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Changes during Storage in Conventional and Ecological Wine: Phenolic Content and Antioxidant Activity

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Polyphenol content, free radical scavenging capacity, and changes during storage over 7 months in the dark were studied in ecological and conventional red and white wines. In red wines, the most changeable components during storage were the anthocyanins since during storage anthocyanins content decreased 88% in conventional wine and 91% in ecological wine. Initially, the total flavonol contents of the conventional and ecological red wines were 163.88 ± 2.69 and 153.58 ± 1.71 mg/L, respectively, and no significant variations occurred during storage. No differences in hydroxycinnamic acid derivatives content between conventional and ecological red and white wines were observed. The flavonol level in white wines was very low, as expected since these compounds are found in grape skin. The initial antioxidant activity was 5.37 ± 0.14 and 5.82 ± 0.31 mM equivalents Trolox for conventional and ecological red wines, respectively; no significant differences were observed ($p = 0.2831$), and these values were 7−8 times higher than the antioxidant activity observed in conventional and ecological white wine. In contrast with other studies, the total concentrations of phenolic compounds in conventional and ecological red and white wines were not related to antioxidant activity ($p > 0.05$). In red wines, no significant differences were observed in the antioxidant activity of ecological and conventional red wine ($p = 0.28$), while in white wine significant differences were observed in the antioxidant activity between conventional and ecological white wine ($p = 0.006$).

KEYWORDS: Antioxidant activity; anthocyanins; DPPH; health promotion; HPLC-DAD; phenolic; storage; wine

INTRODUCTION

Flavonoids in the diet are inversely associated with deaths from heart disease and cancer. Renaud and co-workers (*1*) evaluated the health risks of wine and beer drinking and observed that moderate intake of both (22−32 g of alcohol per day) was related to lower relative risk for cardiovascular disease. The metal-chelating capability of flavonoids, together with their free-radical scavenging properties, has led to the proposal that flavonoids act as dietary antioxidants (*2*). Even the aglycone forms are more active than the glycosides (*3*, *4*). Red wine but not white wine contains abundant polyphenols, which inhibit the oxidation of human LDL in vitro (*5*). Potential antioxidant activity (6), free radical scavenging activity, inhibition of platelet aggregation, and antiulcer activity have been shown for the above compounds (*7*).

Phenolic acids in grape berries are located primarily in the skin and in grape pulp where they are present at much lower concentrations than anthocyanins. In white grape varieties, the concentration of phenolic compounds is much lower in pulp and in the must, whereas benzoic and cinnamic acids are predominant. Catechins and procyanidins are located in solid parts of the berry, particularly in the seeds (*8*). Phenolics have a number of important roles to play in viticulture and enology including UV protection, disease resistance, pollination, color, and defense against predation in plants (*9*), as well as haze formation, hue, and taste in wines (*10*).

Hydroxycinnamic derivatives comprise the greatest part of the nonflavonoid phenolics found in wine (*11*, *12*). Most are individually present below their sensory threshold in wines, although they may contribute to bitterness (*13*) through an additive effect and may be involved in oxidation reactions as they are important substrates for the polyphenol oxidase enzyme. Initially, antioxidant activity was principally associated with flavonoids and stilbenes. Evidence is now increasing that hydroxycinnamates and their conjugates are similarly active. As cinnamates are significant components of the human diet they may provide beneficial health effects (*14*, *15*).

The flavonols are antioxidants that can protect against cancer, heart disease, degenerative illnesses, and the effects of aging.

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The effects of structurally related flavonoids at concentrations from 10 to 500 μM on the lipid peroxidation were examined by Sugihara and co-workers (*16*), where they observed that flavonols such as myricetin, quercetin, fisetin, and kaempferol, but not morin, showed dose-dependent antioxidative activity against metal-induced lipid peroxidation at all metal concentrations. Myricetin, quercetin, and fisetin were the most effective antioxidants, although their efficacies depended on the metal ion. Kaempferol and morin had antioxidative activity equal to the other flavonols in the presence of Cu ions, but were much less effective for the other three metal ions. Furthermore, they are antithrombotic because they are selectively bound to mural platelet thrombi and owing to their free radical scavenging properties resuscitate biosynthesis and action of endothelial prostacyclin and EDRF. Thus, flavonols release the thrombolytic and vasoprotective endothelial mediators only in these vascular segments which are covered by a carpet of aggregating platelets (*17*).

