consumed before the next season), "crianza" (aging at least 6 months in oak and 18 months in bottles), and "reserva" (aging at least 12 months in oak and 24 months in bottles; nevertheless, this can be changed according to each Apellation of Origin) (Ministerio de Agricultura, Pesca y Alimentación, BOE, 1988).

Antioxidant activity is the most studied property in relation to the health benefits of wine consumption, and it has been studied by different in vitro and in vivo methods and related to the presence of polyphenols (Frankel et al., 1995; Fuhrman et al., 1995). Polyphenol antioxidants mainly function as free radical terminators (Shahidi and Wanasundara, 1992), and by this, it is interesting to measure their free radical scavenging capacity. For the evaluation of this property, the concentration of antioxidant needed to decrease by 50% the initial DPPH• substrate concentration (EC_{50}) is widely used (Kanner et al., 1994; Brand-Williams et al., 1995; Vinson et al., 1995); however, we proposed to include a new parameter (T_{EC50}) as the time needed to reach a steady state at the concentration corresponding to EC_{50}. It was also defined the antiradical efficiency (AE), which is more discriminatory than EC_{50} in the comparison of the antiradical capacity of different compounds (Sánchez-Moreno et al., 1998).

The aging of red wines has been studied taking into account the changes in the chemical composition, color, and other sensorial characteristics (such as astringency) in relation to different conditions, such as storage temperature, presence of oxygen, nature and concentration of phenolic compounds, etc. (Andrade et al., 1988; Macheix et al., 1991; Soleas et al., 1997; Gao et al., 1997); however no information was found on the evaluation of the free radical scavenging capacity of wine during this process. By this, the objective of this work was to study the free radical scavenging capacity and main physicochemical properties during the aging of selected red Spanish wines.

MATERIALS AND METHODS

Wines. Red wines [joven or young (Y), crianza (C), and reserva (R)] made from *Vitis vinifera* L. cv. Tempranillo, obtained from four commercial Spanish wineries participating in different Appellation of Origin programs are listed in Table 1.

Polyphenols Analysis by HPLC. Red wine samples were diluted 1:5 with Milli-Q water and filtered through a 0.22 μm filter, and 50 μL aliquots of filtrate were injected. Polyphenols were analyzed by HPLC on a two column system composed of a C-18 (10 × 3 mm) plus LiChrospher 100 RP-18 (4 × 4 mm, 5 μm, Hewlett-Packard) guard columns, a Hypersil BDS-C18 (125 × 3 mm, 3 μm, Hewlett-Packard), and Nucleosil 120 C-18 (250 × 4.6 mm, 5 μm particle size, Tracer Analítica, Madrid, Spain) analytical columns. The columns were eluted in gradient with acetonitrile containing 20% acetic acid (solvent A) and Milli-Q water acidified with acetic acid at pH 2.65 (solvent B), from an initial 25% A:75% B (v/v) ratio to a final A ratio of 100%, with a flow rate of 0.5 mL/min, in 50 min at 45 °C. A diode array detector was set at three wavelengths simultaneously: 280 nm (phenols), 350 nm (flavonoids), and 525 nm

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Table 1. Selected Red Spanish Wines

winery	appellation of origin program	year	samples[a]
1	vinos de Madrid	1996	1Y
		1993	1C
		1992	1R
2	Rioja	1996	2Y
		1994	2C
		1991	2R
3	Ribera del Duero	1996	3Y
		1994	3C
		1992	3R
4	Valdepeñas	1997	4Y
		1995	4C
		1993	4R

[a] Y, joven; C, crianza; R, reserva.

(anthocyanins). Ultraviolet−visible spectra were recorded over the range 200−600 nm. Polyphenols were identified by comparison of retention times and spectra with known standards and quantified by reference of peak areas to the corresponding standard curves.

Some standard polyphenols were purchased from Sigma Chemicals Co. (St. Louis, MO): tannic, gallic, 3,4-dihydroxybenzoic, caffeic, vanillic, syringic, *trans*-*p*-cumaric, synapinic, ferulic, salicylic, and *trans*-cinnamic acids and arbutin, myricetin, quercetin, and catechin. Cyanidin, delphinidin, and oenin chloride were obtained from Extrasynthese (Genay Cedex, France). In DPPH• assay only those standards identified in the samples by HPLC were tested.

