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Antioxidant potential of single-variety red wines aged in the barrel and in the bottle

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ABSTRACT

Wine constitutes a dynamic system in continuous evolution, in which numerous reactions involving polymerization and condensation take place between its phenolic compounds during the ageing process, which undoubtedly affect its structure and, very probably, its antioxidant effect. This study set out to evaluate the effect of ageing on the antioxidant potential of wine. A group of 162 wines were studied, of varying ages, which had undergone different ageing processes, both in the barrel and in the bottle, and which were prepared from different grape varieties and vintages. Total antioxidant capacity (ABTS, DPPH, DMPD, ORAC and FRAP), scavenger activity (HRSA and SRSA) and the biomarkers of oxidative stress (DNA-damage and ABAP-LP) were all analysed. The assay methods showed different behaviours for the same wines, thus the young wines presented higher indices for ABTS, DPPH and DMPD, whereas those that were aged showed higher indices for ABAP-LP and ORAC. Finally, the antioxidant potential of the wines in the study appeared not to be influenced by other factors, such as microoxygenation or grape variety.

1. Introduction

The antioxidant potential of wine, clearly demonstrated in a large number of studies, is largely attributable to its phenolic composition (Burns et al., 2000; Cuevas et al., 2000; Iijima et al., 2002), an effect which is mainly due to flavonoid compounds. Thus, in addition to their having a strong involvement in the organoleptic characteristics of wine, affecting colour, bitterness and astringency (Pérez-Magariño & González-Sanjosé, 2004; Thorngate, 1997), these compounds, also play an important role as antioxidants (Sun, Simonyi, & Sun, 2002).

The study of its antioxidant capacity has been carried out by "in vitro" methods (Fernández-Pachón, Villaño, García-Parrilla, & Troncoso, 2004; Ghiselli, Nardini, Baldi, & Scaccini, 1998; Rivero et al., 2005; Rivero-Pérez, Muñiz, & González-Sanjosé, 2007) that use various assays (ABTS, DPPH, ORAC, FRAP, desoxyribose assay, etc) and allow the antioxidant potential of the wine to be measured in a chemically generated system. Methods have also been used that evaluate its effect in "in vivo" systems using experimental models on laboratory animals (Ates et al., 2007; Mokni, Limam, Elkahoui, Amri, & Aouani, 2007; Soulat et al., 2006), and finally through assays on human beings (Albert et al., 1999; De Lorgeril et al., 2002; Mukamal et al., 2003). These latter studies are found to contribute more realistic information on the effects of wine consumption on the organism. Moreover, studies have also been performed on the bioavailability of wine and its components such as isolated phenolic compounds (Baur & Sinclair, 2006; Rahman & Kilty, 2006; Rathel, Samtleben, Vollmar, & Dirsch, 2007).

However, not all phenolic compounds display the same antioxidant activity and the phenolic composition of wine can be strongly affected, not only qualitatively but also quantitatively, by cultivation methods, grape varieties, maturity, enological techniques, and the ageing process (Gómez-Cordovés & González-Sanjosé, 1995; Ortega-Herás, González-Huerta, Herrera, & González-Sanjosé, 2004; Pérez-Magariño & González-Sanjosé, 2006; Revilla & González-Sanjosé, 2001). It is probably during ageing, whether in the barrel or in the bottle, that the greatest number of polymerization and condensation reactions occur, notably modifying the composition of the wine (Cheynier, Hidalgo, Souquet, & Moutounet, 1997; Dallas, Ricardo-Da-Silva, & Laureano, 1995) and its quality attributes.

The main objective of this work consisted in evaluating the effect of ageing on the antioxidant potential of wine. To do so, a study was performed on the total antioxidant capacity, scavenger activity and the effect on biomolecules (lipids and DNA) of Spanish red wines aged over different periods of time.

Abbreviations: ABAP, 2,2′- diazobis-(2-aminodinopropane)-dihydrochloride; ABAP-LP, ABAP lipid peroxidation; ABTS, 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid; DMPD, *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing/antioxidant power; HAT, hydrogen atom transfer; HRSA, hydroxyl radical scavenging activity; MDA, malondialdehyde; NADH, nicotinamide adenine dinucleotide; NBT, 4-nitroblue tetrazolium chloride; PMS, phenazin methosulfate; SET, single electron transfer; SRSA, superoxide radical scavenging activity; TAC, total antioxidant capacity; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TPTZ, 2,4,6-tris (2-pyridyl)-*S*-triazine; TROLOX, 6-hydroxyl-2,5,7,8-tetramethyl-2-carboxylic acid

