Antioxidant Activity of Grape Extracts in a Lecithin Liposome System

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ABSTRACT: Extracts of 14 different grapes were tested for their antioxidant activities in a copper-catalyzed lecithin liposome oxidation assay and analyzed for their phenolic components by high-performance liquid chromatography (HPLC). The total phenolic contents of the grape extracts varied from 176 to 1236 mg gallic acid equivalents (GAE)/L. Extracts of red wine grape varieties contained higher concentrations of phenolics than other varieties. When compared at the same 20 μM GAE basis, the grape extracts inhibited formation of conjugated diene hydroperoxides by 25.1 to 67.9%, and hexanal formation by 49.3 to 97.8%. Extracts of red table grape varieties Red Globe and Emperor and white wine grape varieties Chardonnay and Sauvignon Blanc gave the highest antioxidant activities. The relative percentage inhibition of conjugated dienes and hexanal correlated with total phenols (r = 0.86 and 0.89). HPLC analyses showed that anthocyanins were the most abundant phenolic compounds in extracts of red grapes, and flavonols were most abundant in extracts of white grapes.

JAOCS 74, 1301–1307 (1997).

KEY WORDS: Antioxidant activity, extraction, grapes, lecithin, liposome, oxidation, phenolics.

Phenolic antioxidants in fruits and vegetables are generally considered to have a protective effect on coronary heart disease and cancer, but the molecular basis of protection is not understood (1). Grapes are the world's largest fruit crop, which approximates an annual production of 65 million metric tons (2) and constitutes the major source of phenolic compounds among different fruits and vegetables (3). About 80% of the total crop is used in wine making, 13% is sold as table grapes, and the balance is grown largely for raisins, juice, and other products.

Considerable interest has been given to the application of natural antioxidants in foods and biological systems because of their potential nutritional and therapeutic effects. The evidence for the benefits of natural antioxidants in inhibiting lipid oxidation in critical food and biological systems has been debated (4). Although the antioxidant activity of wines has been reported in many systems (5–8), little is known about the antioxidant activity of grapes in spite of their high level of phenolic compounds.

Natural antioxidants have been particularly difficult to evaluate in oils and food emulsions owing in part to the complex interfacial phenomena involved. In heterogeneous food systems, the physical properties, such as lipophilicity, solubility, and partition between the aqueous and lipid phases, can become important in determining antioxidant activity (9). The type and polarity of the lipid system used as substrate significantly affect the activity of natural antioxidants (10). Thus, in the evaluation of natural antioxidants, varied results can be obtained with different lipid substrates, and with various methods measuring products at different stages of lipid oxidation. Antioxidants can have different effects in inhibiting hydroperoxide formation and in preventing their decomposition. Thus, α-tocopherol exhibited prooxidant activity with bulk corn oil and with corn oil-in-water emulsions at high concentrations, on the basis of conjugated diene formation, but it had antioxidant activity on the basis of hexanal formation (9). Hexanal determinations, measuring decomposition of hydroperoxides, may be assumed to be closely related to flavor deterioration. Thus, the effects of antioxidants in inhibiting hydroperoxide decomposition may have direct implications in relation to flavor deterioration due to lipid oxidation. Although triglycerides and phospholipids are the major lipids in food systems, linoleic acid has been used commonly to evaluate natural antioxidants (10). We recently showed, however, that linoleic acid may not be a valid substrate for evaluating food antioxidants because it has unique physical properties in aqueous micelles that affect the concentration and location of antioxidants of different polarity (11,12). Unsaturated triglycerides and phospholipids may be considered as being more relevant substrates for evaluating antioxidant activity in lipid food systems.

The objectives of the present study were (i) to test the antioxidant activity of grape extracts in a lecithin liposome system and (ii) to relate their antioxidant activity to the phenolic compositions of extracts analyzed by high-performance liquid chromatography (HPLC). The antioxidant activities of grape phenolics were evaluated in a lecithin liposome assay as a food model test system oxidized in the presence of copper ions.

