Antioxidant Activity of Selected Spanish Wines in Corn Oil Emulsions

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Wines contain phenolic compounds that may be useful for preventing lipid oxidation as dietary antioxidants. This study was aimed at evaluating the antioxidant activity in corn oil emulsions of seventeen selected Spanish wines and two California wines. The inhibition of hydroperoxide formation at 10 μM gallic acid equivalents (GAE) varied from 8.4% to 40.2% with the red wines, from 20.9% to 45.8% with the rosé wines, and from 6.5% to 47.0% with the white wines. The inhibition of hydroperoxide formation at 20 μM GAE varied from 11.9% to 34.1% with the red wines, from 0.1% to 34.5% with the rosé wines, and from 3.3% to 37.2% with the white wines. The inhibition of hexanal formation at 10 μM GAE varied from 23.6% to 64.4% with the red wines, from 42.7% to 68.5% with the rosé wines, and from 28.4% to 68.8% with the white wines. The inhibition of hexanal formation at 20 μM GAE varied from 33.0% to 46.3% with the red wines, from 11.3% to 66.5% with the rosé wines, and from −16.7% to +21.0% with the white wines. The antioxidant effect decreased with increasing concentration. This antioxidant activity was related to the five main groups of phenolic compounds identified in wines by HPLC. The relative antioxidant activity correlated positively with the total phenol content of wines (by the Folin−Ciocalteu method and by HPLC), benzoic acids, anthocyanins, flavan-3-ols, and flavonols, for the inhibition of hydroperoxides and hexanal at 10 and 20 μM GAE.

Keywords: *Wine; corn oil emulsions; hydroperoxides; hexanal; antioxidant activity; prooxidant activity*

INTRODUCTION

There is a growing interest for natural antioxidants found in plants because of the worldwide trend toward the use of natural additives in foods and cosmetics. Plant phenolics present in fruits and vegetables and particularly those in tea and wine have received considerable attention because of their antioxidant activity and potential health benefits (Shahidi and Wanasundara, 1992; Frankel et al., 1993; Kinsella et al., 1993; Pearson et al., 1998). Wine contains large amounts of phenolic compounds, consisting mostly of flavonoids, ranging from 1800 to 4000 mg/L (Frankel et al., 1995). Phenolic compounds in wine have been shown to inhibit the in vitro oxidation of human low-density lipoprotein (LDL) (Frankel et al., 1993, 1995; Kinsella et al., 1993; Kanner et al., 1994; Lavy et al., 1994; Fuhrman et al., 1995; Teissedre et al., 1996; Meyer et al., 1997; Nigdikar et al., 1997, 1998). Oxidative modification of LDL has been considered a primary event in the pathogenesis of atherosclerosis (Steinberg et al., 1989; Esterbauer et al., 1992; Steinberg, 1992).

There is extensive literature on the evaluation of natural antioxidants in different unsaturated lipids (food model systems) (Huang et al., 1994, 1996, 1999; Frankel et al., 1997; Huang and Frankel, 1997) and biological model systems (Frankel et al., 1993, 1995; Meyer et al., 1997; Pearson et al., 1998; Satué-Gracia et al., 1999).

We evaluated the effects of natural antioxidants by measuring both hydroperoxide formation, on the basis of conjugated dienes, and hydroperoxide decomposition, on the basis of hexanal formation (Frankel, 1982; Huang et al., 1994). Hexanal is one of many important volatile products that is a useful marker to determine decomposition of oxidized $n - 6$ polyunsaturated fatty acids (Frankel, 1982). The relative effectiveness of antioxidants is dependent on the lipid substrates, test system, concentration, oxidation time, and method used to determine lipid oxidation (Huang et al., 1996; Frankel, 1998). Antioxidant behavior is more complex when evaluated in emulsion systems than in bulk oil systems because more variables influence lipid oxidation, including the type of emulsifier, pH, and buffer system used (Cillard et al., 1980; Pryor et al., 1988; Barclay and Vinqvist, 1994). The most common emulsifiers used in the food industry are amphiphilic proteins (e.g., from casein, whey, soy, or egg), phospholipids (e.g., egg or soy lecithin) and small-molecule surfactants (e.g., Spans, Tweens, or fatty acids) (Coupland and McClements, 1996). Phospholipids are a very special type of surfactant, possessing a low critical micelle concentration in aqueous solutions, so that they tend to form lamellar mesophases and vesicles in water (Fang and Dalgleish, 1996). Previous studies in this laboratory developed various methods to evaluate antioxidants using different emulsifiers (Frankel et al., 1994, 1996, 1997; Huang et al., 1994, 1996; Hopia et al., 1996). Huang et al. (1997) showed that catechin and tea catechin were prooxidant in oil-in-water emulsions prepared with Tween 20.