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2. Materials and methods

2.1. Chemicals

2,2′-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) purity of 98% minimum, 2,2-diphenyl-1-picrylhydrazyl (DPPH·) purity of 95% minimum, *N*,*N*-Dimethyl-*p*-phenylenediamine dihydrochloride (DMPD) purity of 99% minimum, β-phycoerythrin (PE) content of protein 30% (Lowry), 2,2′-diazobis-(2-aminodinopropane)-dihydrochloride (ABAP) purity of 97% minimum, 6-hidroxyl-2,5,7,8-tetramethyl-2-carboxylic acid (TROLOX) purity of minimum 98% (grade HPLC), 2,4,6-tris (2-pyridyl)-*S*-triazine) (TPTZ) purity of 98% minimum, 2-desoxy-D-ribose purity of 99% minimum, phenazin methosulfate (PMS) purity of 90% minimum and calf thymus DNA from Sigma–Aldrich Co. (St. Louis, MO, USA). Potassium persulphate ($K_2O_8S_2$) purity of 99% minimum, iron(III) chloride 6-hydrate ($FeCl_3 \cdot 6H_20$) purity of 99% minimum, iron(II) sulphate 7-hydrate ($FeSO_4 \cdot 7H_20$) purity of 99% minimum, hydrogen peroxide (H_2O_2) purity of 99% minimum, L-ascorbic acid purity of 98% minimum and trichloroacetic acid (TCA) purity of 99% minimum were obtained from Panreac (Barcelona, Spain). Thiobarbituric acid (TBA) purity of 99% minimum was purchased from Merck (Darmastadt, Germany). Bovine serum albumin (BSA) purity of 98% minimum, nicotinamide adenine dinucleotide disodium salt (NADH) purity of 100% grade I and 4-nitroblue tetrazolium chloride (NBT) purity of 98% minimum from Roche (In, USA). TRIS purity of 97% minimum and EDTA tretrasodium salt purity of 98% minimum, from Amresco (Ohio, USA).

2.2. Samples

A total of 162 Spanish red wines of different ages were studied, which were elaborated from *Vitis vinifera* grapes from the four most characteristic red grape varieties cultivated in various wine-making regions of Castilla y Leon with Apellation of Origin (A.O.), which were Mencía (M) from A.O. Bierzo, Tempranillo (T) from A.O. Cigales and Rueda, Tinta del País (TP) from A.O. Ribera del Duero, and Tinta de Toro (TT) from A.O. Toro. Studied wines were made at the experimental winery of the Enological Station of Castilla y Leon, following a traditional vinification process and a series of control and microoxygenated wines were prepared. Microoxygenation was applied between alcoholic and malolactic fermentations and the dosage and application times were adapted to the phenolic composition and the structure of each wine. This study was carried out with wines made from grape harvested from 2002 to 2004.

The total studied wines (n = 162) were from the next categories

1. Young wines, wines no aged in barrels, and analysis before they were six months old. Twenty wines of this category were analyses, 10 from 2003 vintage and 10 from 2004, with a total number of n = 20. These wines were labelled as young or initial.
2. Aged wines, wines which were aged only in barrel or in barrel and after in bottle for varying periods. This group was subdivided in three sub-categories which were: first, wines aged during four months in barrels, labelled as (4 months). Twenty six samples of this group were analysed, (16 from 2003 and 10 from 2004 vintage). The second subcategory was formed with wines aged during 12 months in barrels, labelled as (12 months). Thirty six wines of this type were analysed including 16 wines from 2002, 16 from 2003 and 4 from 2004 respective vintages. The third subcategory included wines aged in barrels during 24 months, labelled as (24 months). Thirty two wines formed this group, 16 from 2002 vintage and 16 from 2003 vintage. The fourth subcategory grouped wines aged during 12 months in barrels and after storing during six months in bottle, these wines were labelled as (12 + 6b). Sixteen samples were analysed from 2003 vintage. The last subcategory included wines of 12 months of ageing in barrels plus 12 months of storage in bottle, these wines were labelled as (12 + 12b). Thirty two samples were considered, 16 wines from 2002 and 16 from 2003 vintages.

All of the assays were repeated four times.

2.3. $ABTS^{\cdot+}$ method

This assay is based on decoloration that occurs when the radical cation $ABTS^{\cdot+}$ is reduced to ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) (Re, Pellegrini, Proteggente, Pannala, & Yang, 1999). The radical was generated by reaction of a 7 mM solution of ABTS in water with 2.45 mM potassium persulphate (1:1). The assay was made up with 980 μL of $ABTS^{\cdot+}$ solutions and 20 μL of the sample (at a dilution of 1:50 in water). The reaction takes place in darkness at room temperature. Absorbance measurements at 734 nm were made after 15 min of reaction time. The results were expressed in mM of Trolox, using the relevant calibration curve.

2.4. DPPH · Method

This method is based on the reduction of the free radical DPPH· (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams, Cuvelier, & Berset, 1995). The reaction takes place when 980 μL of DPPH· (60 μM) was mixed with 20 μL of the sample (at a dilution of 1:50 in water) at room temperature. After a reaction time of two hours absorbance values at 517 nm were measured. Results were expressed in mM of Trolox using the dose-response curve described by this substance.