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EXPERIMENTAL PROCEDURES

Materials. Catechin, gallic acid, caffeic acid, rutin, Folin & Ciocalteu's phenol reagent (2.0 N), and L-*a*-phosphatidylcholine (lecithin from soybean) were purchased from Sigma Chemical Company (St. Louis, MO). The phosphatidylcholine content of the soybean lecithin was approximately 40%. Malvin chloride (malvidin-3,5-diglucoside chloride) was obtained from Pfaltz and Bauer (Waterbury, CT).

Grape samples. Grapes from 14 varieties of *Vitis vinifera* or *V. labrusca* grown on the campus of the University of California at Davis by the Department of Viticulture and Enology were selected for this study. These samples included seven varieties of table grapes and seven varieties of wine grapes (Table 1). All of the grapes were harvested within optimal commercial maturity on the basis of sugar content: for table grapes within 16.5–18.5 Brix, and for wine grapes within 22–24 Brix. The sugar content (in Brix) was measured in the field directly on the pressed grape juice by a portable refractometer (Fisherbrand, Pittsburgh, PA). After harvest, undamaged and disease-free berries were snipped from clusters and washed several times with distilled water. Seeds were manually separated from whole grapes. Whole grapes without seeds were homogenized in a Waring blender for 1 min at maximal speed and mixed with seeds. Samples of grape extracts containing intact seeds were then divided into smaller aliquots and frozen at –30°C until use.

Preparation of grape extracts. The ground grapes (2.0 g) were homogenized for 1 min with aqueous methanol (10.0 mL). The mixture was filtered (Whatman no. 1 filter; Maidstone, England) and the methanol removed by rotary evaporation under vacuum at 45°C. The samples were then diluted with deionized water to a final volume of 5.0 mL and filtered through a 0.45 μM polytetrafluoroethylene (PTFE) membrane filter (Scientific Resources Inc., Eatontown, NJ).

TABLE 1
Pressed Grape Juices Used in This Study

Grape	Abbreviations	Type	Brix[a]
Calzin	Ca	Red wine	23.3
Petite Sirah	PS	Red wine	23.4
Niabell	Ni	Blush table	18.5
Concord	Co	Red table	18.0
Merlot	Me	Red wine	22.0
Cabernet Sauvignon	CS	Red wine	23.2
Cabernet Franc	CF	Red wine	23.0
Flame seedless	FS	Red table	18.0
Sauvignon Blanc	SB	White wine	22.6
Emperor	Em	Blush table	17.0
Chardonnay	Ch	White wine	23.4
Thompson seedless	TS	White table	19.0
Red Globe	RG	Blush table	17.5
Red Malaga	RM	Blush table	17.0

[a]Brix (percentage sugar content) was measured in the field by pressing the juice of several grapes harvested using a portable refractometer (Fisherbrand, Pittsburgh, PA).

Analyses of phenolic compounds. Total phenols were assayed colorimetrically by a modified procedure of Singleton and Rossi (13) as follows. Samples (0.2 mL) were mixed with 1.0 mL of tenfold diluted Folin-Ciocalteu reagent and 0.8 mL 7.5% sodium carbonate solution. After standing 30 min at room temperature, the absorbance was measured at 765 nm. The content of phenolics was expressed as gallic acid equivalents (GAE) in mg/L.

The phenolic composition of grape extracts was analyzed by HPLC as described by Lamuela-Raventos and Waterhouse (14). A Hewlett-Packard (Santa Clara, CA), model 1090, was used with three low-pressure pumps and a diode array ultraviolet (UV)-visible detector coupled to a Hewlett-Packard Chem Station. A Novapak C18 column, 3.9 × 150 mm, 4 μm particle size (Waters Chromatography, Millipore, Milford, MA) was used with a flow rate of 0.5 mL/min. Based on spectral identifications, the phenolic compounds were classified into four different groups and quantified by using authentic compounds as follows: hydroxycinnamates as caffeic acid equivalents (CFAE) in mg/L, peak area at 316 nm; anthocyanins as malvin equivalents (ME) in mg/L, peak area at 520 nm; flavan-3-ols as catechin equivalents (CCE) in mg/L, peak area at 280 nm; and flavonols as rutin equivalents (RUE) in mg/L, peak area at 365 nm.

