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Influence of enological practices on the antioxidant activity of wines

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Abstract

Wine is a rich source of dietary antioxidants due to its content of phenolic compounds. The purpose of this work is to evaluate the impact of certain enological practices on antioxidant activity of wines. Enological practices included maceration for red wines, pressing degree for white wines, clarification in both types of wines using either albumin or gelatin and membrane filtration. As there is not yet a standard method accepted for the evaluation of the antioxidant activity of wines, the following were adapted and applied, oxygen radical absorbance capacity (ORAC), 2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). As pressure increased, the antioxidant activity of white wines also increased. Maceration time had a positive effect on antioxidant potential of red wines, and behaviour differed, depending on the grape variety (Tempranillo, Syrah, Cabernet Sauvignon). Clarification treatments did not significantly affect the phenolic composition or the antioxidant activity of wines. The information yielded can be used to obtain wines with maximum antioxidant capacity.

Keywords: Antioxidant activity; ORAC; ABTS; DPPH; Wines; Maceration; Filtration; Clarification; Pressing

1. Introduction

Polyphenols of wine have been extensively studied in relation to their protective action in the organism against cardiovascular and degenerative diseases (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Soleas, Diamandis, & Goldberg, 1997). Their chemical structure enables them to scavenge and neutralize free radicals (Kanner, Frankel, Granit, German, & Kinsella, 1994) and they have been demonstrated to inhibit LDL oxidation (Frankel, Kanner, German, Parks, & Kinsella, 1993). They also reduce platelet aggregation and have antiinflamatory properties, acting the eicosanoid metabolism (Laughton, Evans, Hoult, & Halliwell, 1991), and modulate nitric oxide production, which promotes vascular relaxation (Fitzpatrick, Hirschfield, & Coffey, 1993) and have even shown growth-inhibitory effects in cancer cells (Yeh, Herenyiora, & Weber, 1995).

Several studies have been performed on the effect of enological practices on the volatile fraction and phenolic content of wines (Gerbaux, Vincent, & Bertrand, 2002; Poussier, Guilloux-Benatier, Torres, Heras, & Adrian, 2003) and on their impact in the antioxidant activity of wines (Burns et al., 2001; Larrauri, Sánchez-Moreno, Rupérez, & Saura-Calixto, 1999; Netzel et al., 2003). Antioxidant activity of phenolic compounds depends on their chemical structure; hence it is worth studying how the enological practices affect each class of phenolic compounds.

Phenolic composition of white wines can be affected by pressing (Somers & Pocok, 1991). Studies on red wine maceration have also been conducted (Kovac, Alonso, Bourzeix, & Revilla, 1992; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001). These showed that the combination of mass heating and fermentation on skins yielded higher amounts of flavanols, anthocyanins and stilbenes in red wines than with traditional fermentation, as well as an enhanced antioxidant activity as estimated by the ABTS method (Netzel et al., 2003).

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Clarification techniques also affect wine quality. Fining agents are used in order to improve the final appearance of wines and avoid the browning process typical of Sherry and white wines. They have been shown to reduce the phenolic levels of wines (Barón, Mayén, Merida, & Medina, 2000; Paquay, Haenen, Korthouwer, & Bast, 1997). Bentonite has a higher impact on the monomeric than on the polymeric anthocyanins (Bravo-Haro, Rivas-Gonzalo, & Santos-Buelga, 1991) and it is used to reduce the protein content of wines (Main & Morris, 1991). Gelatin provokes small decreases in colour intensity and total phenolic content of red wines (Versari et al., 1998). This effect has also been described with the use of bentonite (Gómez-Plaza, Gil-Muñoz, López-Roca, Cutillas-Martínez, & Fernández-Fernández, 2002). The combined used of activated charcoal, gelatin and bentonite decreased the hydroxycinnamic acid concentrations of Sherry wines by 13–34% (López, Castro, García, Pazo, & Barroso, 2001).

Filtration is a final treatment applied to obtain a limpid wine without organoleptic alterations. Membrane filtration permits clarification, filtration and sterilization in one single step. In practice, both fining and filtration treatments are used in combination.

The objective of the present study is to evaluate the influence of different enological practices on the antioxidant activity and phenolic content of wines. For this purpose, we have studied the techniques commonly used in wine cellars: maceration for red wines, pressing degree for white wines, and clarification, using either albumin or gelatin and membrane filtration, as the final step of production. As the analytical methods for evaluating antioxidant action provide different results, several methods of assessing antioxidant activity of wines are needed (Frankel & Meyer, 2000). In this sense, we have applied three methods commonly used for the measurement of antioxidant activity of foods, ABTS, ORAC and DPPH methods, which have proved to be suitable for the analysis of red, white and Sherry wines (Fernández-Pachón, Villaño, García-Parrilla, & Troncoso, 2004; Villaño, Fernández-Pachón, Troncoso, & García-Parrilla, 2004).

2. Materials and methods

2.1. Chemicals

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in the crystallized diammonium salt form, horseradish peroxidase type VI-A, hydrogen peroxide (30%, v/v), 1,1-diphenyl-2-picrylhydrazyl (DPPH) in free radical form, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and β-phycoerythrin (β-PE), from *Porphydium cruentum*, were obtained from Sigma–Aldrich Quimica (Alcobendas, Spain). Methanol, glycine, ethanol and hydrochloric acid (32%) were provided by Merck (Mollet del Vallés, Spain). 2,2′-Azo-bis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals. Double-distilled water (Millipore Co.) was used throughout. Fining agents (porcine gelatin, ovoalbumin and bentonite) in desiccated form were provided by a wine producer (González Byass, Jerez de la Frontera, Spain) who also provided wine samples. Standards of phenolic compounds were purchased from Sigma, Aldrich and Merck.

2.2. Samples

A total of 35 wines in different stages of production were analysed:

(a) 8 white wines *var.* Palomino, D.O. Jerez-Xères-Sherry, (4 corresponding to vintage 2002 and 4 from vintage 2003) of different degree of pressing, 2 "Yema" (Y) (non-pressed), 2 light press (LP), 2 medium press (MP) and 2 high press (HP).
(b) 27 monovarietal red wine samples: 8 samples *var.* Cabernet Sauvignon (2002), 9 samples from *var.* Tempranillo (2003) and 10 samples from *var.* Syrah (2003) taken at different days of the maceration-fermentation industrial process.