2.5. $DMPD^{\cdot+}$ method

The method described by Fogliano, Verde, Randazzo, and Ritieni (1999) was applied. 1 mL of DMPD solution (200 mM) and 0.4 mL of ferric chloride(III) (0.05 M) were mixed with sodium acetate buffer solution (0.1 M) until final volume of 100 mL and pH was adjusted to 5.25. The reaction took place when 50 μL of the sample (a dilution of 1:10 in water) were added to 950 μL of the $DMPD^{\cdot+}$ solution. Absorbance at 505 nm was measured after 10 min of continuous stirring. The results were quantified in mM of Trolox on the relevant calibration curve.

2.6. FRAP "ferric reducing/antioxidant power"

This method was used to measure the reductive power of a sample (Benzie & Strain, 1996). It is based on increased absorbance at 593 nm due to formation of tripyridyl-*s*-triazine complexes with iron(II) (TPTZ–Fe(II)) in the presence of a reductive agent. The reactive mixture was prepared by mixing 25 mL of sodium acetate buffer solution (0.3 M, pH 3.6), 2.5 mL of TPTZ (10 mM), 2.5 mL of $FeCl_3$ (20 mM) and 3 mL of water. 30 μL of the sample (diluted in water at 1:50) was added to 970 μL of the latter reactive mixture and is incubated at 37 °C for 30 min. The results were expressed as mM of Fe(II), using linear calibration obtained with different concentrations of $FeSO_4$.

2.7. ORAC "oxygen radical absorbance capacity assay"

This method is based on the fluorescent qualities of β-phycoerythrin, which is a pigment extracted from algae (Cao, Alessio, & Cutler, 1993). The presence of an oxidant (ABAP) leads to the formation of peroxyl radicals that directly attack the β-phycoerythrin

protein molecule and reduce its fluorescence. The action of an antioxidant halts the decay of the molecule and its fluorescence is therefore not completely reduced. The reaction was prompted by mixing 553 μL of β-phycoerythrin (16.7 nM) with 20 μL of the sample (diluted at 1:10 with water) and 1177 μL of potassium phosphate buffer solution 75 mM (pH 7.4). After 15 min of incubation at 37 °C 750 μL of ABAP were added (32 mM of the final concentration) and fluorescence was measured. The results were calculated by measuring the difference of net areas under the curve (AUC net) taken from the spectrofluorimeter between the white and the sample colours, expressed as mM of Trolox on an appropriate calibration curve. The fluorescence decay curve was measured for 80 min, using emission and excitation wavelengths of 584 nm and 544 nm, respectively.

2.8. HRSA

Desoxyribose (2-desoxy-D-ribose) decays when exposed to hydroxyl radicals generated by the Fenton reaction (Halliwell, Gutteridge, & Aruoma, 1987). The hydroxyl radicals ($HO^{\cdot}$) were generated through the following system: 10 μL of $FeCl_3$ (0.1 mM), 10 μL of ascorbic acid (0.1 mM), 10 μL of H_2O_2 (1 mM) and 10 μL of EDTA (0.1 mM). 15 μL of the sample (diluted at 1:50 in water) were incubated at 37 °C for 1 h, with 20 μL of desoxyribose (1 mM final concentration) in the presence of $FeCl_3$, ascorbic acid, H_2O_2 and EDTA in the cited amounts. 1.5 mL of TCA (28% w/v) and 1 mL of TBA (1% w/v, NaOH 0.05 M) was added to 1 mL of the sample under incubation and held for 15 min at 100 °C after which it was left to cool to room temperature. The MDA (malondialdehyde) formed from the decay of the desoxyribose is evaluated in reaction with TBA and spectrophotometrically measured at 532 nm. The results were expressed as an inhibition % in relation to a control test (without the sample).

2.9. SRSA

The superoxide radical which reacts with NBT generates a coloured compound with absorbance at 560 nm (Liu, Ooi, & Chang, 1997). The antioxidant scavenging superoxide radical leading the decolorization. The reactive was made up with 50 μL NADH (77 μM), 50 μL NBT (50 μM), 5 μL of PMS (3.3 μM) in a medium of Tris–HCl (16 mM, pH 8) and 10 μL of the sample (diluted at 1:5). The results were expressed as an inhibition % in relation to a control test (without the sample).

2.10. ABAP-LP

The microsomes from the liver of a 250–300 g male Wistar rat were extracted in line with the method of Kessler, Ubeaud, and Jung (2003). Total microsomal protein content is determined using the Bradford method, 1976. A microsomal fraction (1 mg/mL of protein) was incubated with a solution of ABAP (10 mM), prepared in a Tris–HCl buffer solution immediately prior to use, in the presence of 50 μL of the sample (diluted at 1:50). The incubation temperature was set at 37 °C for a period of 90 min. Lipid peroxidation was evaluated with the TBA assay to quantify MDA. Absorbance measured at 532 nm was proportional to the quantity of peroxyl radicals generated and the results were expressed as an inhibition % in relation to a control test (without the sample).