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Table 1. Wines Evaluated for Antioxidant Properties

wine[a]	type	year	total phenols (Folin) GAE (mg/L)
1R (Sp)	Tempranillo	1997	2446
2R (Sp)	Garnacha	1996	1530
3R (Sp)	Garnacha	1997	1277
4R (Sp)	Tempranillo	1996	1455
5R (Sp)	Tempranillo	1996	1887
6R (Sp)	Tempranillo	1997	1678
7R (Ca)	Cabernet Sauvignon	1996	2358
8Ro (Sp)	Garnacha	1997	419
9Ro (Sp)	Garnacha	1997	486
10Ro (Sp)	Garnacha	1997	461
11Ro (Sp)	Tempranillo	1997	330
12Ro (Sp)	Tempranillo	1997	374
13W (Sp)	Malvar	1996	139
14W (Sp)	Malvar	1997	229
15W (Sp)	Verdejo	1996	178
16W (Sp)	Albillo	1997	293
17W (Sp)	Malvar	1997	239
18W (Sp)	Malvar	1997	265
19W (Ca)	Chardonnay	1996	201

[a] Wine numbers: R, red; Ro, rosé; W, white; Sp, Spain; Ca, California.

Previous studies on the antioxidant activity of various phenolic compounds found in wine were based on the in vitro oxidation of LDL to evaluate potential health effects. The objective of the present study was to evaluate the antioxidant activity of phenolic compounds in 19 different commercial wines by testing how they protect against oxidation in a food-relevant model emulsion system. The oil-in-water emulsions were prepared with corn oil stripped of natural tocopherols emulsified with soybean lecithin, a common ingredient in food products (Fang and Dalgleish, 1996). The antioxidant effectiveness of the wines was evaluated at different stages of oxidation by measuring both hydroperoxide formation, on the basis of conjugated dienes, and hydroperoxide decomposition, on the basis of hexanal formation.

MATERIALS AND METHODS

Materials. Corn oil stripped of tocopherols was obtained commercially from Acros Organics (Pittsburgh, PA). Soybean lecithin (~40% of L-α-phosphatidylcholine) was obtained from Sigma Chemical Co. (St. Louis, MO) and potassium phosphate from Fisher Scientific (Fair Lawn, NJ). Catechin, gallic acid, caffeic acid, and rutin were purchased from Sigma Chemical Co. (St. Louis, MO). Malvin chloride was purchased from Pfaltz and Bauer (Waterburg, CT). All chemicals and solvents were of analytical grade.

Wines. The different types of red, rosé, and white wines obtained from various local markets in Spain and California are listed in Table 1. Dealcoholized wine was prepared by removing alcohol with a rotating evaporator at 35 °C under vacuum, followed by purging with nitrogen at room temperature, and replacing the volume lost with distilled water.

Analyses of Phenolic Compounds. Total phenols were determined according to the Folin−Ciocalteu method (Montreau, 1972), using gallic acid as standard and expressing the results as gallic acid equivalents (GAE).

Preparation of Emulsions. A mixture of corn oil triglycerides (10%) and soybean lecithin (1%) in 25 mM potassium phosphate (pH 5.0) was emulsified by homogenization under pressure as described by Huang et al. (1999). Dealcoholized wines were added to screw-capped 50 mL Erlenmeyer flasks to reach a final concentration of total phenol of either 10 or 20 μM GAE in the 30 g emulsion samples.

Oxidation. The oxidation of emulsions was carried out at 50 °C in a shaker-oven (Lab-Line Instrument, Inc., Melrose Park, IL). The oxidative stability of these samples was determined by measuring conjugated diene hydroperoxides spectrophotometrically and hexanal by headspace gas chromatography (GC).

Measurement of Conjugated Diene Hydroperoxides. Samples (0.1 mL) were dissolved in 5 mL of methanol and diluted with more methanol to obtain measurable absorbance. The absorbance was measured at 234 nm and calculated as mmol of linoleate hydroperoxide/kg of oil as described previously (Frankel et al., 1994).

Measurement of Hexanal by Static Headspace GC. The procedures used for hexanal measurements were described previously (Frankel et al., 1994), except that all samples were equilibrated at 60 °C for 15 min before GC analyses were carried out in an Autosystem gas chromatograph equipped with a headspace autosampler, model HS-40 (Perkin-Elmer, Norwalk, CT).