Lecithin liposome oxidation assay. Lecithin was suspended in doubly deionized water at a concentration of 8 mg/mL by sonication and stirring with a glass rod in a bath-type sonicator (Ultrasonics Inc., New York, NY). For the antioxidant assay, liposome samples were weighed into screw-capped 50-mL Erlenmeyer flasks and diluted with doubly deionized water to a final concentration of 0.8% by weight of lecithin. The particle sizes of liposomes were 0.03–0.1 μM (Microtrac Ultrafine Particle Analyzer; Leeds & Northrup, North Wales, PA). To test antioxidant activity, grape extracts were added to the liposome system to a final concentration of 20 μM GAE. After addition of grape extracts, the liposome suspension was sonicated again for 1 min (15) and oxidized with cupric acetate (3.0 μM) and shaking (120 rpm) at 37°C in the dark. Liposome oxidation was monitored by determining production of conjugated diene hydroperoxides and hexanal (9,16).

Measurement of conjugated diene hydroperoxides. Liposome samples (0.1 g) were dissolved in methanol (5.0 mL), and conjugated dienes were measured at 234 nm. Results were calculated as hydroperoxides in millimoles per kg of phosphatidylcholine by using a molar absorptivity of 26,000 for linoleate hydroperoxides (17).

Measurement of hexanal. Liposome samples (1.0 g) were weighed into 22-mL headspace vials and sealed with silicone rubber-Teflon caps (SunBrokers, Wilmington, NC). Hexanal was measured by static headspace gas chromatography by a rapid procedure as described previously (9,16,18).

Percentage inhibition and relative percentage inhibition. Antioxidant activities of grape extracts were calculated as percentage inhibition of conjugated diene and hexanal production and expressed as:

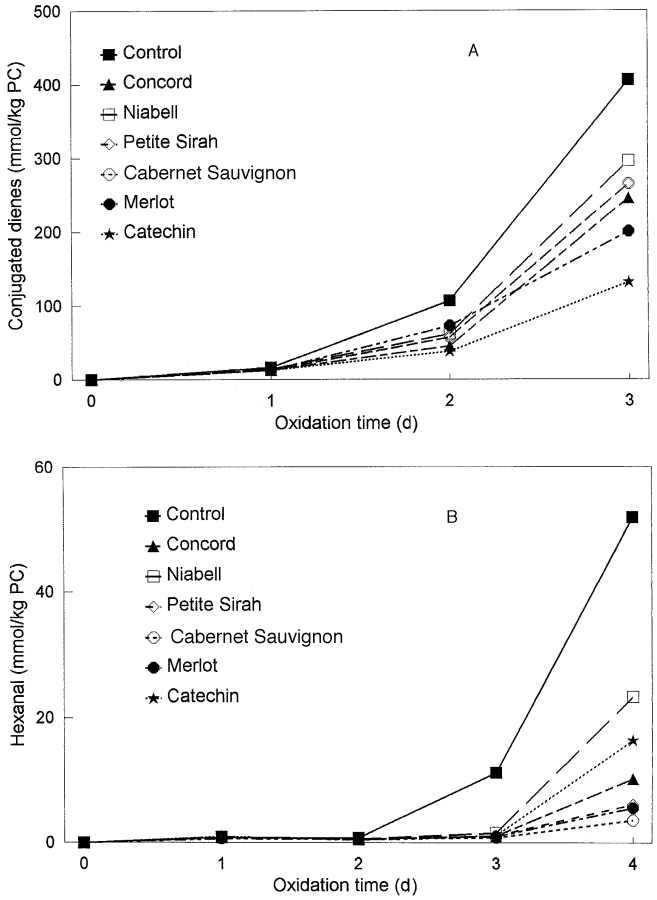

FIG. 1. Antioxidant activity of grape extracts in inhibiting the oxidation of lecithin liposomes at 37°C in the presence of 3 μM copper acetate. (A) Formation of conjugated dienes, (B) formation of hexanal. PC, phosphatidylcholine.