2.11. Damage to DNA

2.11.1. Incubation of DNA calf thymus

The DNA from calf thymus (200 μg) in the absence and the presence of 200 μL of undiluted wine was exposed to the action of hydroxyl radicals generated by the mixture of 100 μL of ascorbic acid (1 mM final concentration) and 10 μL of copper sulphate (II) (100 μM final concentration). The mixture was incubated at 37 °C for 1 h after which the fragments were separated by electrophoresis.

2.11.2. Agarose gel electrophoresis

Electrophoresis was carried out with 1% agarose at room temperature using a BIO-RAD power–Pac 1000 (Hercules, CA, USA) electrophoresis system. Subsequently, the gel containing 15 μL of ethidium bromide (10 mg/mL) was observed under ultraviolet light, using a transilluminator, and photographed. The results were expressed as DNA bp fragments, calculated using the standard molecular weight markers such as the 100 bp DNA Ladder of 100–1500 bp and the λ DNA/Hind III of 125 bp–23.1 Kb, both from Invitrogen (CA, USA).

2.12. Statistical analysis

The statistical analysis of the data was carried out by analysis of the variance (ANOVA) and the LSD test (least significant difference) which showed the values statistically different. A significance level of $\alpha \leqslant 0.05$ was used. Statgraphics Plus software programme (Manugistic Inc., 1999) was used.

3. Results and discussion

Prior to an analysis of the results, as a first point it should be made clear that the ABTS, DPPH, DMPD and HRSA assays were applied to all of the wines, whereas, because of logistical reasons in the design of the experiment, owing to the great number of samples, the remainder of the assays (FRAP, ORAC, SRSA, ABAP-LP and DNA-damage) were only applied to the young wines and those that have been aged for 12 and 24 months in the barrel.

The second point is that published works that have sought to cover this same field do exist, but they include a smaller number of samples and assay methods (Echeverry et al., 2005; Landrault et al., 2001; Larrauri, Sanchez-Moreno, Rupérez, & Saura-Calixto, 1999; Pellegrini et al., 2000; Roginsky et al., 2006; Yamaguchi, Yoshimura, Nakazawa, & Ariga, 1999), and their results are not always consistent. So, studies by Larrauri et al. (1999), Yamaguchi et al. (1999), Echeverry et al. (2005) showed that ageing of wines increased the antioxidant potential of the wine, giving higher indices for DPPH, SRSA and ABAP-LP. On the contrary, in the works of Pellegrini et al. (2000), Landrault et al. (2001), Roginsky et al. (2006), it was noted that young wines presented greater antioxidant effect, with higher indices for ABTS and inhibition of lipidic peroxidation in a methyl-linoleate medium, than aged ones. This lack of consensus has called into question the effect of ageing on the antioxidant potential of wine.

The need for various methods has already been shown by many authors (Aruoma, 2003; Huang, Ou, & Prior, 2005; Prior, Wu, & Schaich, 2005; Rivero-Pérez et al., 2007), who attribute it to the multiple mechanisms involved in the various methods and the wide variety of antioxidant substances with different properties that are present in wine. Obtaining a global profile of the antioxidant potential of wine may only be achieved by using assay methods that reflect hydrophilic and lipophylic antioxidant capabilities, which use single-electron transfer mechanisms (SET) and hydrogen atom transfer (HAT) to evaluate scavenger activity in the face of certain free radicals, and which measure biomolecular damage.

According to the previous comments, this work was carried trying to approach the question of ageing in wine and its antioxidant potential, using a great number of methods. Furthermore, a large number of samples were considered. To the best of the authors' knowledge, no published works cover such a wide spectrum of methods or such a large number of samples.

The third and final point is that all the wines under study were made under similar conditions, for which reason the indices of their antioxidant capacity should not, in principle, be affected by this variable.

A global analysis of the data sets proves that the effect of ageing on the antioxidative properties of wine differs according to the method used in its evaluation, (Fig. 1). Thus, the wines presented indices for ABTS and DPPH that tended to descend with age, and although in quantitative terms it was not a marked decline this was statistically significant ($p \leqslant 0.05$). The behaviour was similar in both methods probability due to their being methods that work with the same antioxidant mechanism, mediated by electron transfer (SET). This not-at-all sharp drop in the antioxidant activity indices of ABTS coincide with the findings of Roginsky et al. (2006), who argue that the set of condensations that take place in wine during ageing do not affect the number of active groups directly involved in electron transfer mechanisms. Moreover, Pellegrini et al. (2000), obtained similar results, attributing greatest responsibility for the antioxidative capacity of young wines to the anthocyanins, as was also demonstrated by Ghiselli et al. (1998).

Contrary to these results, the reductive capacity of the wines evaluated in FRAP assays showed higher indices in wines aged for 12 months in the barrel. One explanation for that might be offered for this phenomenon is the greater transfer of ellagitannins from the wood to the wine, during the early stages of ageing. These compounds that are peculiar to wood regulate oxidation mechanisms in wine (Del Alamo Sanza, Nevares Domínguez, & Garcia Merino, 2004) and it is possible that they increase its reductive potential. It is well known that the transfer of compounds peculiar to the wood is stopped in wine that is aged for longer periods (Brouillard, George, & Fougerousse, 1997), for which reason these two-year-old wines would revert to having a lower reductive potential after losing the contribution made by the wood, amongst other things.