Color Measurement. A Tristimulus reflectance Colorimeter (HunterLab, model D25) calibrated with a white standard tile ($X = 82.45$; $Y = 84.46$; $Z = 101.44$) was used. Samples were placed in Petri dishes and color was recorded using the CIE-L^*, a^*, b^* uniform color space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (−) to red (+) axis, and b^* indicates hue on a blue (−) to yellow (+) axis. Two CIE-Lab values were used to express the sample extracts color: hue angle $H° = (\tan b^*/a^*)^{-1}$ and saturation (or chroma) $S = (a^{*2} + b^{*2})^{0.5}$.

UV−vis Spectra. Wines were diluted 1:30 with ethanol/water 50:50 (v/v) and filtered through Whatman No. 41 filter paper, and pH < 2 was adjusted with a few drops of 35% hydrochloric acid (Panreac Química SA, Spain). Spectra were recorded in a DU-640 Beckman spectrophotometer over the range 250−600 nm at 1200 nm/min using ethanol/water as a blank reference.

Free Radical Scavenging Method. The DPPH• method reported by Brand-Williams et al. (1995) and modified in our laboratory (Sánchez-Moreno et al., 1998) was used. Briefly, 0.1 mL of appropriately diluted wine was added to 3.9 mL of DPPH• (0.025 g/L) in methanol. A DU-640 Beckman spectrophotometer with 6 cell holders was used to measure the absorbance at 515 nm at 0.25 min intervals until the reaction reached a plateau (time at the steady state). Wine concentration, expressed as grams of dry sample per gram of DPPH• in the reaction medium, ranged from 0.44 to 4.42.

DPPH• concentration in the medium at different time intervals (t) was calculated from the following calibration curve:

$$A_{515\ nm} = 2935.68[DPPH^•]_t - 2.18 \times 10^{-3}$$

where $[DPPH^•]_t$ was expressed as grams per liter and correlation coefficient (r) = 0.999.

The percentage of remaining DPPH• (% $DPPH^•_{REM}$) at the steady state was calculated as follows:

$$\%\ DPPH^•_{REM} = \{[DPPH^•]_t/[DPPH^•]_{t=0}\} \times 100$$

The antiradical efficiency (AE) of the wines was calculated according to a previous procedure: $AE = 1/EC_{50}T_{EC50}$.

The amount of sample needed to decrease by 50% the initial DPPH• concentration (EC_{50}) and the time needed to reach the steady state to EC_{50} concentration (T_{EC50}) were calculated graphically (Sánchez-Moreno et al., 1998).

Table 2. Tristimulus Measurement of Color and Spectral Absorbance 420/520 nm Ratio from Red Wine Samples

	CIELAB color parameters			
wine samples[a]	lightness (L^*)	hue angle (deg)	saturation (S)	spectra ($A_{420/520nm}$)
1Y	6.7 ± 0.9	15.7 ± 1.4	9.8 ± 0.7	0.39
1C	4.4 ± 0.3	24.8 ± 1.9	9.4 ± 0.5	0.59
1R	5.1 ± 0.4	21.9 ±1.8	9.7 ± 0.6	0.61
2Y	5.6 ± 0.6	11.7 ± 0.8	11.0 ± 0.8	0.24
2C	4.0 ± 0.4	14.9 ± 0.9	9.4 ± 0.4	0.34
2R	4.0 ± 0.5	16.1 ± 1.0	9.4 ± 0.5	0.36
3Y	4.2 ± 0.4	16.4 ± 0.9	8.7 ± 0.4	0.33
3C	4.0 ± 0.5	14.7 ± 1.1	8.9 ± 0.5	0.54
3R	3.7 ± 0.4	22.3 ± 1.7	7.9 ± 0.4	0.67
4Y	9.9 ± 0.5	28.4 ± 2.0	7.8 ± 0.4	0.39
4C	6.5 ± 0.5	35.8 ± 2.3	10.1 ± 0.7	0.56
4R	8.3 ± 0.4	38.5 ± 2.5	9.4 ± 0.6	0.63

[a] Y, joven; C, crianza; R, reserva.

Statistical Analysis. Results were processed by the following computer programs: Excel 97 and Statgraphics Plus ver. 2.1.

RESULTS AND DISCUSSION

The aging of red wines was accompanied by a remarkable change in color (Table 2), mainly by a decrease in lightness (L^*) and an increase in hue angle values (losses in the red color). The saturation values (S) were between 7.8 and 11.0, and they were not related to wine aging. Bakker et al. (1986) found a similar behavior in port wines during aging.