HPLC Analyses. The wine samples were analyzed by the HPLC method described by Lamuela-Raventós and Waterhouse (1994). A Hewlett-Packard model 1090 (Santa Clara, CA) was used with a diode array UV−vis detector coupled with an HP Chem station. All classes of phenolic compounds were identified by analyzing the UV spectra in relation to their retention times. The phenolic compounds were thus quantified by classifying them into five groups and by calibrating with individual authentic compounds as follows: benzoic acids, as GAE, peak area 280 nm; hydroxycinnamates, as caffeic acid equivalents (CFAE), peak area 316 nm; anthocyanins, as malvin equivalents (ME), peak area 520 nm; flavan-3-ols, as catechin equivalents (CE), peak area 280 nm; flavonols, as rutin equivalents (RUE), peak area 365 nm (Meyer et al., 1997).

Statistical Analyses. Significant differences between the samples were calculated by analysis of the variance (ANOVA). One-way analysis of variance was calculated on the two duplicate measurements of both hydroperoxides and hexanal using a significance level of $p < 0.05$. All statistical analyses were performed using Minitab Statistical Software, release 9 (Addison-Wesley Publishing Co., Reading, MA).

RESULTS

The concentration of total phenol as determined by the Folin−Ciocalteu method varied from 1277 to 2446 mg/L GAE, averaging 1804 mg/L GAE, for red wines, from 330 to 486 mg/L GAE, averaging 409 mg/L GAE, for rosé wines, and from 139 to 293 mg/L GAE, averaging 221 mg/L GAE, for white wines (Table 1).

Oxidation of the control corn oil emulsion samples showed that the formation of conjugated diene hydroperoxides and the formation of hexanal accelerated after approximately 3 days (Figures 1−3). Oxidized samples were compared on day 4 during the propagation phase of the oxidation, and percentage inhibition values were calculated for both hydroperoxides and hexanal.

The corn oil emulsion oxidation assay for antioxidant activity was carried out at the same total phenol concentration equivalent to 10 and 20 μM GAE, as determined by the Folin−Ciocalteu assay. Both hydroperoxide formation and hexanal formation were inhibited by wine in corn oil emulsified with soybean lecithin in phosphate at pH 5.0 and oxidized at 50 °C. The inhibition of hydroperoxide formation at 10 μM GAE varied from 8.4% to 40.2% with the red wines, from 20.9% to 45.8% with the rosé wines, and from 6.5% to 47.0% with the white wines (Table 2). The inhibition of hydroperoxide formation at 20 μM GAE varied from 11.9% to 34.1% with the red wines, from 0.1% to 34.5% with the rosé wines, and from 3.3% to 37.2% with the white wines. The inhibition of hydroperoxide formation

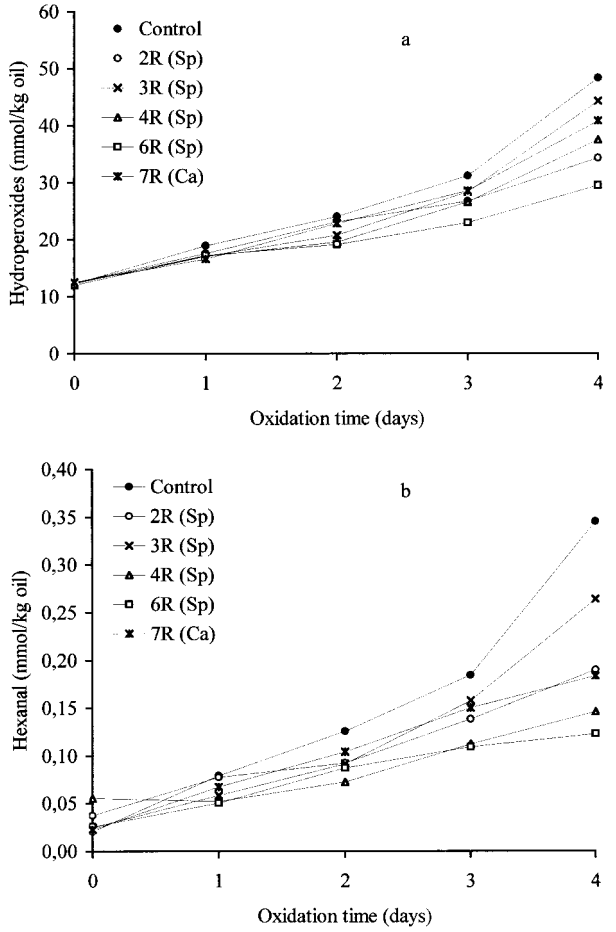

Figure 1. Effect of red wines at 10 μM on the oxidative stability of a stripped corn oil emulsion at pH 5.0 and 50 °C: (a) hydroperoxide formation; (b) hexanal formation.