The DMPD indices of the wines that had been aged were much lower than those of the young wines. These results seem to suggest that the transference of hydrogen atoms (HAT), a mechanism that predominates in this assay method, is sharply reduced when it coincides with the formation of more complex and stable compounds, such as newly formed pigments (vitisins, pyro-anthocyanins, aril-anthocyanins and so on) (Bakker et al., 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2003; Pérez-Magariño & González-Sanjosé, 2004).

Values of hydroxyl radical scavenger activity (HRSA) showed similar results in close agreement with those described by FRAP and, therefore, perhaps also attributable to certain compounds extracted from the wood. Thus, wines aged in the barrel for four months presented a higher scavenger capacity than the young wine. This capacity dropped to reach minimum indices in wines aged for 12 and 24 months.

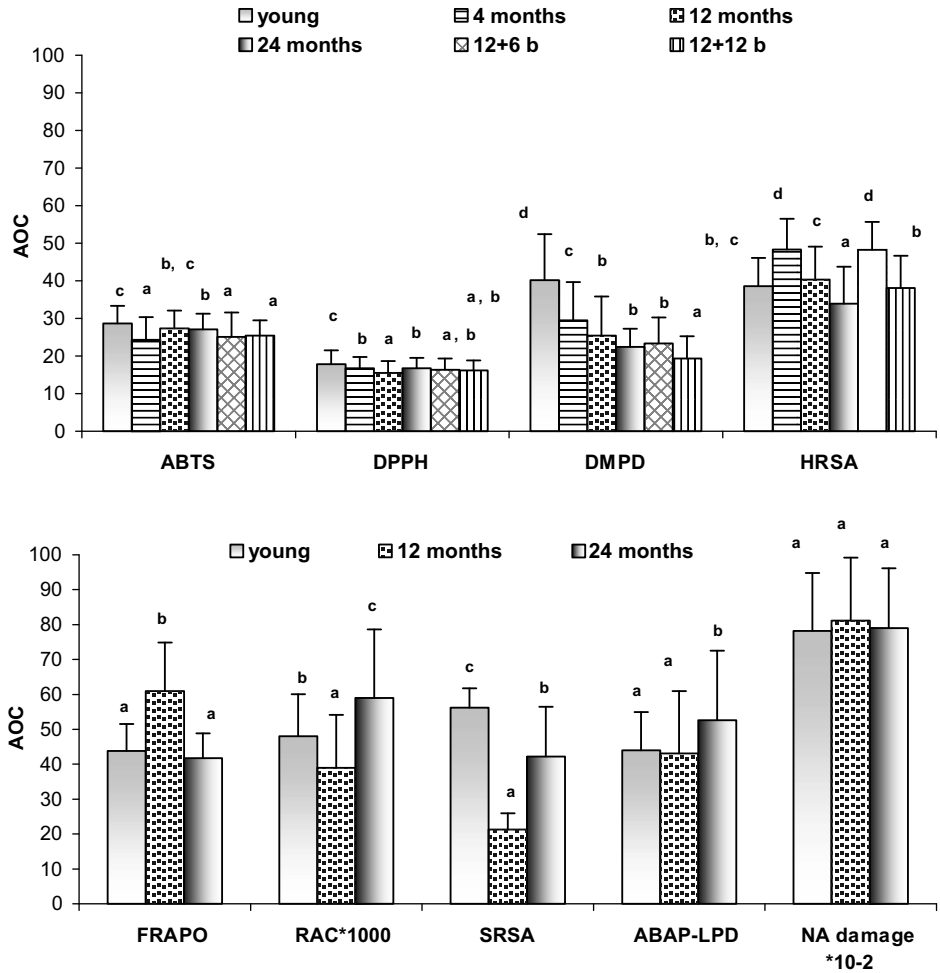

Fig. 1. Antioxidant potential of Spanish red wines of different ages. Averages values and standard desviation (error bars) were showed (n_T = 162 wines, measured with four assays replicates). Values with different letters are significantly different (LSD test, p = 0.05). 12 + 6b: 12 months in barrel and six months in bottle; 12 + 12b: 12 months in barrel and 12 months in bottle.

On the contrary, superoxide radical scavenger activity (SRSA) presented minimum indices in wines aged for 12 months in the barrel and maximum indices for the young wines. The differences between wines aged in the barrel for 12 and 24 months are in agreement with the results described by Ariga (1990) and Saito, Hosoyama, Ariga, Kataoka, and Yamaji (1998), which associate the increase in superoxide radical scavenger activity with the increase in the degree of proanthocyanidin polymerization that occurs during the ageing of the wine in the barrel.