In recent years, ecological produce has increased by more than 20%. In the field of ecological wine, the elaboration of the product from grapes grown without the use of chemical fertilizers, insecticides, and other pest control substances is important. Avoiding their use means avoiding the presence of any chemical residue in the wine. The main objective is to preserve healthy and biologically active soil by means of natural fertilizers such as manure. As far as the use of pesticides is concerned, the ecological alternative is to encourage natural predators to combat plagues rather than use poisonous insecticides. One of the ever more extended theories regarding ecological vineyards is that these have a greater resistance to the inclemency of the weather or pests, resulting in better performance in poor years with respect to conventional vineyards. Furthermore, another of their benefits is that in ecological vineyards the grapes are collected by hand instead of using machinery. This permits the collection of only the most ripe and healthy bunches, reducing to a minimum damage to the vine, fruit, or soil. For these reasons, there is an increase in consumption of ecological foodstuffs due to the increase in demand for safe products.

The aim of the present work is to study the phenolic composition and antioxidant activity in ecological and conventional white and red Spanish wines and changes during the first 7 months of storage in the dark.

MATERIALS AND METHODS

Reagents. Formic acid and methanol (MeOH) were of analytical grade supplied by Merck (Darmstad, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure was used throughout this research. Cyanidin 3-rutinoside was purchased from Polyphenols A.S (Sandnes, Norway). Rutin was purchased from Merck (Darmstadt, Germany). Chlorogenic acid was purchased from Sigma (Madrid, Spain). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,2-diphenylpicrylhydrazyl (DPPH$^•$) were purchased from Sigma (Madrid, Spain).

Wine Samples. The ecological and conventional red and white wines used in the present study were prepared from ripe grapes from *Vitis vinífera* var. "Monastrell" for red wine and "Airen" for white wine harvested in September 2001 in Jumilla (Murcia, Spain) and stored for 7 months at 20 °C in glass bottles and in the dark. Conventional grapes (red and white) were treated with plaguicides (Benomilo: 1-(butylcarbamoyl) bency-imidazole 2-yl carbamate de methyl), fenarimol: 2,4-dichloro α-(pyrimidine-5-yl) benzhydrylic), triclorfon: (2,2,2-trichloro-1-hydroxyethyl phosphate methylfostato), whereas organic grapes were treated with natural pesticide such as sulfur and treatment with feromonas, allowed by organic agriculture.

HPLC-DAD Analysis. Ecological and conventional red and white wines were filtered through a 0.45 μ filter (type Millex HV13, Millipore Corp, Bedford, MA) before HPLC analysis.

Twenty microliters of every sample was injected for HPLC analysis on equipment using a Merck-Hitachi pump L-6200 (Merck-Hitachi, Darmstadt, Germany) and a diode array detector Shimadzu SPD-M6A UV (Shimadzu, Kyoto, Japan) using a reversed-phase column Lichrochart RP-18 column (Merck, Darmstadt, Germany) (25 × 0.4 cm, 5 μm particle size), using as solvents water plus 5% formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL min^{-1}. Elution was performed with a gradient starting at 2% B to reach 32% B at 30 min, 40% B at 40 min, and 95% B at 50 min, and became isocratic for 5 min. Chromatograms were recorded at 510, 320, and 360 nm.

Phenolic Compounds Identification and Quantification. The phenolic compounds in wine were identified by their UV−Vis spectra, recorded with a diode-array-detector and, wherever possible, by chromatographic comparison with authentic markers. Individual anthocyanins were quantified by comparisons with an external standard of cyanidin 3-rutinoside at 510 nm. Flavonols were quantified as rutin at 360 nm and the hydroxycinnamic acid derivatives at 320 nm as chlorogenic acid. All analyses were repeated three times, and the results were expressed as mean values in milligrams per liter of wine ± SD. The reproducibility of the HPLC analyses was 5%. The total phenolic compounds were calculated by addition of the amounts of the anthocyanins, flavonols, and hydroxycinnamic acids detected in each chromatogram, as previously reported (*18*).