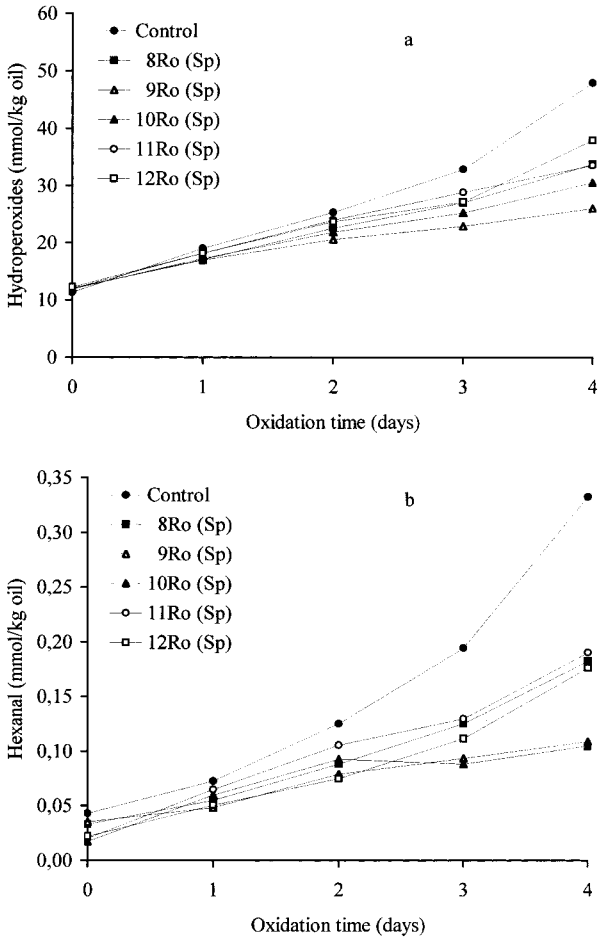

Figure 2. Effect of rosé wines at 10 μM on the oxidative stability of a stripped corn oil emulsion at pH 5.0 and 50 °C: (a) hydroperoxide formation; (b) hexanal formation.

by red wines decreased with increasing concentration, except for the 3R and 7R wines. The inhibition of hexanal formation at 10 μM GAE varied from 23.6% to 64.4% with the red wines, from 42.4% to 68.5% with the rosé wines, and from 28.4% to 68.8% with the white wines (Table 2).The inhibition of hexanal formation at 20 μM GAE varied from 33.0% to 46.3% with the red wines, from 11.3% to 66.5% with the rosé wines, and from −16.7% to +21.0% with the white wines. The inhibition of hexanal formation by red wines decreased with increasing concentration, except for the 3R wine. The inhibition of hydroperoxide and hexanal formation by rosé and white wines always decreased with increasing concentration (Table 2). In general, the antioxidant activity of the wine tested decreased with increasing concentration. The nonphenolic compounds of the wines may include prooxidant components such as trace metals that decrease the antioxidant activity at higher concentrations of the wines.

Catechin and gallic acid inhibited hydroperoxide formation by 28.6% and 21.8%, respectively, at 10 μM GAE, and by 41.4% and 40.5%, respectively, at 20 μM GAE. Catechin and gallic acid inhibited hexanal formation by 47.0% and 17.3%, respectively, at 10 μM GAE, and by 46.7% and 59.3%, respectively, at 20 μM GAE. In contrast to the wine samples, the inhibition of hydroperoxide and hexanal formation increased with increasing concentration of catechin and gallic acid. This result supports the assumption that prooxidant nonphenolic components may be present in the wines that counteract the antioxidant effects of the phenolic compounds.

Total phenols measured by the Folin−Ciocalteu method showed a significant correlation with the relative inhibition of hydroperoxides ($r = 0.86$, $r = 0.94$; in both cases $p < 0.001$) and hexanal ($r = 0.96$, $r = 0.98$; in both cases $p < 0.001$) at 10 and 20 μM GAE (Table 4). Other authors also found a significant correlation between total phenols and antioxidant activity of wines, but with different systems, such as the LDL oxidation system (Frankel et al., 1995) and free radical scavenging system (Simonetti et al., 1997; Sánchez-Moreno et al., 1998, 1999; Larrauri et al., 1999).

To relate the antioxidant activity of wines to phenolic components, the phenolic compositions of wine samples were analyzed by HPLC (Table 3). The five main groups of phenolic compounds identified included benzoic acids, cinnamic acids, anthocyanins, flavan-3-ols, and flavonols and were expressed, respectively, as equivalents of gallic acid, caffeic acid, malvin, catechin, and rutin. The HPLC analyses show that cinnamic acids were the most abundant compounds (136−911 mg/L, averaging 431 mg/L). The second most abundant compounds were flavan-3-ols (23−401 mg/L, averaging 133 mg/L). The concentration of anthocyanins ranged from 9 to 158 mg/L, averaging 64 mg/L. Benzoic acids ranged from 17 to 92 mg/L, averaging 39 mg/L. Flavonols ranged from 2 to 68 mg/L, averaging 17 mg/L (Table 3).