White wine samples were from different degrees of pressing and fulfilled the specifications of the Denomination of Origin Jerez-Xères-Sherry. In the first stages, a pneumatic horizontal press is used, thus minimizing the contact with the solid parts of the grape, which allows wineries to obtain the so called "Yema" wine (Y). Light (LP) and medium press (MP) wines are collected as pressure increases. High press wine (HP) is obtained with a conventional continuous press that reaches maximum pressures.

Vinification of red grape varieties was carried out in stainless steel tanks and samples were collected on different days during the maceration and fermentation process. A traditional maceration with addition of SO_2 was performed. The wine was pumped over the cap twice a day. The initial fermentation temperature was 22 °C and it was controlled to a maximum of 28 °C. No pectolytic enzymes were added.

Clarification was performed on a laboratory scale similarly to the process carried out in the wine cellar. Clarified samples corresponded to two red wines of 2003 (Tempranillo and Syrah) and 4 white Palomino wines (different pressing degrees). The fining agents, commonly used in the wine industry, were albumin and gelatin, at concentrations of 5 g compound hl^{-1} wine. Bentonite (40 g hl^{-1}), as a stabilization step, was finally added.

These products were first dissolved in water, at concentrations of 10 l kg^{-1} for albumin and gelatin and 15 l kg^{-1} for bentonite. 200 ml of wine sample were clarified with albumin and 200 ml of the same wine with gelatin, for comparison purposes, leaving them 1 day to act. Afterwards, bentonite was added and left standing for at least 1 more day, to obtain a properly clarified and limpid wine. Finally, the wine was filtered through a No. 1 Whatman paper and its antioxidant activity determined by the methods described.

2.3. Filtration

All samples clarified were divided into two equal parts and one of them was filtered with Durapore® (Millipore, Co.) membrane filters. These are hydrophilic membranes of polyvinylidene fluoride, with a pore size of 0.45 μm.

2.4. Instrumental

Absorbance measurements were recorded on a UV-2800® Spectrophotometer (Hitachi), thermostatized with a Peltier system at 25 °C. Fluorimetric measurements were recorded in a F-2500 Hitachi Fluorometer thermostatted at 37 °C.

2.5. ABTS method

Antioxidant activity was determined using the ABTS method described by Cano, Hernández-Ruíz, García-Cánovas, Acosta, and Arnao (1998), with some modifications for the special case of wine samples (Villaño et al., 2004). The radical is generated by an enzymatic system between 1.5 mM ABTS, 15 μM hydrogen peroxide and 0.25 μM peroxidase in 50 mM glycine–HCl buffer (pH 4.5). The reaction yields 30 μM of the $ABTS^{\cdot+}$ radical cation (final concentration). These concentrations had to be checked by measuring their absorbances and using their molar extinction coefficients. Glycine–HCl buffer was used as blank.

Once the radical was formed, 0.1 ml of test sample was added to 2 ml of $ABTS^{\cdot+}$ radical cation and absorbance at 414 nm was measured for 15 min. All measurements were performed in duplicate. Standard trolox solutions (40–200 μM) were also evaluated against the radical in order to obtain a calibration curve.

Results were expressed as trolox equivalent antioxidant capacity (TEAC). The TEAC value of a wine expresses the concentration of a trolox solution whose antioxidant activity is identical to that of the wine itself. It is obtained by interpolating the decrease in absorbance (corresponding to a diluted wine sample) on the calibration curve, thus obtaining a concentration of trolox. TEAC values were obtained at 2 (rapid) and 15 min (total reaction). Appropriate corrections were made, taking into account the dilution (Van den Berg, Haenen, Van den Berg, & Bast, 1999; Villaño et al., 2004).

2.6. DPPH method

The procedure used is described by Sánchez-Moreno, Larrauri, and Saura-Calixto (1998). Briefly, 0.1 ml of different sample concentrations were added to 3.9 ml of $DPPH^{\cdot}$ methanolic solution (25 mg l^{-1}). Absorbance at 515 nm was measured at different time intervals until the reaction reached a plateau. The blank reference cuvette contained methanol. All measurements were performed in duplicate. TEAC values were obtained at the steady state, as previously reported for the ABTS method.

In both the ABTS and the DPPH methods, dilutions were prepared in 15% ethanol aqueous solution and were selected depending on the TPI of the wine (Villaño et al., 2004). The radical was prepared daily and protected from the light. Absorbance was recorded to check the stability of the radical throughout the time of analysis. A linear relationship between radical concentration and absorbance was obtained.

2.7. Oxygen radical absorbance capacity assay (ORAC)

This method (Cao & Prior, 1999) was briefly as follows: 150 μl of the diluted wine were mixed with 150 μl (68 mg l^{-1}) of β-phycoerythrin and 75 μl AAPH (160 mM). Fluorescence was recorded at 37 °C for 60 min until the final value was less than 5% of the initial value (λ_{ex} = 540 nm; λ_{em} = 565 nm). 150 μl phosphate buffer saline (PBS) (75 mM, pH 7) were used as blank and a trolox solution (20 μM) was used as standard. Results were calculated as ORAC values using the differences of areas under the β-PE decay curve between the blank and the sample and expressed as trolox equivalents (μM). Trolox fluorescence decay curves were registered for every new solution of β-PE. Wine samples were dealcoholized under vacuum at 38° in order to avoid interferences with ethanol (Fernández-Pachón et al., 2004). PBS was added to reconstitute the dealcoholized wine sample to reach the initial volume. White wines were diluted 1:100 and red wines 1:500 to achieve an adequate response.

2.8. Total phenol index

Total phenol index (TPI) was determined by the Folin–Ciocalteau method (Singleton & Rossi, 1965) and results were expressed as gallic acid equivalents (GAE, mg l^{-1}).