Statistical Data Treatment. The relationships occurring between the compounds quantified and the antioxidant properties was assessed using SPSS 11.0 (SPPS Inc., Chicago, IL).

Antioxidant Activity. The samples were analyzed according to the technique reported by Brand-Williams et al. (1995) (*19*). Briefly, a volume of sample [5 μL for red wine (1:1, v:v with methanol) and 20 μL of white wine] was added to a volume of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^•$) (Sigma, Steinheim, Germany) 0.094 mM in methanol up to completing 1 mL. The free radical scavenging activity using the free radical DPPH$^•$ reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20 °C in a spectrophotometer (Varian Cary 50-Bio, Victoria, Australia). The reaction was carried out in closed eppendorf tubes shaken at 20 °C. The results were expressed as mmol/L Trolox equivalents, a vitamin E analogue (*20*).

RESULTS AND DISCUSSION

The following compounds were identified in the ecological and conventional red and white wines:

Anthocyanins. Six different anthocyanins were detected in the ecological and conventional red wines: delphinidin-3-glucoside, cyanidin 3-glucoside, peonidin 3-glucoside, petunidin 3-glucoside, malvidin 3-glucoside, and malvidin-3-*p*-cumaroyl-glucoside. Initially, mean total anthocyanins content was 386 ± 18.95 mg/L (**Table 1**) and 350.9 ± 20.51 mg/L (**Table 1**) for conventional and ecological red wine, respectively. Malvidin 3-glucoside was the major anthocyanin, with concentrations of up to 248.3 ± 12.7 mg/L for conventional red wine and 228.5 ± 5 mg/L for ecological red wine. These data exceded by far the levels measured in 50 different French red wines (mean 164 mg/L) by Teidresse and co-workers (*21*) and were similar as compared with other red wines (*22*).

During the 7 months of storage of wine in the dark, the anthocyanins content decreased 88% in conventional red wine and 91% in ecological red wine. The higher loss in pigment composition was observed during the first month, and the most stable anthocyanin was cyanidin 3-glucoside. The results were in accordance with general findings in which monomeric pigment concentrations decreased during storage (*23, 24*). It has also been demonstrated that the concentration of polymeric

Table 1. Anthocyanins Composition of Conventional and Ecological Red Wine and Changes during Storage over 6 Months at 20 °C in the Dark[a]

month	delphinidin 3-GLC		cyanidin 3-gluc		petunidin 3-gluc		peonidin 3-gluc		malvidin 3-gluc		malvidin 3-*p*-Cumaroyl GLC		totals anthocyanins	
	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol
initial	27.32 ± 1.39	25.11 ± 1.17	5.38 ± 1.25	4.94 ± 0.73	51.49 ± 1.64	46.51 ± 1.73	22.22 ± 1.86	19.26 ± 2.10	248.34 ± 12.7	228.5 ± 3.96	31.20 ± 1.90	26.56 ± 3.67	386.0 ± 18.95	350.90 ± 20
1	12.05 ± 0.81	14.77 ± 2.47	3.26 ± 1.46	3.07 ± 0.89	22.97 ± 3.03	26.48 ± 3.82	8.17 ± 0.86	10.95 ± 1.32	111.99 ± 6.76	126.0 ± 13.2	15.39 ± 6.66	15.26 ± 2.09	173.9 ± 5.86	196.6 ± 19.18
2	8.39 ± 0.23	11.55 ± 0.34	1.39 ± 0.15	2.56 ± 0.95	16.32 ± 2.67	19.35 ± 1.54	5.42 ±!! 0.24	7.74 ± 0.37	73.60 ± 3.88	96.62 ± 6.14	26.75 ± 2.58	22.13 ± 3.56	131.9 ± 16.34	148.4 ± 7.04
3	5.99 ± 0.89	8.51 ± 0.29	1.10 ± 0.30	1.49 ± 0.14	11.10 ± 0.29	15.45 ± 1.19	3.77 ± 0.89	8.70 ± 2.10	49.86 ± 8.41	75.88 ± 5.12	19.91 ± 2.58	14.82 ± 1.61	91.8 ± 14	124.9 ± 9.23
4	4.37 ± 0.45	8.33 ± 1.45	0.86 ± 0.07	3.11 ± 0.64	8.0 ± 0.93	12.50 ± 0.28	2.84 ± 0.34	5.19 ± 0.50	39.83 ± 4.72	59.94 ± 2.83	21.63 ± 5.36	21.84 ± 1.07	77.6 ± 9.29	110.9 ± 10.58
5	6.05 ± 0.97	5.58 ± 0.65	1.19 ± 0.21	1.06 ± 0.23	10.96 ± 1.13	10.09 ± 1.44	3.93 ± 0.98	4.77 ± 2.18	51.00 ± 6.95	50.08 ± 9.40	ND	ND	73.2 ± 9.6	71.6 ± 13.06
6	4.72 ± 0.55	5.67 ± 1.32	1.28 ± 0.28	1.27 ± 0.39	9.32 ± 2.42	10.33 ± 0.94	3.10 ± 0.26	3.82 ± 0.23	39.24 ± 2.66	47.10 ± 7.31	ND	ND	57.7 ± 5.66	68.2± 9.92
7	3.53 ± 0.48	2.29 ± 0.95	1.32 ± 0.97	2.02 ± 0.02	6.41 ± 0.71	5.32 ± 0.39	2.33 ± 0.24	1.41 ± 0.25	32.29 ± 2.60	22.45 ± 0.74	ND	ND	45.9 ± 5.00	32.2 ± 2.49