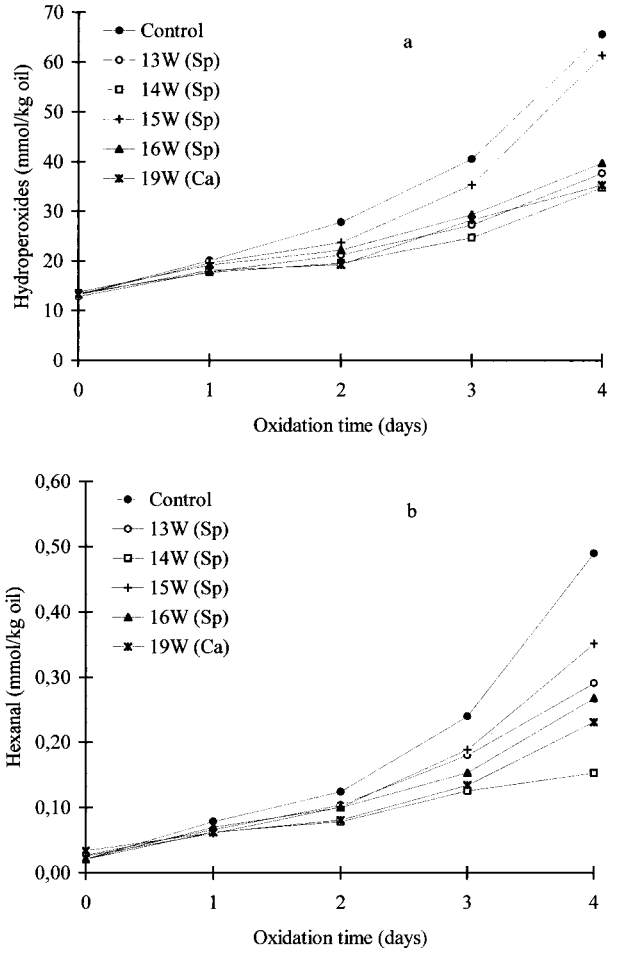

Figure 3. Effect of white wines at 10 μM on the oxidative stability of a stripped corn oil emulsion at pH 5.0 and 50 °C: (a) hydroperoxide formation; (b) hexanal formation.

Regression analyses of the relative antioxidant activity for hydroperoxides and hexanal at 10 and 20 μM GAE with the total phenol content by HPLC, benzoic acids, anthocyanins, flavan-3-ols, and flavonols, gave a significant correlation (Table 4).

Total phenols measured by HPLC showed a significant correlation for hydroperoxides ($r = 0.63$, $p = 0.004$; $r = 0.61$, $p = 0.005$) and hexanal ($r = 0.69$, $p = 0.001$; $r = 0.78$, $p < 0.001$) at 10 and 20 μM GAE with the relative antioxidant activity. This correlation was lower compared to that for total phenols by the Folin−Ciocalteu method.

Benzoic acids showed a low but significant correlation with the relative antioxidant activity in all cases (hydroperoxides and hexanal at 10 and 20 μM GAE). This is in accordance with the correlation found between gallic acid and antioxidant activity of wines (Frankel et al., 1995; Teissedre et al., 1996; Simonetti et al., 1997).

Cinnamic acids did not correlate with the relative antioxidant activity, except for hexanal at 20 μM GAE (in this case the correlation was low but significant, $r = 0.40$, $p = 0.088$). This result agrees with the results of a study on sparkling wines as inhibitors of in vitro human LDL oxidation (Satué-Gracia et al., 1999) and with the results of a study on grape extracts (Meyer et al., 1997).

The level of anthocyanins showed a significant correlation with the relative antioxidant activity for red and rosé wine samples for hydroperoxides ($r = 0.79$, $p = 0.002$; $r = 0.71$, $p = 0.010$) and for hexanal ($r = 0.84$, $p = 0.001$; $r = 0.76$, $p = 0.004$) at 10 and 20 μM GAE, respectively. These anthocyanins were absent in white wines, which are made only with white grapes. Satué-Gracia et al. (1997) studied the antioxidant activity of several anthocyanins on human low-density lipoprotein and lecithin−liposome systems. Other studies showed the antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape (Tamura and Yamagami, 1994) and the contribution of anthocyanins present in wine to the free radical scavenging activity, inhibiting LDL oxidative modification and platelet aggregation (Ghiselli et al., 1998).