There were the following correlations between the tristimulus parameters of wines tested: $L^* = -0.64 + 50.83/a^*$, $r = 0.667$*; $L^* = 1.19 + 1.25b^*$, $r = 0.863$**; $L^* = 2.02 + 0.15$H, $r = 0.867$**; $S = 1/[0.16 - 0.0068a^*]$, $r = -0.868$**, where the significance level was $p < 0.05$ (*) and $p < 0.01$ (**). These correlations indicate that redness is the most important color of the wines and they agree with other works (Bakker et al., 1986; Gómez-Cordovés and González-San José, 1995), meaning that in aging, red wine becomes darker (lower L^* value) with a decrease in the red component (higher hue angle).

The ratio of absorbance at 420 to 520 nm ($A_{420/520}$) was defined as the "tint or nuance" of the wines (Sudraud, 1958; Bakker et al., 1986). Young wines have a large absorbance peak at 520−535 nm, and as the wines age, the absorbance decreases in this region, increasing that in the yellow/brown region at 400−420 nm. By this, the $A_{420/520}$ ratio increased during aging in all of the red wines tested (Table 2). This behavior is characteristic in the aging process of red wines (Bakker et al., 1986; Macheix et al., 1991; Brouillard and Dangles, 1994; Gómez-Cordovés and González-San José, 1995). Figure 1 illustrates an example of the spectra of wines (Y, C, and R) from winery 2, and a decrease of absorbances at 520−535 nm (anthocyanins) and an increase in the region of 280 nm (phenols) during red wine aging were noted.

Phenolics, especially anthocyanins, are the main compounds responsible for the color changes in red wines (Gómez-Cordovés and González-San José, 1995). Table 3 shows that the main polyphenolics in the wines tested were tentatively identified as tannic acid, oenin, and gallic acid; tannic acid was the major compound identified. Another unidentified component, which was present

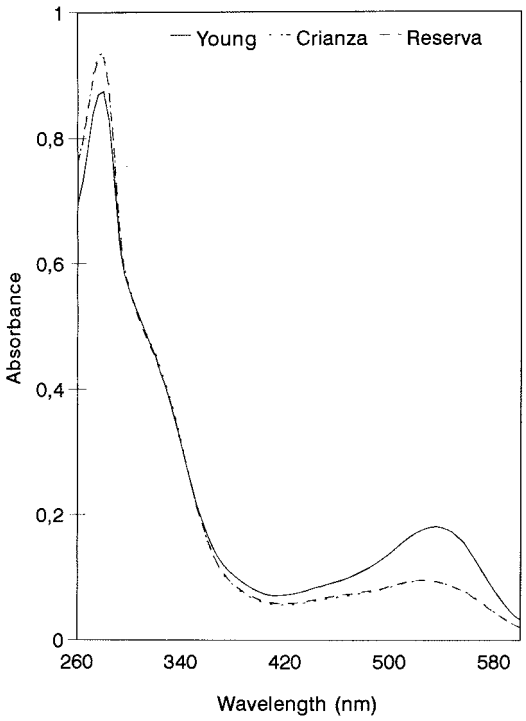

Figure 1. UV−vis spectra of red wines with different aging time: Y = young; C = crianza; R = reserva.

Table 3. Concentration of Main Polyphenolic Constituents (mg/L) of Red Wine Samples As Determined by HPLC

wine samples[a]	tannic acid	gallic acid	oenin
1Y	250.5	36.7	35.9
1C	378.6	*b*	4.6
1R	403.9	*b*	9.9
2Y	372.8	*b*	92.9
2C	438.2	1.01	46.5
2R	323.4	1.1	47.1
3Y	525.0	3.6	69.4
3C	597.2	3.2	12.9
3R	619.4	1.3	7.6
4Y	298.2	*b*	19.2
4C	528.1	*b*	12.3
4R	524.4	*b*	15.0

[a] Y, joven; C, crianza; R, reserva. [b] Not detected.

in higher concentrations in wines from winery 1 than in those from the rest of wineries, was also detected.