[a] Values are expressed as mg/L wine ± standard deviation (SD) ($n = 3$). ND, not detected; conv, conventional wine; ecol, ecological wine.

pigments increased with temperature and storage time. This fact has an important effect on color of juices in red wine (*24*) and is characteristic during the aging process of red wines (*25*−*28*), since during the aging of red wines the absorbance decreases in the region of 520−535 nm and increases in the yellow/brown region at 400−420 nm. In addition, 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 (*29*). Ghiselli and co-workers (*30*) observed that among the wine fractions, anthocyanins were the most effective against peroxyl and hidroxyl radicals and are the main compound responsible for the color changes in red wine.

Hydroxycinnamic Acids. The hydroxycinnamic acid derivatives most quantified and identified in conventional and ecological red and white wines were *trans*-caffeoyltartaric acid and *trans*-*p*-cumaroyltartaric. The initial concentration of *trans*-caffeoyltartaric acid was 31.1 ± 1.22 mg/L in conventional red wine and 35 ± 3.04 mg/L in ecological red wine (**Table 2**), accounting for more than 50% of the total hydroxycinnamates, and the initial content of *trans*-*p*-cumaroyltartaric acid was 15.4 ± 0.55 and 14.6 ± 1.04 mg/L in conventional and ecological red wine, respectively (**Table 2**); these results were higher than those obtained by Pellegrini and co-workers (*31*) in young red wine and Alonso and co-workers (*32*) in different white and red wines and lower than the values observed by Gómez-Plaza and co-workers (*33*) in Monastrell red wines. Two hydroxycinnamic acids were also detected in small not identified amounts that once they are quantified produce hydroxycinnamic derivatives, 65.8 ± 2.25 and 64.10 ± 2.32 mg/L in conventional and ecological red wine, respectively (**Table 2**).

During the 7-month storage period at 20 °C in the dark in ecological red wine, no variation in the total concentration of hydroxycinnamic acids was observed. However, in conventional red wine a decrease in the esters of caffeic acid is observed, possibly due to the hydrolysis of these compounds, and the concentration of caffeic acid increases, not initially quantified, having been detected in traces amounts.

The nonflavonoids phenolics are almost equally distributed in conventional and ecological white wine and conventional and ecological red wine. The total initial concentration of hydroxycinnamic acids derivatives is 63.6 ± 2.14 mg/L in conventional white wine and 64 ± 2.19 mg/L in ecological white wine. During storage, no significant variations are produced (**Table 3**)

Flavonols. In the white wines, the flavonol amounts were very low, as expected since these compounds are found in grape skin. Only red wine contained appreciable values: initially, myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, and rutin were the most abundant, both in ecological red wines and in conventional red wines, followed by quercetin, myricetin, and kaempferol aglycon. Initially, total flavonol contents of the conventional and ecological red wines were 163.88 ± 2.69 and 153.58 ± 1.71 mg/L, respectively (**Table 4**). The total concentration of flavonol at the end of the conservation period of 7 months of storage at 20 °C in the dark does not suffer significant variations, the concentration of flavonol being 156 ± 5.02 mg/L in conventional red wine and 155 ± 1.04 mg/L in ecological red wine (**Table 4**), although it was observed that the glycosides flavonols were more unstable than aglycon flavonols which increased during storage; presumably, it was due to enzymatic hydrolysis of flavonol conjugates during vinification and/or maturation of the wines (*34*).