The flavan-3-ol group showed strong correlation with the relative antioxidant activity. This is in accordance with the results of various studies on antioxidant activity of wines and grape juices (Frankel et al., 1995; Meyer et al., 1997; Simonetti et al., 1997).

The flavonol group showed strong correlation with the relative antioxidant activity. In another study quercetin-3-glucuronide showed low but significant correlation with the relative inhibition of LDL oxidation (Satué-Gracia et al., 1999).

Table 2. Inhibition of Hydroperoxide and Hexanal Formation by Wines in Corn Oil Emulsified with Soybean Lecithin in 25 mM Potassium Phosphate at pH 5.0 and 50 °C (Percent Inhibition, Mean ± SD)[a,b]

	hydroperoxides (day 4)		hexanal (day 4)	
wine	10 μM	20 μM	10 μM	20 μM
1R (Sp)	40.2 ± 3.0^{c}	30.6 ± 0.4^{f}	61.0 ± 0.3^{c}	46.1 ± 1.8^{c}
2R (Sp)	29.0 ± 0.4^{e}	19.5 ± 0.4^{j}	45.0 ± 1.9^{gh}	43.2 ± 2.1^{cde}
3R (Sp)	8.4 ± 0.4^{h}	11.9 ± 0.7^{l}	23.6 ± 2.2^{k}	33.0 ± 1.5^{f}
4R (Sp)	22.5 ± 0.3^{f}	21.3 ± 0.4^{hij}	57.8 ± 1.0^{d}	40.6 ± 2.6^{e}
5R (Sp)	39.8 ± 0.8^{c}	23.0 ± 0.5^{h}	60.4 ± 1.7^{cd}	45.3 ± 0.4^{cd}
6R (Sp)	39.0 ± 1.1^{c}	20.4 ± 0.3^{ij}	64.4 ± 2.1^{b}	41.7 ± 3.6^{de}
7R (Ca)	15.7 ± 0.4^{g}	34.1 ± 1.8^{d}	46.9 ± 0.7^{fg}	46.3 ± 1.8^{c}
8Ro (Sp)	29.5 ± 1.3^{e}	26.8 ± 0.8^{g}	45.1 ± 0.1^{gh}	43.1 ± 5.8^{cde}
9Ro (Sp)	45.8 ± 0.4^{a}	33.9 ± 1.6^{de}	67.3 ± 0.1^{a}	66.5 ± 0.7^{a}
10Ro (Sp)	36.3 ± 0.4^{d}	34.5 ± 0.8^{cd}	68.5 ± 0.7^{a}	63.7 ± 1.3^{a}
11Ro (Sp)	30.0 ± 0.4^{e}	11.0 ± 1.7^{l}	42.7 ± 0.0^{hi}	28.9 ± 1.5^{f}
12Ro (Sp)	20.9 ± 0.7^{f}	0.1 ± 1.8^{n}	47.0 ± 0.7^{fg}	11.3 ± 2.2^{h}
13W (Sp)	42.5 ± 0.1^{b}	22.3 ± 0.4^{hi}	40.7 ± 1.2^{i}	2.0 ± 0.6^{j}
14W (Sp)	47.0 ± 0.7^{a}	36.1 ± 0.4^{bc}	68.8 ± 0.6^{a}	7.3 ± 0.4^{hi}
15W (Sp)	6.5 ± 2.1^{h}	3.3 ± 0.1^{m}	28.4 ± 0.2^{j}	-16.7 ± 0.7^{k}
16W (Sp)	39.4 ± 0.1^{c}	37.2 ± 0.3^{b}	45.4 ± 0.8^{fgh}	21.0 ± 0.9^{g}
17W (Sp)	46.0 ± 0.2^{a}	32.0 ± 0.4^{ef}	47.1 ± 1.1^{fg}	8.9 ± 0.6^{hi}
18W (Sp)	45.7 ± 0.3^{a}	30.3 ± 0.4^{f}	48.0 ± 1.0^{f}	4.9 ± 0.9^{ij}
19W (Ca)	46.1 ± 0.1^{a}	17.2 ± 0.6^{k}	53.0 ± 1.0^{e}	2.9 ± 0.3^{j}
Catechin	28.6 ± 0.7^{e}	41.4 ± 1.6^{a}	47.0 ± 2.5^{fg}	46.7 ± 2.9^{c}
Gallic acid	21.8 ± 0.2^{f}	40.5 ± 0.4^{a}	17.3 ± 2.3^{l}	59.3 ± 0.7^{b}

[a] Percent inhibition = $[(C - S)/C] \times 100$, where C = hydroperoxides or hexanal formed in the control and S = hydroperoxides or hexanal formed in the sample. Negative values indicate prooxidant activity. SD = standard deviation, $n = 2$. [b] Values within each column followed by the same superscript letter are not significantly different ($p < 0.05$).