$$\% \text{ inhibition} = [(C - S)/C)] \times 100 \quad [1]$$

where C was the amount of conjugated diene hydroperoxides (or hexanal) formed in the control sample and S was the amount of conjugated diene hydroperoxides (or hexanal) formed in the sample containing grape extract. Relative percentage inhibition of oxidation was calculated by multiplying the values of percentage inhibition at 20 μM total phenols by the dilution factor and by taking the highest values as 100% (6). All analyses were done in duplicate. Typical oxidation curves are shown in Figure 1.

Differences in antioxidant activities were tested statistically by one-way analysis of variance (Minitab Statistical Software, Addison-Wesley, Reading, MA). Significance level was $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Extraction of grapes. The efficiency of the solvent extraction was determined by testing the effect of different mixtures of water and methanol on total phenolic contents of the extracts. The efficiency of extraction as measured by total phenols increased with decreasing methanol content of different water–methanol mixtures (Fig. 2). The concentration of total phenols reached a maximum between 50 and 70% methanol. Because the rate of filtration was very slow at 50% methanol, the water–methanol mixture containing 60% methanol was chosen to prepare all grape extracts.

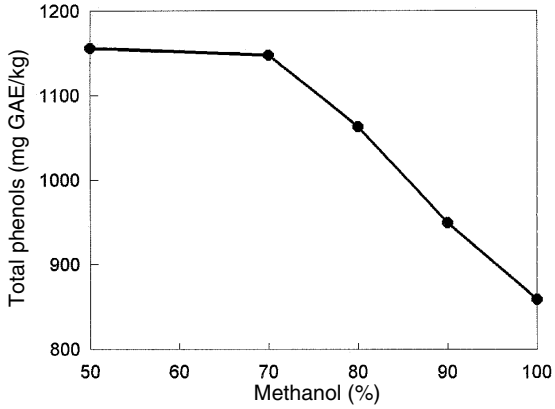

FIG. 2. Effect of methanol contents of water–methanol mixtures on extraction of total phenols in black seedless grapes. GAE, gallic acid equivalents.

Alonso *et al.* (19) investigated the effects of a number of mixtures of ethanol–water to extract catechins and proanthocyanidins from grape seeds. They reported that the extraction was more efficient when the ethanol content of the extractant was increased and the extraction time increased from 3 to 72 h. On the other hand, Kallithraka *et al.* (20) reported that methanol was the best solvent for the quantitative extraction of (+)-catechin, (−)-epicatechin, and epigallocatechin from grape seeds. Obviously, extraction yields will be expected to depend on the type of solvent and extraction time employed since grapes contain a great variety of different phenolic compounds having different polarity and solubility. Longer extraction times increase the possibility of oxidation of phenolics unless reducing agents are added to the solvent system (21). However, the addition of antioxidative reducing agents is precluded in the present study of the antioxidant potency of endogenous grape phenolics.

Because the present study was aimed at simulating the actual antioxidants consumed in grapes and at minimizing the oxidation of phenolic antioxidants, we limited our evaluations to a short 1-min extraction of grapes. A contact time of 1 min was used to extract fresh grapes with aqueous methanol solution containing 40% water. No reducing agent was added.