Similar results were detected for the ORAC assay with maximum indices in wines aged for 24 months in the barrel. These results could be in agreement with those obtained by Yilmaz and Toledo (2006), who described higher ORAC indices in polymer and oligomeric proanthocyanidin grape extracts than in monomer extracts.

The methods that evaluate biomarkers of oxidative stress ABAP-LP and DNA-damage, also presented a somewhat odd behaviour, perhaps due to the biomolecules involved or to the protection mechanisms of each case. Thus, although no significant differences are appreciated between the capacities of the different types of wines to protect against damage to DNA, the results indicated a greater capacity for the inhibition of lipidic peroxidation in wines aged in the barrel for 24 months; results which are in agreement with those of Echeverry et al. (2005) and Roginsky et al. (2006). The latter authors attribute this fact to the polymers formed by the ageing of the wine. The involvement of polymeric tannins in the prevention of lipidic oxidation has been studied "in vivo" (Tebib, Rouanet, & Besancon, 1997), as well as through "in vitro" assays (De Whalley, Rankin, Hoult, Jessup, & Leake, 1990; Frankel, Kanner, German, Parks, & Kinsella, 1993; Kanner, Frankel, Granit, German, & Kinsella, 1994).

From what has been said, it is clear that defining the effect of ageing on the antioxidant capacity of the wine is no easy task, as contradictory results may arise depending on the measurement method that is used, which gives an idea of the complexity of this field.

It might be considered that the results as shown could hide inconsistent information because the wines under consideration present many variable sources as vintage, variety and microoxygenation treatment, and not only the age. Hence, any possible interference by one or more of these factors was evaluated.

The results of the wines grouped under vintages were similar to those previously described, for which reason it cannot be said that this factor either notably influenced or distorted the previously mentioned global results.

On the other hand, it is well known that microoxygenation produces significant changes in the structure of wines (Parish, Wollan, & Paul, 2000) directly linked to changes in the phenolic fraction. Accordingly, it was decided to carry out a comparative study of the ages distinguishing between both types of wine: the microoxygenated and the non-microoxygenated control group (Fig. 2).

No great differences were found with respect to those described for the global indices. Differences were only found between the control and the microoxygenated wines for the ORAC and DNA-damage assays. The microoxygenated wines aged for 12 months in the barrel presented the greatest ability to protect DNA, whereas, their corresponding control groups were the worst protectors. Moreover, although ORAC indices increased moving from the young control wines to those aged for 24 months in the barrel, in the microoxygenated wines a marked drop was only observable after 12 months in the barrel. These differences are difficult to explain and may be random.