The concentration of tannic acid increased during aging, while that for oenin decreased. This fact is in agreement with the previous spectral data and may be explained by the breakdown of hydrolyzable tannins and the degradation of lignins in wood, which increases the levels of benzoic acid, cinnamic, and benzoic aldehyde derivatives (Soleas et al., 1997). On the other hand, different oxidation, condensation, and polymerization reactions during red wine aging produce the color changes in the aged wines and a gradual transition from monomeric anthocyanins through oligomers to the more stable polymeric pigments, leading by losses of anthocyanin compounds such as oenin (Macheix et al., 1991; Brouillard and Dangles, 1994; Mazza, 1995).

The content of anthocyanins (oenin) in Crianza wines varied from 4.6 to 46.5 mg/L and was close to that reported by Bakker et al. (1986), 10−39 mg/L; nevertheless, this fact is influenced by different factors such as winemaking regions, ecology, and varietal conditions (McCloskey and Yengoyan, 1981).

Table 4. Free Radical Scavenging Parameters of Wine Samples and Main Polyphenolic Constituents

samples[a]	EC_{50} (g/g of DPPH•)[b]	T_{EC50} (min)[c]	AE × 10^{-3} [d]
1Y	2.45 ± 0.02	39.9 ± 2.5	10.2 ± 0.6
1C	2.31 ± 0.08	31.5 ± 2.3	13.7 ± 0.4
1R	2.29 ± 0.01	32.5 ± 1.2	13.5 ± 0.4
2Y	1.53 ± 0.07	31.5 ± 1.3	20.7 ± 1.1
2C	1.36 ± 0.03	28.0 ± 1.4	26.3 ± 1.9
2R	1.41 ± 0.01	26.0 ± 1.9	27.2 ± 2.2
3Y	1.26 ± 0.08	31.0 ± 1.4	25.5 ± 2.6
3C	1.16 ± 0.02	30.0 ± 1.4	28.8 ± 0.7
3R	1.30 ± 0.07	26.3 ± 0.7	29.1 ± 1.5
4Y	1.74 ± 0.04	29.8 ± 1.1	19.3 ± 0.6
4C	1.23 ± 0.00	30.5 ± 1.2	26.6 ± 1.3
4R	1.03 ± 0.04	32.2 ± 1.0	30.0 ± 1.1
standards			
tannic acid	0.059 ± 0.003	29.6 ± 1.6	571.6 ± 30.2
oenin	0.334 ± 0.005	20.3 ± 0.9	147.7 ± 8.9
gallic acid	0.026 ± 0.001	14.7 ± 1.1	2628.2 ± 130.1

[a] Y, joven; C, crianza; R, reserva. [b] EC_{50}, amount of sample needed to decrease by 50% the initial DPPH• concentration. [c] T_{EC50}, time needed to reach the steady state at EC_{50} concentration. [d] AE, antiradical efficiency.

The amount of wine sample necessary to decrease by 50% the initial DPPH• concentration (EC_{50}) decreased between young (Y) and crianza (C) wines (Table 4), meaning a higher antioxidant activity (AA) in crianza wines. Older wines (R) did not increase their AA compared to those of crianza wines. The times at the steady state of the samples corresponding to EC_{50} concentration (T_{EC50}) were between 25 and 40 min, and they can be classified as "slow", taking into account a previous kinetic classification proposed for some polyphenolic compounds (Sánchez-Moreno et al., 1998).

The antiradical efficiency (AE) is a new parameter for the measurement the free radical scavenging of samples, and it combines the potency ($1/EC_{50}$) and the reaction time (T_{EC50}) (Sánchez-Moreno et al., 1998). The aged wines (C and R) had more ability for scavenging free radicals than the young (Y) wines (higher AE). These differences may be explained by the increase in tannic acid content with the aging process, because this polyphenol had higher AE than oenin. This fact may be explained by the following correlationship:

$$EC_{50} = 1/(0.18 + 0.0011[\text{tannic acid}]_{mg/L}) r = 0.744^{**}$$

which means that an increase in the tannic acid content as the wines were aging decreased their EC_{50} and consequently increased the AE of the wines.

In summary, a decrease of the anthocyanin and an increase of the phenol content were observed during the aging of red wines, and the increase of the tannic acid could be related to a higher antioxidant efficiency of the aged wines, compared to the young wines. Nevertheless, besides polyphenols, there are many other bioactive compounds such as vitamins and minerals (Brun, 1995), as well as their synergistic effect, which also may be related to the free radical scavenging capacity of red wines.

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