2.9. Liquid chromatographic analysis of phenolic compounds

The phenolic composition of white wines was determined by high performance liquid chromatography (HPLC), as already described (Betés-Saura, Andrés-Lacueva, & Lamuela-Raventós, 1996). Samples were filtered through a Millex-GV13 0.22 μm filter before injection. Volume injected was 50 μl. The column was a Merck Superspher 100 RP-18 (250 × 4 mm) protected by a guard cartridge Nova-Pak C_{18} module (Waters). The flow rate was 1.5 ml min^{-1} and the temperature was set at 40 °C. The mobile phase consisted of two solvents: A (acetic acid in water adjusted to pH 2.65) and B (20% A + 80% acetonitrile) programmed as follows: $t = 0$ 100% A; $t = 35'$ 70% A, 30% B; $t = 40'$ 100% B. Duplicate analyses were performed for each sample. The identification was carried out by retention time and spectra matching, while quantification was performed by external calibration with standards.

2.10. Statistical analysis

Analysis of variance and linear correlations tests were performed using the STATISTICA'99® version software package.

3. Results and discussion

3.1. Pressing

Total phenolic content increased during skin contact and pressing, reflecting the extraction of phenols with the increasing pressure. The increase is up to 14-fold when comparing yema and high press wines from the same year (2002) (Table 1).

Antioxidant activity increased to a larger extent (55-fold with $ABTS_{2\ min}$ method, 35 with DPPH and 11 with ORAC) than total phenolic content. The ABTS method was the most sensitive for evaluating the pressing effect.

Yema and light press wines have values similar to commercial finished white and Sherry wines whilst medium press and high press wines are comparable to rosé and red wines (Landrault et al., 2001). We have not found significant differences from the values previously reported, either in TPI or in antioxidant activity (Fernández-Pachón et al., 2004).

To investigate the influence of pressing on the phenolic content of white wines, samples were analysed by HPLC and a total of 15 polyphenolic compounds have been identified. Fig. 1 shows the chromatogram obtained at 280 nm for sample MP03.

As expected, phenolic acids with a C6–C3 chemical structure are the most abundant in all the samples from both vintages. Grape pulp is rich in hydrosoluble phenolic acids (hydroxybenzoic and hydroxycinnamic) that are extracted with the juice during pressing. Gallic acid is the only benzoic acid detected, with a maximum concentration of 89.2 mg l^{-1}, in HP02 (Table 2). The most abundant compound in yema and light press wines is caftaric acid, which is also present at high levels in all the samples analyzed (up to 203 mg l^{-1}, in HP02). Tartaric derivatives from *p*-coumaric acid (*p*-coumaroyltartaric acid and its glucoside) are also important. As can be seen in Table 2, concentrations generally increase with the degree of pressing. *p*-Coumaric acid, in the free form, is present at very low levels compared with the ester derivatives. This also occurs for caffeic acid. Ferulic acid is only present in Y02 and at a low level (0.2 mg l^{-1}). Phenolic acids and derivatives increase in concentration as pressing degree increases. Hence, the levels of hydroxycinnamic acids in press wines are similar to those reported for red wines (Arnous, Makris, & Kefalas, 2001). If they were the only phenols involved in the antioxidant activity, these values would be expected to be 4 times higher. However, a marked increase in flavan-3-ols takes place (Table 3). Flavan-3-ols, such as (−)-epicatechin, procyanidins B1 and B2 and (−)-epigallocatechin gallate, are present in medium and high press wines. Procyanidin B1 is the most abundant flavanol, ranging from 8.7 to 89.3 mg l^{-1}. These compounds can explain the high values obtained for antioxidant activity.

3.2. Maceration

During 14 days of maceration, total phenol content increased progressively, reflecting the extraction of phe-

Table 1
Effect of pressing: TPI and antioxidant activity values of white wines (*var.* Palomino)

Sample[a]	TPI gallic acid equiv. (mg l^{-1})	ORAC (μM)	TEAC $ABTS_{2\ min}$ (mM)	TEAC $ABTS_{15\ min}$ (mM)	TEAC DPPH (mM)
Y02	230	800 ± 61	0.18 ± 0.01	0.27 ± 0.01	0.51 ± 0.05
LP02	344	1418 ± 114	0.38 ± 0.04	0.54 ± 0.01	0.97 ± 0.05
MP02	837	3111 ± 122	1.94 ± 0.19	2.55 ± 0.28	3.35 ± 0.18
HP02	3293	8628 ± 1264	9.77 ± 1.42	12.60 ± 1.90	17.90 ± 1.22
Y03	222	1643 ± 25	0.15 ± 0.01	0.21 ± 0.00	0.57 ± 0.03
LP03	348	3931 ± 539	0.46 ± 0.08	0.69 ± 0.04	0.96 ± 0.10
MP03	872	5370 ± 81	2.23 ± 0.00	2.73 ± 0.04	4.02 ± 0.07
HP03	1738	15101 ± 101	5.06 ± 0.26	6.18 ± 0.27	9.00 ± 0.27

[a] Y, Yema wine; LP, light press wine; MP, medium press wine; HP, high press wine; 02, vintage 2002; 03, vintage 2003.