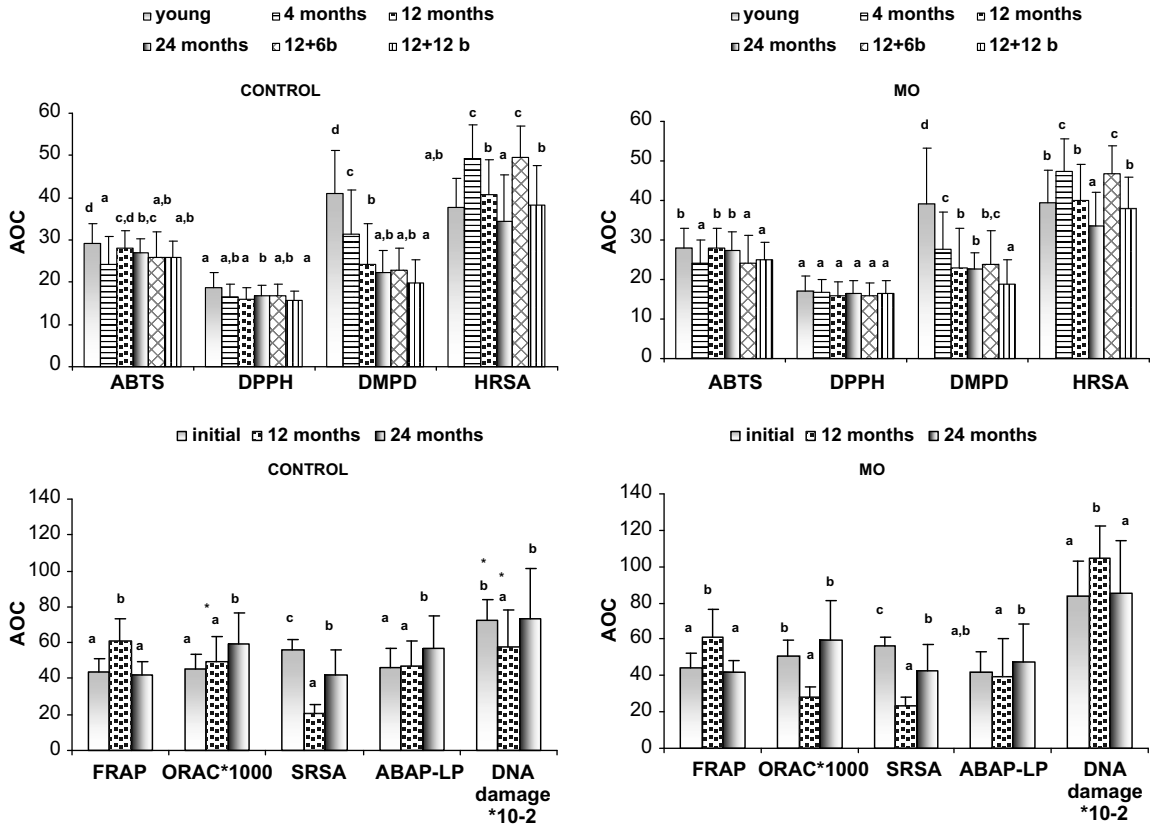

Fig. 2. Antioxidant potential of non microoxygenated (CONTROL) and microoxygenated (MO) Spanish red wines of different ages. Averages values and standard desviation (error bars) were showed ($n_T = 162$ wines, measured with four assays replicates). Values with different letters are significantly different (LSD test $p = 0.05$). * This symbol indicates values statistically different between CONTROL and MO wines of the same age ($p \leqslant 0.05$). 12 + 6b: 12 months in barrel and six months in bottle; 12 + 12b: 12 months in barrel and 12 months in bottle.

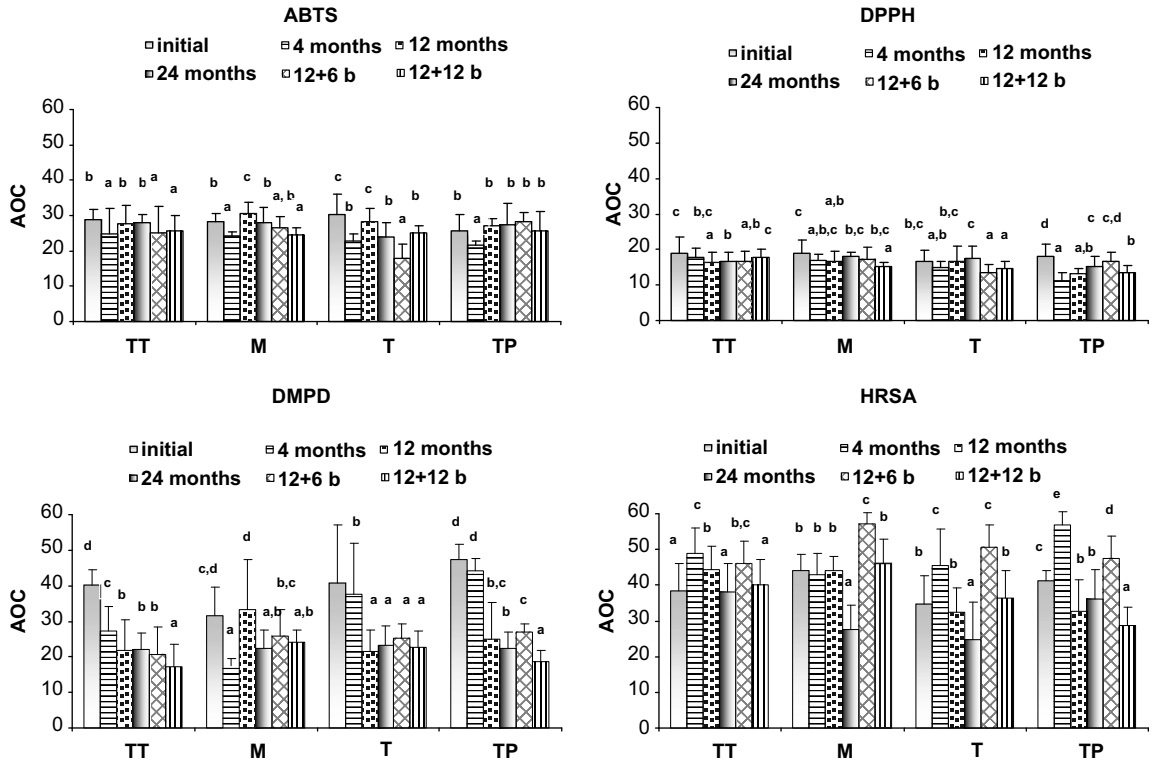

Fig. 3. Antioxidant potential of different varieties of Spanish aged red wines. Averages values and standard desviation (error bars) were showed (n_T = 162 wines, measured with four assays replicates).Values with different letters are significantly different (LSD test p = 0.05). TT (Tinta de Toro); M (Mencía); T (Tempranillo); TP (Tinta del País). 12 + 6b: 12 months in barrel and six months in bottle; 12 + 12b: 12 months in barrel and 12 months in bottle.