DISCUSSION

The total phenol content varied in different types of wine, depending on the grape variety, environmental factors in the vineyard, and wine processing techniques. The total phenol as determined by the Folin−Ciocalteu method varied from 139 to 2446 mg/L GAE. These values are in accordance with the values reported by other authors also determined by the Folin−Ciocalteu method (Kanner et al., 1994; Frankel et al., 1995; Simonetti et al., 1997; Sánchez-Moreno et al., 1999). The

Table 3. Classes of Phenolic Compounds Identified in Wine Samples by HPLC[a]

wine sample	total phenols by HPLC (mg/L)	benzoic acids, GAE (mg/L)	cinnamic acids, CFAE (mg/L)	anthocyanins, ME (mg/L)	flavan-3-ols, CCE (mg/L)	flavonols, RUE (mg/L)
1R (Sp)	953	62 (6)	403 (42)	158 (17)	285 (30)	45 (5)
2R (Sp)	1287	92 (7)	911 (71)	24 (2)	236 (18)	23 (2)
3R (Sp)	1136	59 (5)	833 (73)	36 (3)	176 (16)	31 (3)
4R (Sp)	1068	68 (7)	561 (53)	132 (12)	282 (26)	25 (2)
5R (Sp)	1190	51 (4)	559 (47)	153 (13)	377 (32)	50 (4)
6R (Sp)	870	17 (2)	526 (61)	63 (7)	247 (28)	17 (2)
7R (Ca)	856	44 (5)	266 (31)	78 (9)	401 (47)	68 (8)
average		56	580	92	286	37
8Ro (Sp)	822	20 (3)	714 (87)	18 (2)	61 (7)	9 (1)
9Ro (Sp)	841	26 (3)	690 (82)	31 (4)	85 (10)	8 (1)
10Ro (Sp)	964	70 (7)	805 (84)	48 (5)	31 (3)	10 (1)
11Ro (Sp)	278	25 (9)	175 (63)	24 (9%)	51 (18)	4 (1)
12Ro (Sp)	314	24 (8)	239 (76)	9 (3%)	36 (11)	5 (2)
average		33	524	24	53	7
13W (Sp)	188	20 (11)	143 (76)		23 (12)	2 (1)
14W (Sp)	340	26 (7)	255 (75)		57 (17)	2 (1)
15W (Sp)	269	17 (6)	212 (79)		36 (13)	4 (2)
16W (Sp)	325	36 (11)	242 (75)		42 (13)	5 (1)
17W (Sp)	329	28 (8)	269 (82)		25 (8)	7 (2)
18W (Sp)	324	28 (8)	259 (80)		31 (10)	6 (2)
19W (Ca)	202	28 (14)	136 (67)		34 (17)	4 (2)
average		26	217		36	4

[a] Values in percent in parentheses are relative to the total phenols as measured by HPLC. GAE, gallic acid equivalents; CFAE, caffeic acid equivalents; ME, malvin equivalents; CCE, catechin equivalents; RUE, rutin equivalents.

Table 4. Regression Analyses of Relative Inhibition of Hydroperoxides and Hexanal at 10 and 20 μM GAE with Total Phenol Content (Folin−Ciocalteu and HPLC) and the Five Main Groups of Phenolic Compounds Identified

	hydroperoxides (day 4)				hexanal (day 4)			
	10 μM		20 μM		10 μM		20 μM	
compound	r	p	r	p	r	p	r	p
total phenols (Folin−Ciocalteu)	0.86	0.000	0.94	0.000	0.96	0.000	0.98	0.000
total phenols (HPLC)	0.63	0.004	0.61	0.005	0.69	0.001	0.78	0.000
benzoic acids	0.43	0.068	0.46	0.046	0.48	0.038	0.57	0.011
cinnamic acids	0.28	0.237	0.20	0.407	0.29	0.226	0.40	0.088
anthocyanins	0.79	0.002	0.71	0.010	0.84	0.001	0.76	0.004
flavan-3-ols	0.79	0.000	0.89	0.000	0.92	0.000	0.94	0.000
flavonols	0.69	0.001	0.92	0.000	0.84	0.000	0.92	0.000

total phenols found in these wine samples (188−1287 mg/L) measured by HPLC were lower than those reported in red, rosé, and white wines in this study and by other authors, on the basis of the Folin−Ciocalteu method (Frankel et al., 1995; Sánchez-Moreno et al., 1999). The difference may be due to the retention of high molecular weight wine components by the HPLC method. The levels of total phenol in wines determined according to the Folin−Ciocalteu method are also not absolute measurements of the amounts of phenolic materials but are based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid.