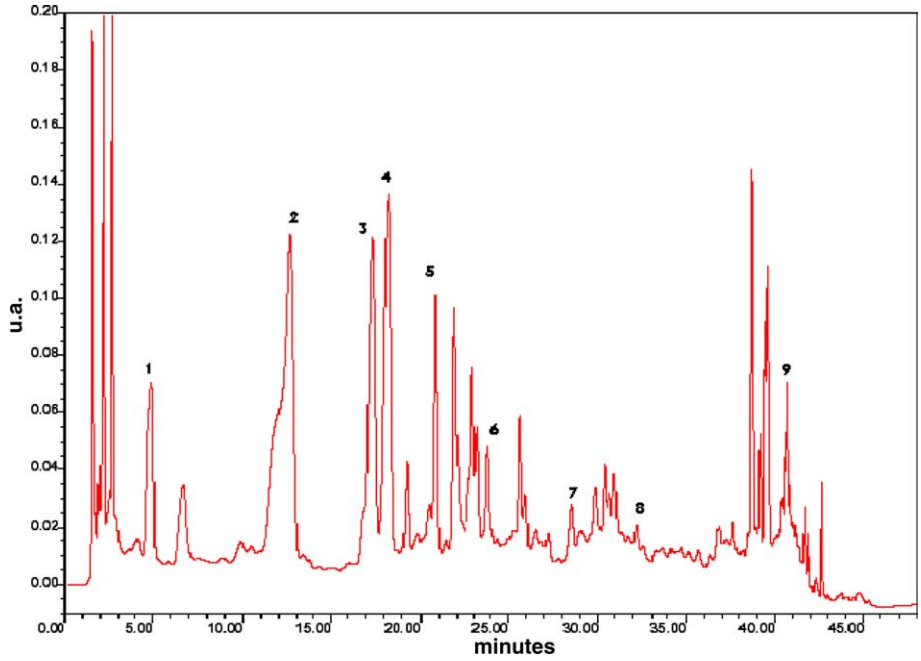

Fig. 1. Chromatogram of white wine MP03 at 280 nm: 1, gallic acid; 2, caftaric acid; 3, coutaric glucoside; 4, coutaric acid; 5, procyanidin B1; 6, procyanidin B2; 7, (−)-epicatechin; 8, tryptophol; 9, quercetin.

Table 2
Effect of pressing, clarification and filtration on the phenolic composition of white wines determined by HPLC: phenolic acids (mg l^{-1})

Sample[a]	Gallic acid	Caftaric acid	Coutaric glucoside	Coutaric acid	Ethyl caffeate	Caffeic acid	*p*-Coumaric acid	Ferulic acid
Palomino 2002								
Y	16.5 ± 0.6	64.7 ± 2.3	9.3 ± 1.1	13.3 ± 0.6	2.5 ± 0.0	1.7 ± 0.1	0.5 ± 0.2	0.2 ± 0.0
LP	4.0 ± 0.4	50.9 ± 2.8	30.7 ± 0.5	15.8 ± 0.2	2.6 ± 0.3	1.2 ± 0.0	0.1 ± 0.1	n.d.
MP	16.6 ± 0.2	124 ± 5.0	51.3 ± 1.9	50.4 ± 1.9	n.d.	5.6 ± 0.8	2.8 ± 0.2	n.d.
HP	89.2 ± 13.5	203 ± 57.1	45.1 ± 0.6	96.5 ± 9.9	n.d.	n.d.	n.d.	n.d.
Palomino 2003								
Y0	0.9 ± 0.0	64.4 ± 0.7	11.6 ± 3.1	7.3 ± 0.2	2.3 ± 0.1	1.6 ± 0.2	0.2 ± 0.0	n.d.
YA	3.3 ± 0.0	66.3 ± 4.2	9.7 ± 0.4	9.9 ± 0.3	2.3 ± 0.2	2.1 ± 0.0	0.4 ± 0.2	n.d.
YG	n.d.	63.4 ± 2.9	10.4 ± 0.7	7.9 ± 0.8	2.3 ± 0.2	2.0 ± 0.1	n.d.	n.d.
YAF	1.5 ± 0.5	65.8 ± 1.6	9.6 ± 0.1	7.8 ± 0.7	2.8 ± 0.3	1.3 ± 0.4	0.3 ± 0.0	n.d.
YGF	0.3 ± 0.0	64.1 ± 1.1	10.9 ± 1.1	8.4 ± 0.6	2.2 ± 0.0	1.9 ± 0.1	0.3 ± 0.0	n.d.
LP0	1.2 ± 0.4	57.4 ± 6.3	24.2 ± 5.4	11.0 ± 1.0	3.1 ± 0.0	1.6 ± 0.0	0.3 ± 0.1	n.d.
LPA	2.8 ± 0.0	52.8 ± 2.7	31.0 ± 1.7	11.8 ± 0.8	3.5 ± 0.0	1.9 ± 0.3	0.5 ± 0.1	0.4 ± 0.0
LPG	3.0 ± 0.1	53.6 ± 1.1	30.4 ± 1.9	12.5 ± 0.0	2.9 ± 0.2	1.9 ± 0.1	0.5 ± 0.0	n.d.
LPAF	1.5 ± 0.2	51.1 ± 0.3	28.0 ± 1.0	11.7 ± 1.1	3.0 ± 0.2	1.9 ± 0.1	0.5 ± 0.0	n.d.
LPGF	2.6 ± 0.7	55.0 ± 1.6	31.3 ± 1.2	13.3 ± 0.5	3.0 ± 0.3	1.8 ± 0.2	0.5 ± 0.0	n.d.
MP0	15.8 ± 1.3	123.7 ± 5.7	48.5 ± 3.3	44.6 ± 5.2	n.d.	n.d.	n.d.	n.d.
MPA	14.4 ± 1.5	113.5 ± 1.5	39.8 ± 0.8	40.7 ± 4.0	n.d.	n.d.	n.d.	n.d.
MPG	16.2 ± 0.3	120.5 ± 7.7	44.1 ± 0.4	44.8 ± 0.8	n.d.	n.d.	n.d.	n.d.
MPAF	13.2 ± 0.1	113.2 ± 0.2	46.0 ± 0.2	45.9 ± 0.0	n.d.	n.d.	n.d.	n.d.
MPGF	14.2 ± 0.6	119.6 ± 0.8	43.8 ± 0.1	40.5 ± 1.3	n.d.	n.d.	1.2 ± 0.0	n.d.
HP0	44.8 ± 3.6	152.2 ± 9.7	46.0 ± 3.0	33.2 ± 2.5	n.d.	5.1 ± 0.0	3.5 ± 0.0	n.d.
HPA	47.1 ± 1.3	145.7 ± 7.1	37.5 ± 4.6	42.1 ± 2.1	n.d.	n.d.	4.1 ± 0.0	n.d.
HPG	45.4 ± 3.5	149.9 ± 7.0	37.7 ± 4.7	35.7 ± 1.5	n.d.	n.d.	n.d.	n.d.
HPAF	43.1 ± 3.2	147.2 ± 4.4	32.2 ± 7.7	40.0 ± 4.4	n.d.	n.d.	4.3 ± 0.0	n.d.
HPGF	47.0 ± 1.7	147.7 ± 4.7	40.6 ± 3.9	41.4 ± 2.9	n.d.	n.d.	n.d.	n.d.