Total phenolic contents. The total phenolic concentrations varied from 176 to 738 mg GAE/L in the table grapes, and from 230 to 1236 mg GAE/L grapes in the wine grapes (Table 2). The red wine Calzin and Petite Sirah grapes had the highest phenol content. Our data are in agreement with the findings of Kanner *et al.* (7) who reported that the total phenolic concentrations of Thompson seedless and Flame seedless grapes extracted with 80% ethanol were 260 mg/kg and 850 mg/kg, respectively, expressed in molar equivalents of quercetin. Kanner *et al.* (7) also showed that the red wine Cabernet Sauvignon and Petite Sirah grapes contain higher

TABLE 2
Total Phenolic Contents of Grapes and Grape Extracts

Grape	Total phenols	
	Extracts (mg GAE/L)[a]	Fresh materials (mg GAE/kg)[b]
Calzin	1236 ± 32 a	3090
Petite Sirah	1115 ± 89 b	2787
Niabell	738 ± 27 c	1845
Concord	693 ± 70 c	1732
Merlot	569 ± 33 d	1422
Cabernet Sauvignon	565 ± 47 d	1412
Cabernet Franc	550 ± 36 d,e	1365
Flame seedless	432 ± 2 e	1080
Sauvignon Blanc	264 ± 11 f	660
Emperor	259 ± 18 f	647
Chardonnay	230 ± 37 f	575
Thompson seedless	198 ± 27 f	495
Red Globe	179 ± 18 f	447
Red Malaga	176 ± 11 f	440

[a]Values followed by the same letters are not significantly different at $P < 0.05$. Values are mean ± standard deviation, $n = 3$.
[b]Calculated from analyses of extracts. GAE, gallic acid equivalents.

concentrations of phenolics than the table varieties Thompson, Flame, and black seedless grapes.

Antioxidant activity. The antioxidant activities of the grape extracts were estimated by determining conjugated diene hydroperoxide and hexanal formation in lecithin liposomes oxidized at 37°C in the presence of cupric acetate. The formation of conjugated diene hydroperoxides in the liposome system exhibited an induction period of 1 d followed by significant increases in rates varying according to the samples tested (Fig. 1A). The rate of hexanal formation followed the production of conjugated dienes by about 1 d with an induction period of 2 d for the control and 3 d for the samples containing grape extracts (Fig. 1B). From these results, the antioxidant activity of grape extracts was determined after 3 d based on conjugated diene formation, and after 4 d based on hexanal formation. All the grape extracts tested showed good antioxidant activity in inhibiting liposome oxidation (Table 3). When compared at 20 μM GAE, the grape extracts inhibited conjugated diene formation between 25.1 and 67.9% and hexanal formation between 49.3 and 97.8%. On the basis of conjugated diene formation, pure catechin had the highest antioxidant activity. However, this antioxidant activity was not significantly greater than the activities of the Emperor and Red Globe grape extracts. The grape extracts showed the following decreasing trend in antioxidant activity: Red Globe, Emperor > Thompson seedless, Cabernet Sauvignon, Chardonnay, Sauvignon Blanc > Flame seedless > Red Malaga, Merlot >Concord > Petite Sirah, Cabernet Franc > Calzin, Niabell.

On the other hand, on the basis of hexanal formation, all the grape extracts except Niabell, Red Malaga, and Calzin were better antioxidants than pure catechin. Extracts from Red Globe, Emperor, and Chardonnay retarded the hexanal formation by more than 95% (Table 3). The order of antioxidant activity was: Chardonnay, Red Globe > Sauvignon Blanc, Emperor > Cabernet Sauvignon > Flame seedless > Petite Sirah, Thompson seedless, Merlot > Concord > catechin, Cabernet Franc > Red Malaga > Niabell > Calzin.