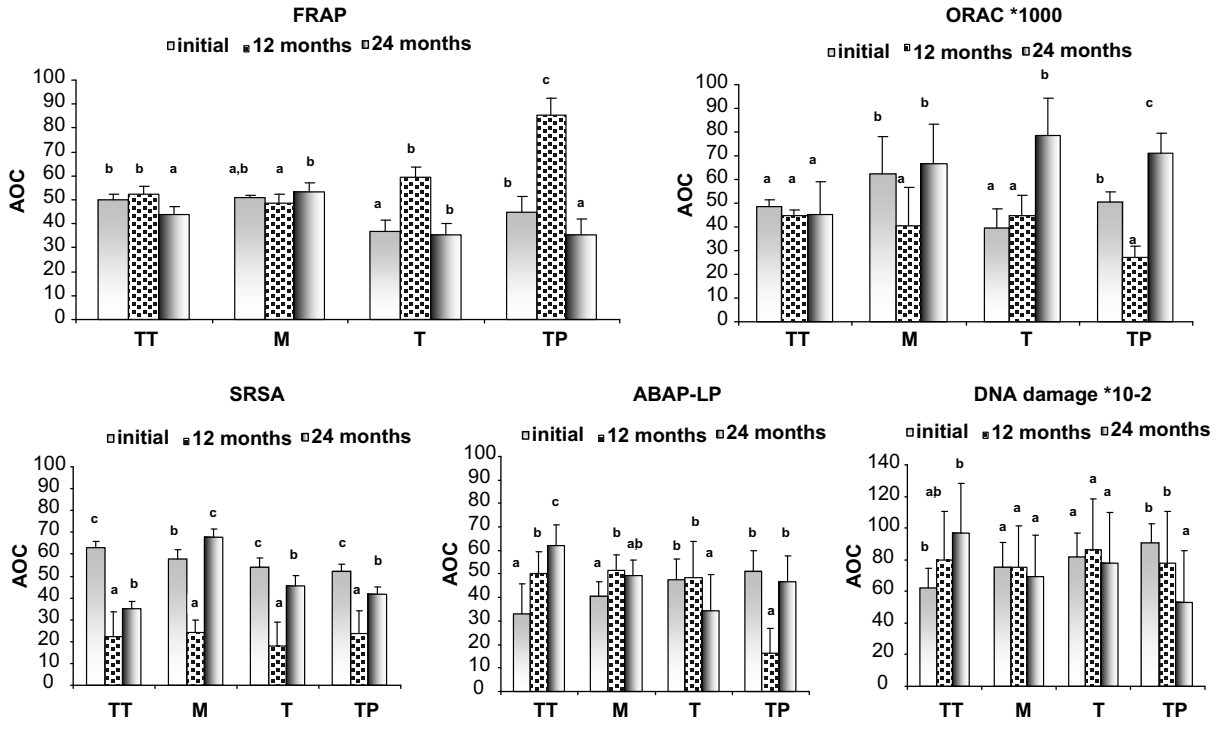

Fig. 4. Antioxidant potential of different varieties of Spanish aged red wines. Averages values and standard desviation (error bars) were showed (n_T = 88 wines, measured with four assays replicates). Values with different letters are significantly different (LSD test p = 0.05). TT (Tinta de Toro); M (Mencía); T (Tempranillo); TP (Tinta del País).

It can be deduced from the above that although microoxygenation is a technique that provokes changes in the global phenolic composition of the wines, it does not induce substantial changes in their antioxidant capacity; a fact which hitherto has neither been proven nor published.

The varietal effect undoubtedly affects the phenolic content and composition of the wines. For this reason it was also decided to study the joint effect of variety and age (Figs. 3 and 4).

There are some works that have grappled with varietal factor and the antioxidant potential of wines (Kedage, Tilak, & Dixit,

2007; Roginsky et al., 2006; Villaño, Fernández-Pachón, Trocoso, & García-Parrilla, 2006). They attribute the differences in the antioxidant capacity of wines to the diverse phenolic composition of the different varieties. The general tendencies observed earlier repeat themselves once again, which is to say, a slow drop in ABTS and DPPH indices, a more pronounced one for DMPD, higher HRSA indices at four months (Fig. 3) and higher FRAP indices at 12, coinciding with the minimum indices for SRSA (Fig. 4).

However, greater differences were found between wine varieties than between microoxygenated and non-microoxygenated wines. The varietal differences showed up most clearly in the FRAP, ORAC, ABAP-LP and DNA-damage assays. In addition, it is notable that the Mencía (M) wines presented greater differences for the effects of age (for example, DMPD indices at 12 months were greater than those at four months; HRSA indices were no greater for four month-old wines than for the young wines). These results could be explained by multiple factors, however, the most noteworthy being, as initially stated, that not even the varietal factor seems to be able to annul the general results that have been commented on. As a result, on the basis of this study, we may therefore conclude that the process of ageing, whether in the barrel or the bottle, does not lead to significant changes in the antioxidant potential of red wines.

The results need to be treated with caution and are not comparable with data on the antioxidant potential of wine evaluated by other assay methods. Variables such as the variety, the vintage, or microoxygenation, neither cover up nor distort the effect of the age of the wine.

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