[a] 0, non-clarified; A, albumin; G, gelatin; F, membrane filtered; n.d., not detected.

nolic compounds. Cabernet Sauvignon and Tempranillo wines reached their final values at the 4th day of maceration, whilst Syrah wines increased the content until day 14 (Fig. 2). Maximum values of TPI were achieved at the end of maceration time and varied, depending on the variety of grape (Table 4), although they are in the range

Table 3
Effects of pressing, clarification and filtration on the phenolic composition of white wines determined by HPLC: flavonoids and other compounds (mg l^{-1})

Sample[a]	(−)-Epicatechin	(−)-Epigallocatechin gallate	Procyanidin B1	Procyanidin B2	Quercetin	Tyrosol	Tryptophol
Palomino 2002							
Y	n.d.	n.d.	n.d.	n.d.	n.d.	d.	0.7 ± 0.0
LPM	n.d.	n.d.	8.7 ± 0.6	n.d.	n.d.	5.6 ± 2.3	7.1 ± 0.5
MP	n.d.	27.3 ± 0.0	37.1 ± 0.4	n.d.	2.6 ± 0.0	6.2 ± 4.2	17.1 ± 1.7
HP	n.d.	n.d.	89.3 ± 7.3	172 ± 0.0	3.4 ± 0.1	n.d.	n.d.
Palomino 2003							
Y0	n.d.	n.d.	n.d.	n.d.	n.d.	0.5 ± 2.1	n.d.
YA	n.d.	n.d.	n.d.	n.d.	n.d.	4.1 ± 1.1	1.04 ± 0.0
YG	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
YAF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7 ± 0.0
YGF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LP0	n.d.	n.d.	n.d.	n.d.	n.d.	6.2 ± 1.0	1.9 ± 0.2
LPA	n.d.	1.3 ± 0.0	n.d.	n.d.	1.9 ± 0.0	5.9 ± 2.2	2.2 ± 0.5
LPG	n.d.	n.d.	n.d.	n.d.	1.8 ± 0.0	4.3 ± 0.3	n.d.
LPAF	n.d.	3.7 ± 0.0	n.d.	n.d.	1.9 ± 0.1	9.1 ± 0.9	2.3 ± 0.1
LPGF	n.d.	n.d.	n.d.	n.d.	2.2 ± 0.2	6.9 ± 1.8	2.1 ± 0.0
MP0	5.4 ± 0.0	n.d.	47.7 ± 9.2	14.6 ± 10.6	2.6 ± 0.1	8.5 ± 4.8	1.0 ± 0.0
MPA	n.d.	n.d.	49.0 ± 3.8	n.d.	2.5 ± 0.2	8.8 ± 0.5	n.d.
MPG	n.d.	n.d.	48.8 ± 2.2	15.2 ± 0.0	2.9 ± 0.0	8.5 ± 1.4	n.d.
MPAF	n.d.	n.d.	55.0 ± 8.1	23.1 ± 0.0	2.7 ± 0.1	8.7 ± 0.0	n.d.
MPGF	n.d.	n.d.	52.0 ± 6.4	22.9 ± 1.1	2.9 ± 0.1	7.2 ± 0.5	3.1 ± 0.0
HP0	n.d.	69.6 ± 6.8	79.7 ± 7.0	38.5 ± 1.4	3.2 ± 0.3	9.8 ± 2.9	4.0 ± 0.0
HPA	n.d.	91.1 ± 4.0	95.9 ± 7.5	59.2 ± 7.0	2.8 ± 0.2	13.2 ± 0.5	n.d.
HPG	n.d.	80.1 0.0	89.0 ± 6.2	n.d.	2.7 ± 0.1	14.3 ± 1.4	n.d.
HPAF	n.d.	80.5 ± 0.0	101 ± 4.1	64.5 ± 20.6	2.9 ± 0.1	9.1 ± 0.0	n.d.
HPGF	n.d.	111 ± 42.9	109 ± 7.3	63.0 ± 0.0	2.2 ± 0.1	18.3 ± 0.5	n.d.

[a] 0, non-clarified; A, albumin; G, gelatin; F, membrane-filtered; n.d., not detected; d., detected, not quantified.

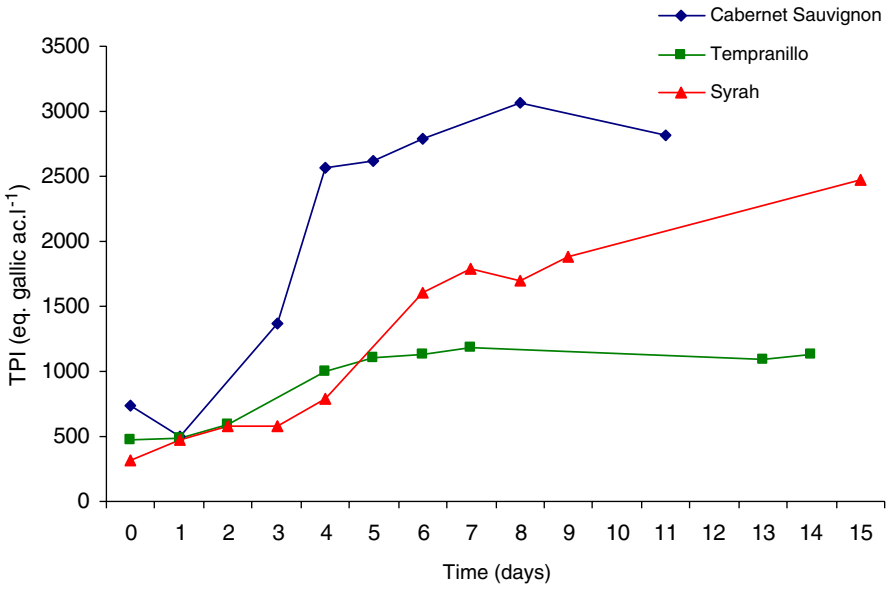

Fig. 2. Effect of maceration on the TPI of red wines.

of finished red wines (Landrault et al., 2001). Notable are the high values of Cabernet Sauvignon throughout all the maceration time, reaching a final TPI of 2813 mg gallic acid equiv l^{-1}.