Table 2. Hydroxycinnamic Acid Derivatives Composition of Ecological and Conventional Red Wine and Changes during Storage over 6 Months at 20 °C in the Dark[a]

	trans-Caffeoyl tartaric		compound **2**		compound **3**		*trans*-*p*-coumaroyltartaric		totals	
month	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol
initial	31.1 ± 1.22	35.0 ± 3.04	8.6 ± 0.35	7.5 ± 0.67	6.6 ± 0.32	6.8 ± 0.72	15.4 ± 0.55	14.6 ± 1.04	65.80 ± 2.25	64.10 ± 2.32
1	35.4 ± 1.21	30.9 ± 3.33	6.3 ± 0.31	7.4 ± 0.95	6.3 ± 0.31	5.6 ± 0.75	14.8 ± 0.67	13.9 ± 1.40	63.1 ± 2.61	57.9 ± 6.39
2	33.7 ± 0.28	35.3 ± 0.60	6.0 ± 0.17	8.0 ± 0.70	6.8 ± 0.16	6.0 ± 0.34	14.8 ± 0.17	16.4 ± 0.50	61.4 ± 0.70	65.7 ± 2.11
3	31.4 ± 2.96	33.7 ± 1.34	6.5 ± 0.35	7.1 ± 0.28	5.4 ± 0.75	5.6 ± 0.30	14.7 ± 1.24	15.7 ± 0.46	58.0 ± 4.96	62.1 ± 1.76
4	25.1 ± 5.14	34.0 ± 0.72	6.8 ± 0.46	7.6 ± 0.23	3.8 ± 0.96	5.4 ± 0.26	12.2 ± 2.46	16.9 ± 0.38	48.0 ± 8.98	63.9 ± 1.39
5	34.9 ± 1.06	35.7 ± 1.39	6.1 ± 0.03	7.7 ± 0.83	5.5 ± 0.04	5.7 ± 0.23	16.0 ± 0.04	17.7 ± 0.51	62.5 ± 1.17	66.7 ± 2.08
6	32.9 ± 0.06	34.1 ± 0.70	7.3 ± 0.13	7.7 ± 1.13	5.0 ± 0.04	5.4 ± 0.17	15.6 ± 0.05	17.7 ± 0.73	60.8 ± 0.20	64.9 ± 2.01
7	18.7 ± 11.15	35.2 ± 1.21	7.1 ± 0.55	7.7 ± 0.50	2.6 ± 1.58	5.3 ± 0.36	9.3 ± 5.17	17.5 ± 1.42	37.7 ± 18.22	65.7 ± 2.75

[a] Values are expressed as mg/L wine ± standard deviation (SD) ($n = 3$). ND, not detected; conv, conventional wine; ecol, ecological wine.

Table 3. Hydroxycinnamic Acid Derivatives Composition of Ecological and Conventional White Wine and Changes during Storage over 6 Months at 20 °C in the Dark[a]