When evaluated at the same total phenol concentration as GAE, the phenol components in Red Globe, Chardonnay, Emperor, and Sauvignon Blanc grapes were thus more active antioxidants than those in other grapes. The antioxidant activities of the undiluted extracts inhibited conjugated diene

TABLE 3
Inhibition of CD and Hx Formation and Phenolic Composition by HPLC of Grape Extracts[a]

Grape	% Inhibition of CD	% Inhibition of Hx	Dilution factor	OH cinnamates (CFAE mg/L)[b]	Anthocyanins (ME mg/L)[c]	Flavan-3-ols (CCE mg/L)[d]	Flavonols (RUE mg/L)[e]
Ca	25.1 ± 1.3 a	49.3 ± 0.9 a	373.5	3.5 ± 0.2 (0.4)	851 ± 6.3 (96.6)	15.2 ± 0.2 (1.7)	11.0 ± 0.1 (1.2)
PS	34.6 ± 2.1 b	88.3 ± 0.1 f	345.4	10.4 ± 0.2 (0.6)	1685 ± 10 (97.6)	10.6 ± 0.2 (0.6)	19.8 ± 0.0 (1.1)
Ni	27.0 ± 1.8 a	55.1 ± 0.1 b	207.9	7.2 ± 0.2 (1.0)	684 ± 5.6 (99.0)	0.0 (0)	0.0 (0)
Co	39.5 ± 2.1 c	80.4 ± 0.2 e	180.8	12.5 ± 0.2 (2.5)	468 ± 4.6 (93.6)	0.0 (0)	19.5 ± 0.1 (3.9)
Me	50.7 ± 1.7 d,e	89.4 ± 0.1 f,g	157	5.3 ± 0.1 (1.1)	437 ± 4.5 (93.2)	0.0 (0)	26.8 ± 0.0 (5.7)
CS	55.7 ± 0.6 f	93.2 ± 0.1 h	151.8	0.8 ± 0.2 (0.1)	640 ± 5.4 98.4)	0.0 (0)	9.3 ± 0.1 (1.4)
CF	37.1 ± 2.6 b,c	68.7 ± 0.4 d	165.5	0.9 ± 0.2 (0.1)	922 ± 6.7 (98.3)	0.0 (0)	15.3 ± 0.1 (1.6)
FS	51.3 ± 1.7 e	90.6 ± 0.2 g	127.7	5.8 ± 0.2 (4.9)	89.1 ± 3.0 (75.6)	6.5 ± 0.5 (5.5)	16.4 ± 0.1 (13.9)
SB	63.2 ± 0.2 f,g	94.8 ± 0.1 i	81	6.5 ± 0.2 (39.4)	0.0 (0)	0.0 (0)	10.0 ± 0.1 (60.6)
Em	67.9 ± 1.3 g	97.1 ± 0.1 ij	75.2	4.0 ± 0.2 (22.0)	11.4 ± 2.5 (62.6)	0.0 (0)	2.8 ± 0.1 (15.4)
Ch	57.7 ± 1.0 f	97.8 ± 0.1 j	56.9	1.4 ± 0.2 (25.0)	0.0 (0)	0.0 (0)	4.2 ± 0.1 (75.0)
TS	58.5 ± 1.0 f	89.3 ± 0.2 f,g	50.5	3.8 ± 0.2 (29.9)	0.0 (0)	0.0 (0)	8.9 ± 0.1 (70.1)
RG	66.5 ± 0.6 g	97.8 ± 0.1 j	46.6	2.7 ± 0.2 (10.8)	19.6 ± 2.6 (78.7)	0.0 (0)	2.6 ± 0.1 (10.4)
RM	47.2 ± 0.7 d	62.4 ± 0.8 c	48.1	3.7 ± 0.2 (14.1)	21.1 ± 2.6 (80.5)	0.0 (0)	1.4 ± 0.1 (5.3)
Catechin	67.6 ± 2.2 g	68.5 ± 0.4 d					

[a]See Tables 1 and 2 for other abbreviations. Inhibition of conjugated dienes (CD) and hexanal (Hx) determined at 20 μM total phenols as GAE in mg/L. Values are mean ± SD ($n = 2$). Values followed by the same letter are not significantly different at $P < 0.05$. Number in parentheses is the relative percentage of each compound. HPLC, high-performance liquid chromatography.
[b]CFAE, caffeic acid equivalents.
[c]ME, malvin equivalents.
[d]CCE, catechin equivalents.
[e]RUE, rutin equivalents.