The three varieties were cultivated in the same area of the south of Spain. Cabernet Sauvignon has perfectly adapted to this sunny and hot climate, while Tempranillo is the most difficult to adapt; this could explain the low phenolic contents observed.

On the other hand, the characteristics of the variety itself are important. Cabernet Sauvignon is characterized by small, thick-skinned grapes, with a high skin:vol-

Table 4
Effects of maceration: TPI and antioxidant activity values of red wines

Days of maceration	TPI gallic acid equiv. (mg l^{-1})	ORAC (μM)	TEAC $ABTS_{2\ min}$ (mM)	TEAC $ABTS_{15\ min}$ (mM)	TEAC DPPH (mM)
Cabernet Sauvignon 2002					
0	734	4290 ± 647	1.07 ± 0.12	1.44 ± 0.19	2.47 ± 0.10
1	500	4708 ± 1309	0.60 ± 0.04	0.82 ± 0.03	1.28 ± 0.31
3	1373	9473 ± 660	3.32 ± 0.10	4.17 ± 0.13	5.80 ± 0.28
4	2561	11051 ± 519	6.73 ± 0.33	8.90 ± 0.50	11.0 ± 0.46
5	2624	12464 ± 463	7.06 ± 0.50	9.43 ± 0.64	11.2 ± 1.07
6	2795	13563 ± 335	8.37 ± 0.38	10.7 ± 0.24	13.2 ± 1.08
8	3065	13130 ± 156	8.37 ± 0.38	10.8 ± 0.39	14.8 ± 0.23
11	2813	11765 ± 1121	7.65 ± 0.55	9.80 ± 0.61	12.3 ± 0.69
Tempranillo 2003					
0	470	3416 ± 448	0.20 ± 0.03	0.27 ± 0.03	1.01 ± 0.10
1	487	6104 ± 1089	0.46 ± 0.05	0.61 ± 0.04	1.37 ± 0.15
2	598	3494 ± 184	0.53 ± 0.24	0.70 ± 0.33	2.15 ± 0.10
4	1000	8275 ± 132	1.61 ± 0.06	1.98 ± 0.13	4.03 ± 0.24
5	1103	8231 ± 8	2.01 ± 0.22	2.50 ± 0.12	4.45 ± 0.27
6	1129	9905 ± 225	2.08 ± 0.18	2.59 ± 0.08	4.96 ± 0.81
7	1188	9878 ± 808	2.55 ± 0.35	2.96 ± 0.39	4.70 ± 0.46
13	1086	9477 ± 110	2.38 ± 0.15	3.10 ± 0.07	4.59 ± 0.45
14	1137	5811 ± 467	2.41 ± 0.12	3.08 ± 0.08	5.08 ± 0.83
Syrah 2003					
0	320	7591 ± 704	0.48 ± 0.03	0.64 ± 0.03	1.35 ± 0.03
1	477	7912 ± 641	0.60 ± 0.02	0.80 ± 0.03	1.74 ± 0.10
2	577	8586 ± 0.00	0.67 ± 0.03	0.90 ± 0.03	1.91 ± 0.17
3	585	7762 ± 979	0.80 ± 0.02	1.13 ± 0.02	2.06 ± 0.08
4	784	10049 ± 231	1.03 ± 0.06	1.50 ± 0.10	2.65 ± 0.12
6	1611	12864 ± 214	3.09 ± 0.06	4.22 ± 0.06	6.30 ± 0.30
7	1785	12469 ± 69	3.86 ± 0.24	5.27 ± 0.28	7.91 ± 0.14
8	1694	13296 ± 506	4.10 ± 0.13	5.49 ± 0.09	7.42 ± 0.22
9	1885	13282 ± 366	4.11 ± 0.32	5.51 ± 0.37	7.54 ± 0.42
15	2472	14294 ± 547	5.72 ± 0.23	7.55 ± 0.23	10.4 ± 1.15

ume ratio and this results in a higher release of phenolic compounds into the wine. Some authors have attributed the high levels of flavonols (Mc Donald et al., 1998), catechins (Pérez-Magariño & González-Sanjosé, 2004) and anthocyanins (Burns et al., 2001) to the high proportion of solid parts of this grape as compared to pulp.

Cabernet Sauvignon and Tempranillo wines showed a similar trend towards antioxidant activity and TPI (Figs. 3–5). It is noteworthy that values obtained for Cabernet Sauvignon, i.e., $ABTS_{2\ min}$ are up to 7-fold higher than the initial one. Wines from this variety of grape have a higher-than-average total phenolic content, vasodilatant properties and antioxidant activity (Burns et al., 2000). It has been hypothesized that this could be due to the higher catechin content found in this variety (Landrault et al., 2001). In contrast, Tempranillo samples show the lowest antioxidant potential of the three varieties analyzed.

Statistical analyses have demonstrated a strong correlation between antioxidant activity and TPI over time (Table 5). The relationship between ORAC and TPI values is closer when the three varieties are considered separately.

Absolute values of TEAC at 2 (rapid) and 15 min (total) of reaction in the ABTS method are different, the increases in antioxidant activity over time are very similar, which confirms the validity of measurements at 2 min of reaction.

The increases in antioxidant activity are different, depending on the method used. The ABTS method is the most sensitive to maceration time. When the capacity for scavenging free radicals is determined by means of the ABTS and DPPH methods, the increases in TEAC values, due to maceration of, e.g., Tempranillo, are 12 and 5 times, respectively. With the ORAC method, the final value was 2-fold the initial one whilst with TPI, it was 2.5 times higher at the end of maceration. This is due to the different reactivities of the polyphenols with each method applied; thus, the relationships between the three methods and the TPI are qualitative and not quantitative.