	trans-Caffeoyl tartaric		compound **2**		compound **3**		*trans*-*p*-coumaroyl tartaric		total hydroxy-cinnamic acid derivatives	
month	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol
initial	40.2 ± 1.87	42.0 ± 1.37	11.2 ± 0.51	9.2 ± 0.33	4.4 ± 0.03	4.8 ± 0.27	7.9 ± 0.22	8.1 ± 0.24	63.6 ± 2.14	64.0 ± 2.19
1	38.9 ± 0.23	42.5 ± 1.18	10.5 ± 0.38	9.6 ± 1.03	4.2 ± 0.19	4.6 ± 0.36	7.4 ± 0.05	7.9 ± 0.16	60.9 ± 0.69	64.6 ± 0.46
2	41.0 ± 1.66	42.5 ± 2.63	10.4 ± 0.59	8.3 ± 0.83	4.3 ± 0.42	4.6 ± 0.45	7.6 ± 0.63	7.8 ± 0.73	63.4 ± 3.25	63.2 ± 4.62
3	40.7 ± 1.75	39.9 ± 2.24	10.2 ± 0.48	7.7 ± 0.44	4.3 ± 0.14	4.3 ± 0.16	7.5 ± 0.50	7.3 ± 0.33	62.6 ± 2.87	59.1 ± 3.18
4	42.3 ± 0.69	40.9 ± 0.99	10.3 ± 0.39	7.6 ± 0.24	4.1 ± 0.25	4.2 ± 0.11	7.9 ± 0.46	7.2 ± 0.19	64.7 ± 1.60	59.9 ± 1.48
5	42.4 ± 0.65	39.7 ± 7.01	10.3 ± 0.25	7.4 ± 1.32	4.6 ± 0.31	4.1 ± 0.70	8.0 ± 0.41	6.9 ± 1.10	65.3 ± 1.22	58.0 ± 10.0
6	42.4 ± 0.53	40.9 ± 1.09	9.9 ± 0.27	7.5 ± 0.23	4.2 ± 0.12	4.3 ± 0.15	7.9 ± 0.15	7.6 ± 0.73	64.4 ± 0.56	60.4 ± 2.18
7	43.4 ± 0.41	41.0 ± 1.01	9.7 ± 0.18	7.6 ± 0.23	4.0 ± 0.14	4.4 ± 0.13	7.5 ± 0.12	7.8 ± 0.62	64.6 ± 0.48	60.8 ± 1.99

[a] Values are expressed as mg/L wine ± standard deviation (SD) ($n = 3$). ND, not detected; conv, conventional wine; ecol, ecological wine.

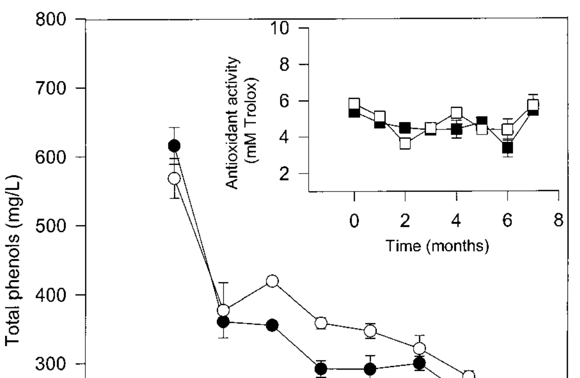

Figure 1. Total phenols and antioxidant activity in conventional and ecological red wine. (●) Total phenols in conventional red wine. (○) Total phenols in ecological red wine. Inset: (■) Antioxidant activity in conventional red wine, (□) Antioxidant activity in ecological red wine.

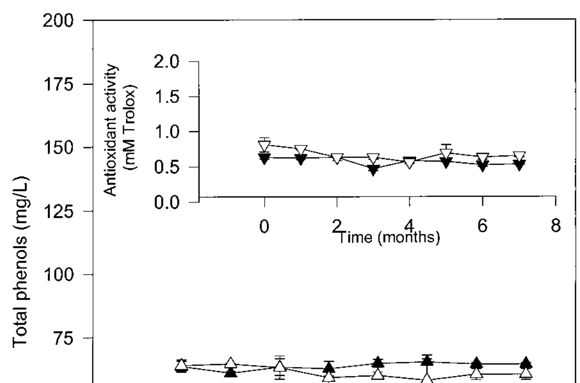

Figure 2. Total phenols and antioxidant activity in conventional and ecological white wine. (▲) Total phenols in conventional white wine. (△) Total phenols in ecological white wine. Inset: (▼) Antioxidant activity in conventional white wine. (▽) Antioxidant activity in ecological red wine.

The flavonol content in conventional and ecological red wines is superior to the concentrations observed in the study carried out by McDonald and co-workers (*34*) with 65 types of red wine of different geographical origins which ranged from 4.6 to 41.6 mg/L in Italian red wine (*30*). These differences can be supported by previous studies in geographic regions where cultivars are grown in which the level of ripening of grape berries and practiced winemaking techniques are determining factors in the modulation of phenolic types or their content (*5, 8, 31*).