The amount of total phenolics is influenced by various factors. The relative antioxidant activity of wines correlated better with total phenols measured by the Folin−Ciocalteu method than with total phenols measured by HPLC (Table 4). In our study, the differences in antioxidant activity among the wines tested were not always in accordance with the differences in total phenols.

This study demonstrates that the wines tested were antioxidants in corn oil emulsions prepared with lecithin as emulsifier. The inhibition of hydroperoxide formation by wines decreased with increasing concentration, except for the 3R and 7R wines (Table 2), and the inhibition of hexanal formation by wines decreased with increasing concentration except for the 3R wine (Table 2). This effect could be explained by the presence of prooxidant components in the wines, such as trace metals, which would decrease the antioxidant activity of the phenolic compounds at higher concentrations.

HPLC analyses of the wine samples showed that the major phenolic compounds were cinnamic acids and flavan-3-ols, followed by anthocyanins (in red and rosé wines), benzoic acids, and flavonols (Table 3). Similar results were obtained in other studies with Spanish white wines from different areas and grape varieties (Betés-Saura et al., 1996; Satué-Gracia et al., 1999). Cinnamic acids were the major phenolics in all wines, except in the red Cabernet Sauvignon wine 7R from California. California wine 7R had higher levels of flavan-3-ols compared to Spanish wines; this agrees with the data from a study on 20 California wines (Frankel et al., 1995).

To explain the relations among the five classes of phenolic compounds identified in wine samples and the antioxidant activity of these wines is difficult because in different wines we need to consider possible interaction effects (synergism and antagonism) of these phenolic compounds (Meyer et al., 1998). In red wines, the 1R, 5R, 6R, and 7R wines gave better results in the inhibition of hydroperoxide and hexanal formation.

Among them, in the 1R wine the concentration of anthocyanins was higher than that in the other wines. The 2R wine had higher levels of benzoic and cinnamic acids than the other red wines. In addition, the red Cabernet Sauvignon wine 7R (from California) had higher levels of flavan-3-ols and flavonols than the other red wines.

In rosé wines, the rosé Garnacha wines 9Ro and 10Ro gave better results on antioxidant activity. The 10Ro wine had the highest levels of benzoic and cinnamic acids and flavonols. The 9Ro wine had the highest level of flavan-3-ols. The higher antioxidant activity of rosé wines from the Garnacha grape variety than that for rosé wines from the Tempranillo grape variety agrees with other results on free radical scavenging capacity (Sánchez-Moreno et al., 1999).

In white wines, the 14W, 16W, 17W, and 19W wines gave better results on the inhibition of hydroperoxide and hexanal formation compared to the other white wines tested. The 14W wine had the highest level of flavan-3-ols. The 16W wine had the highest levels of benzoic acid. The 17W wine had the highest level of cinnamic acids and flavonols.

In this study, catechin and gallic acid had high activity in the inhibition of hydroperoxide and hexanal formation at 20 μM. This result agrees with our previous study on the antioxidant activities of pure phenolic compounds in inhibiting LDL oxidation, showing that catechin was the most active antioxidant of the phenolic compounds tested (Teissedre et al., 1996). In the same way, the antioxidant activities of 14 different grape extracts were compared at the same molar phenol concentrations. Pure catechin used as a reference was consistently more active than the grape extracts (Meyer et al., 1997). However, catechin and gallic acid had low activity at 10 μM.

Although the phenolic compounds have similar chemical properties, their reducing capacity is not a very precise predictor of their antioxidant activity. In the inhibition of hydroperoxide and hexanal formation and other tests for antioxidant activity, the system is typically heterogeneous and physical properties, such as lipophilicity, solubility, and partitioning between the aqueous and lipid phases, can become important in determining antioxidant activity (Frankel, 1993; Frankel et al., 1994).

This study confirms the potential antioxidant activities of the wine phenolics in corn oil emulsion systems. In addition, the results demonstrate that rosé and white wines, although with a polyphenolic concentration lower than that of red wine, could have a greater antioxidative capacity when compared at the same phenolic concentration. The different activities of the samples can be ascribed to their different phenolic compositions. Further studies are needed with individual phenolic compounds of wines to elucidate the different antioxidant mechanisms, and possible synergism.

ABBREVIATIONS USED

CE, catechin equivalents; CFAE, caffeic acid equivalents; GAE, gallic acid equivalents; GC, gas chromatography; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; ME, malvin equivalents; r, correlation coefficient; RUE, rutin equivalents; Tween 20, polyoxyethylene sorbitan monolaurate.

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