At day 0 of maceration, wine is mainly composed of phenolic acids from the pulp. This composition and the antioxidant activity are quite similar to those of white wines and there are no statistical differences from works previously reported (Fernández-Pachón et al., 2004). During maceration, major changes occur within the first week and affect to the concentrations of flavan-3-ols and anthocyanins (Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández,

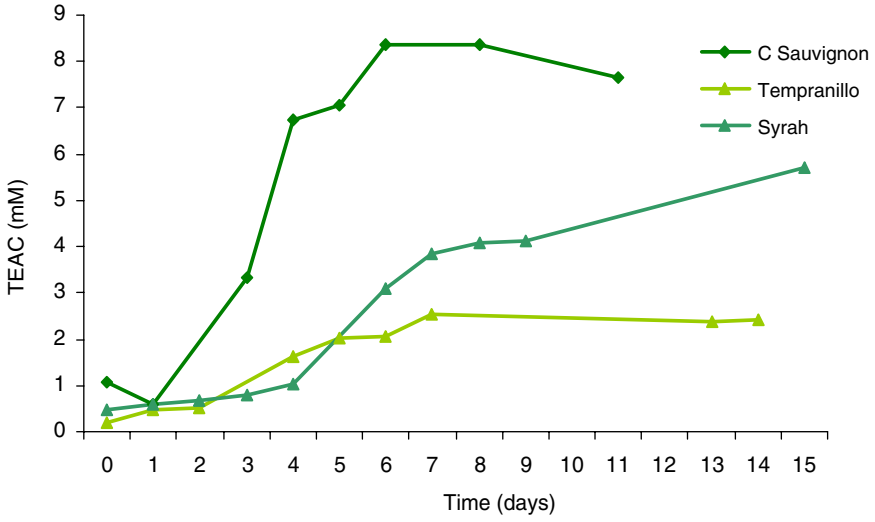

Fig. 3. Effect of maceration on $TEAC_{2\ min}$ values of red wines: ABTS method.

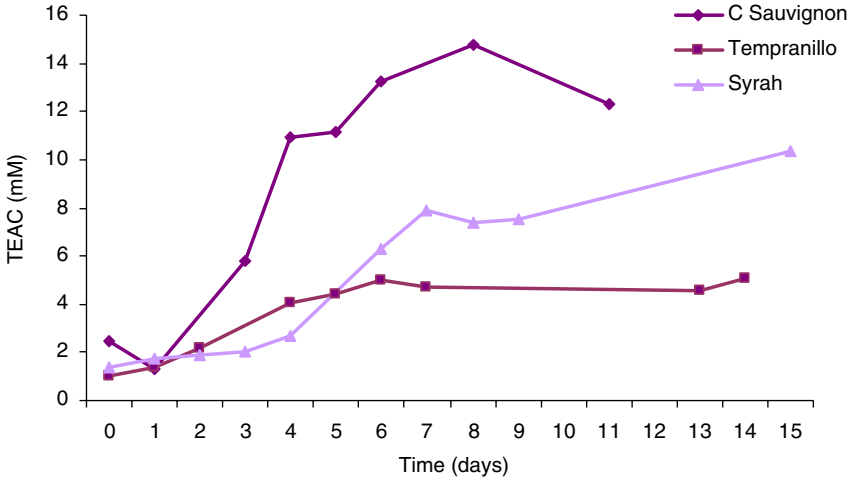

Fig. 4. Effect of maceration on TEAC values of red wines: DPPH method.

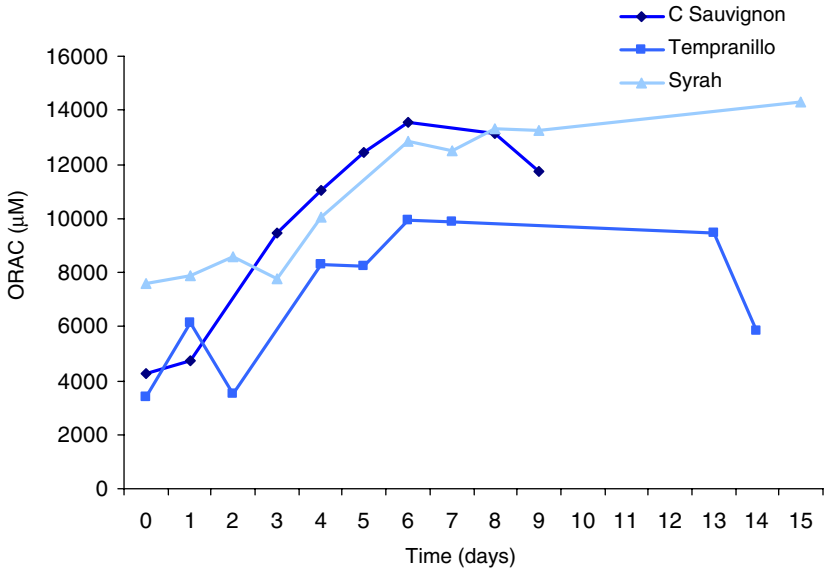

Fig. 5. Effect of maceration on ORAC values of red wines.

Table 5
Correlation coefficients between TPI and antioxidant activity values of red wine samples (*var.* Cabernet Sauvignon, Tempranillo, Syrah)

	TPI	ORAC	$ABTS_{2\ min}$	$ABTS_{15\ min}$	DPPH
TPI	1.0000				
ORAC	0.8019	1.0000			
$ABTS_{2\ min}$	0.9905	0.7740	1.0000		
$ABTS_{15\ min}$	0.9919	0.7816	0.9991	1.0000	
DPPH	0.9949	0.7953	0.9932	0.9929	1.0000

2001, 2002). There is an enrichment of this phenolic fraction, which has proved to be the most potent in terms of antioxidant activity (Arnous et al., 2001; Burns et al., 2001; Simonetti, Pietta, & Testolin, 1997) and is responsible of 50–60% of total antioxidant activity of red wines as estimated by the three methods (Fernández-Pachón et al., 2004). This fact could explain the considerable increase in the antioxidant activity of wine at the end of the maceration period, compared with the non-macerated samples.