Changes in Antioxidant Activity during Storage over 7 Months at 20 °C in the Dark. In addition to analyzing the phenolic content of each sample, the antioxidant capacity was also measured. The antioxidant activity shown in **Figures 1** and **2** are equivalent to those of Trolox solution with the indicated concentrations in millimolar. The initial antioxidant activity

Table 4. Flavonols Composition of Conventional and Ecological Red Wine and Changes during Storage over 7 Months at 20 °C in the Dark[a]

month	myricetin-3-glc		quercetin 3-gluc		Q-3-glc/rut		kaempferol-3-glc		myricetin		quercetin		total flavonols	
	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol	conv	ecol
initial	51.63 ± 2.29	49.89 ± 0.53	44.28 ± 1.82	40.05 ± 0.88	35.18 ± 1.06	33.42 ± 0.77	8.03 ± 1.29	7.95 ± 0.49	2.86 ± 0.72	2.70 ± 0.18	21.87 ± 2.45	19.55 ± 1.1	163.88 ± 2.69	153.58 ± 1.71
2	37.36 ± 3.26	44.52 ± 1.57	41.43 ± 0.36	54.06 ± 2.03	ND	14.49 ± 0.45	6.22 ± 0.46	7.12 ± 0.17	17.78 ± 2.2	14.93 ± 0.45	59.48 ± 4.3	59.07 ± 3.8	162.29 ± 6.61	194.21 ± 1.36
3	27.75 ± 1.32	39.44 ± 4.03	40.22 ± 5.30	53.75 ± 1.81	1.20 ± 0.05	2.42 ± 0.51	5.52 ± 0.51	6.25 ± 0.39	15.99 ± 1.8	12.15 ± 2.71	50.97 ± 2.4	56.87 ± 10.4	141.26 ± 5.00	170.91 ± 14.76
4	32.18 ± 0.66	37.85 ± 3.27	40.12 ± 1.05	50.89 ± 2.55	ND	2.05 ± 0.62	7.06 ± 0.28	6.41 ± 0.52	23.19 ± 1.5	17.17 ± 2.66	62.51 ± 7.6	63.41 ± 1.28	161.1 ± 9.81	177.11 ± 5.73
5	30.10 ± 3.0	35.85 ± 2.42	43.04 ± 5.60	48.91 ± 0.82	ND	1.76 ± 0.02	6.71 ± 0.68	7.41 ± 0.38	23.38 ± .51	21.26 ± 1.89	67.57 ± 4.4	69.57 ± 5.25	170.8 ± 2.87	184.20 ± 10.03
6	25.80 ± 1.87	31.87 ± 1.28	34.71 ± 4.41	46.16 ± 2.11	ND	ND	6.12 ± 0.06	7.28 ± 1.40	24.86 ± 3.52	24.17 ± 0.99	60.43 ± 5.5	65.72 ± 3.06	151.9 ± 15.06	175.22 ± 1.87
7	26.40 ± 1.08	30.89 ± 0.21	30.71 ± 3.00	33.48 ± 0.40	3.00 ± 1.98	6.23 ± 0.46	6.97 ± 0.55	6.73 ± 0.13	25.30 ± 1.09	24.14 ± 0.47	63.58 ± 2.3	53.62 ± 0.23	155.98 ± 5.02	155.09 ± 1.04

[a] Values are expressed as mean values mg/L wine ± standard deviation (SEM) ($n = 3$). Quercetin 3-gluc, quercetin 3-glucoside; Q-3 glc/rut, quercetin 3-glucoside + quercetin 3-rutinoside; kaempferol 3-glc, kaempferol 3-glucoside; ND, not detected; conv, conventional wine; ecol, ecological wine. In the first month analysis the absorbance at 360 nm was not recorded.

average was 5.37 ± 0.14 and 5.82 ± 0.31 mM equivalents Trolox for conventional and ecological red wines, respectively, and these values are 7−8-fold higher than the antioxidant activity observed in conventional and ecological white wines, which present values of 0.63 ± 0.02 and 0.81 ± 0.1 mM equivalents Trolox, respectively. These values are inferior to those in previous reports (*31*, *35*, *36*) in conventional red wine using the Randox kit, and these results are in the range of previously published data in white wines analyzed by Tubaro and co-workers (*37*), from 0.08 to 1.2 mM equivalents Trolox, and lower than those observed by Alonso and co-workers (*32*) in white wines electrochemical method using Trolox as the reference substance. During storage of wine over 7 months, the antioxidant activity in conventional and ecological red and white wines were not significantly different, except that conventional red wine observes a decrease in antioxidant capacity during the sixth month of storage which is then regained during the seventh month (**Figures 1** and **2**). This decrease coincides with the hydrolysis of caffeic acids esters which is produced in some bottles of conventional red wine and which is not observed in ecological red wine.

The antioxidant activity of a wine is due to polyphenolic compounds (*38*, *39*). The free flavonols are much more active than their conjugated derivatives in both antioxidant and antiplatelec aggregation assay (*4*, *5*, *40*), and it could be a contributory factor in explaining the slight decrease in antioxidant activity during storage.

In red wine, no significant differences were observed between the antioxidant activity of the ecological and conventional wines ($p = 0.28$). The concentration of total phenols in conventional and ecological red wines has no relation to antioxidant activity ($p = 0.09$ $r = 0.34$; $p = 0.58$ $r = 0.11$). In **Figure 1**, it can be observed that the decrease in the concentration of total phenols is greater than the variation in antioxidant activity which is produced during storage over 7 months in conventional and ecological red wine. These results are not in agreement to those observed by Alonso and co-workers (*32*) and Frankel and co-workers (*5*) who noted that there was a high degree of correlation between the polyphenolic content of the wine and the results of the antioxidant activity.

On carrying out a lineal regression analysis, it was observed that there was correlation between antioxidant activity and anthocyanin content in conventional red wine ($p = 0.0052$), but there was no correlation in ecological red wine, a result that coincides with that obtained by Burns and co-workers (*41*).

Similarly, in white wine significant differences in antioxidant activity between conventional and ecological white wines are observed ($p = 0.006$), and the antioxidant activity in ecological white wine was the greater. The antioxidant activity was not significantly correlated with the concentration of total phenols in either conventional or ecological white wine ($p = 0.92$, $r = 0.02$; $p = 0.87$, $r = 0.03$) (**Figure 2**).

It is important to bear in mind that the polyphenols identified in this paper only represent a small proportion of the total polyphenols of wine, indicating that other nonidentified compounds, phenolic acids and possibility polymers, contribute in a significant manner to antioxidant activity in wine. There has been conflicting evidence on the effect of aging on the antioxidant activity of wines. As wine ages, anthocyanins and other complex compounds along with the proanthocyanidins contribute to the formation of tannins (*42*). Tannins may be responsible for the fact that antioxidant activity did not decrease to a significant degree during conservation of the wine as might be expected given that total phenols decrease, since the antioxidant capacity of high molecular weight polyphenolics (tannins) was reported to be 15−30 times more effective at quenching peroxyl radicals than simple phenolics or Trolox (*43*). A recent study observed a significant relationship between the polymeric pigment content and antioxidant activity of New World wines ($p = 0.014$, $r = 0.52$). Although a significant proportion of the antioxidant activity of a wine may be attributed to large complexes such as the condensed tannins, few information is available on the extent of their absorption and bioavailability.

According to Ghiselli and co-workers (*30*), the protective effect of red wine is mainly due to the anthocyanic fraction, quantitatively the more abundant phenolic subclass in red wine; however, our results do not exclude the possibility of a synergistic action among different classes of polyphenols, since the total anthocyanin content decreases during storage and the antioxidant activity does not vary significantly during storage.

Nevertheless, besides polyphenols, there are many other bioactive compounds such as vitamins and minerals (*44*) as well as their synergistic effect, which also may be related to the free radical scavenging capacity of red wines. The phenols in wine are hypothesized to act synergistically as antioxidants in a mechanism in which the easily oxidized phenols are regenerated by less active phenols (*45*). Consumption of phenols from wine, grape juice, or grapes should provide an excellent means of increasing antioxidants in the diet.

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