3.3. Clarification

Results obtained for Palomino wines, Tempranillo and Syrah, clarified either with albumin or gelatin, are shown in Table 6. Total phenolic contents and antioxidant activities of wines decreased with the fining treatments, both with albumin and gelatin, and in all cases analyzed. However, ANOVA analyses showed no significant differences between clarified and non clarified wines, either in the TPI ($p < 0.9893$) or in the antioxidant activity estimated by ABTS ($p < 0.9953$ at 2 min; $p < 0.9514$ at 15 min), DPPH ($p < 0.9756$) and ORAC methods ($p < 0.7731$). There are no significant differences

Table 6
Effects of clarification and filtration: TPI and antioxidant activity of wines

Sample	TPI (mg gallic acid l^{-1})	ORAC (μM)	$ABTS_{2\ min}$ (mM)	$ABTS_{15\ min}$ (mM)	DPPH (mM)
Tempranillo					
T0	1137	5811 ± 467	2.41 ± 0.12	3.08 ± 0.08	5.08 ± 0.83
TA	984	3907 ± 302	1.84 ± 0.09	2.30 ± 0.06	4.03 ± 0.25
TG	993	4461 ± 51	2.14 ± 0.13	2.83 ± 0.15	4.42 ± 0.20
TAF	963	2924 ± 529	1.79 ± 0.13	2.20 ± 0.14	4.02 ± 0.26
TGF	964	3652 ± 302	2.00 ± 0.06	2.56 ± 0.06	4.10 ± 0.15
Syrah					
S0	2472	14294 ± 547	5.72 ± 0.23	7.55 ± 0.23	10.4 ± 1.15
SA	2258	10755 ± 280	5.39 ± 0.03	7.08 ± 0.06	10.1 ± 0.50
SG	2215	10249 ± 1060	5.42 ± 0.01	7.09 ± 0.01	9.52 ± 0.11
SAF	2241	9495 ± 167	5.27 ± 0.12	6.93 ± 0.15	10.1 ± 0.33
SGF	2156	9860 ± 423	5.20 ± 0.03	6.91 ± 0.10	9.11 ± 0.55
Palomino Yema wine					
Y0	222	1643 ± 25	0.15 ± 0.01	0.21 ± 0.00	0.57 ± 0.03
YA	209	ND	0.13 ± 0.02	0.17 ± 0.02	0.52 ± 0.02
YG	214	ND	0.12 ± 0.02	0.16 ± 0.02	0.51 ± 0.02
YAF	212	1525 ± 134	0.12 ± 0.01	0.18 ± 0.01	0.52 ± 0.03
YGF	207	1365 ± 24	0.12 ± 0.01	0.16 ± 0.01	0.56 ± 0.00
Palomino light press wine					
LP0	348	3931 ± 539	0.53 ± 0.03	0.69 ± 0.04	0.96 ± 0.10
LPA	339	ND	0.48 ± 0.02	0.63 ± 0.02	0.86 ± 0.11
LPG	348	ND	0.46 ± 0.02	0.65 ± 0.01	0.83 ± 0.04
LPAF	338	3001 ± 12	0.43 ± 0.06	0.57 ± 0.00	0.92 ± 0.02
LPGF	344	2619 ± 18	0.45 ± 0.05	0.66 ± 0.06	0.95 ± 0.04
Palomino medium press wine					
MP0	872	5370 ± 81	2.23 ± 0.00	2.73 ± 0.04	4.02 ± 0.07
MPA	838	ND	2.35 ± 0.10	2.24 ± 0.69	3.55 ± 0.18
MPG	847	ND	2.27 ± 0.15	1.69 ± 0.05	3.74 ± 0.13
MPAF	857	5002 ± 58	2.21 ± 0.00	2.72 ± 0.00	3.54 ± 0.07
MPGF	843	5365 ± 93	2.04 ± 0.00	2.45 ± 0.00	3.26 ± 0.16
Palomino high press wine					
P0	1738	15101 ± 101	5.24 ± 0.05	6.18 ± 0.27	9.00 ± 0.27
PA	1646	ND	5.17 ± 0.15	5.27 ± 1.35	8.80 ± 0.11
PG	1629	ND	4.97 ± 0.09	5.47 ± 1.41	8.05 ± 0.79
PAF	1621	11345 ± 18	4.83 ± 0.13	6.01 ± 0.03	8.97 ± 0.14
PGF	1604	13057 ± 302	4.68 ± 0.22	5.50 ± 0.32	8.53 ± 0.05

ND, not determined.

between the two fining agents in all varieties under study.

In order to check if there were differences between the initial wine and those clarified, the concentrations of different phenolic compounds were determined by HPLC. From the results of Tables 2 and 3 and ANOVA analysis it can be concluded that the concentrations of phenolic compounds do not change significantly with the fining treatments.

3.4. Filtration

A slight reduction of the total phenolic content and antioxidant activity, measured by the three methods, can be appreciated after membrane filtration, when comparing with non-treated wine and clarified and non-filtered wines (Table 6). However, there are no statistical differences, due to the filtration, in the TPI ($p < 0.9037$) and antioxidant activity estimated by ABTS ($p < 0.8291$; $p < 0.9666$), DPPH ($p < 0.8994$) and ORAC methods ($p < 0.3514$).

Phenolic composition of samples filtered is shown in Tables 2 and 3. ANOVA analysis shows that it is not significantly affected by the process of filtration.

4. Conclusions

The antioxidant potential of wine increases with the degree of pressing. There is a good correlation between TPI and the antioxidant activity of red wines during maceration and the evolution varies, depending on the variety studied. Fining treatments had only minor effects on phenolic levels in both white wines and red wines.

From the results discussed above, it is difficult to propose the use of other fining agents which may imply an additional cost to the producer. The products analyzed in the present study do not greatly affect the antioxidant potential of wines and their use may not decrease the potential health effects of wine after ingestion.

Membrane filtration can be considered suitable for wine clarification in terms of maintaining its antioxidant activity.

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