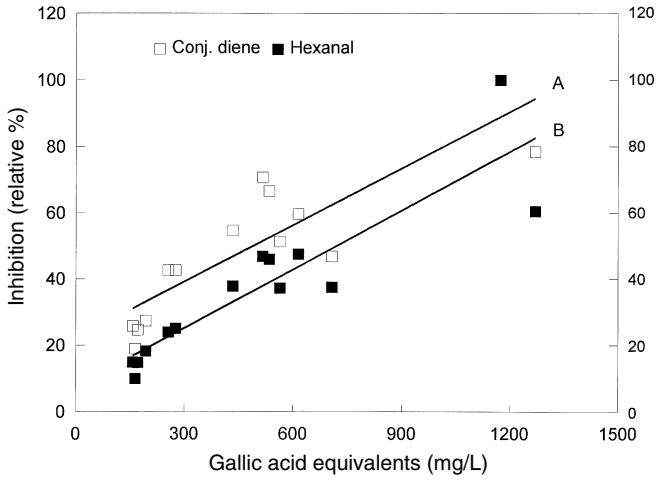

FIG. 3. Relative percentage inhibition of liposome oxidation by grape extracts vs. phenol content as gallic acid equivalents (relative percentage inhibition was calculated by multiplying the inhibition values by the dilution factor and by taking the highest value as 100%). (A) calculated conjugated (conj.) diene; (B) calculated hexanal.

formation at levels ranging from 19.0 to 59.7% with the table grapes, and from 27.4 to 100% with the wine grapes (Fig. 3). The corresponding relative inhibition of hexanal formation varied from 9.9 to 47.6% with the table grapes, and from 18.3 to 100% with the wine grapes. As expected the red table and, especially, red wine grape varieties such as Petite Sirah, Calzin, and Cabernet Sauvignon had higher relative antioxidant activity than the white varieties when evaluated on the basis of their total phenol content. The relative percentage inhibition of conjugated diene and hexanal formation correlated highly with total phenolic contents, and the calculated *r* were 0.86 and 0.89, respectively.

Phenolic composition of grape extracts. The major classes of phenolic compounds in grape extracts were analyzed by HPLC (Table 3). The concentration of hydroxycinnamates, expressed as caffeic acid equivalents, ranged from 0.8 to 12.5 mg/L, averaging 4.9 mg/L. The Concord grapes had the highest hydroxycinnamate content followed by the Petite Sirah, Niabell, Sauvignon Blanc, and Merlot. As expected, anthocyanins were the most abundant phenolic components in the red grapes, ranging from 684 to 1685 mg/L in the extracts of Petite Sirah, Cabernet Franc, Calzin, and Niabell. Cabernet Sauvignon, Merlot, and Petite Sirah were earlier reported to have a high percentage of the malvidin-3-monoglucoside, malvidin-3-monoglucoside-acetate, and malvidine-3-monoglucoside-*p*-coumarate (22). The amount and composition of the anthocyanins present in red grapes vary greatly with the species, varieties, maturity, seasonal conditions, production area, and yield of fruit (23,24).

Small amounts of flavan-3-ols were found only in the extracts of Petite Sirah, Calzin, and Flame seedless, ranging from 6.5 to 15.2 mg/L (Table 3). However, catechin was found to be one of the most abundant monomeric phenolics in wines, averaging 144 mg/L (6). This difference can be attributed to the short 1-min extraction used in this study with intact seeds. To determine the effect of crushing the seeds, experiments were carried out to compare the grapes of Cabernet Sauvignon and Petite Sirah with either intact or crushed seeds. Grapes extracted with crushed seeds produced higher initial total phenols than grapes extracted with intact seeds. However, the antioxidant activity of these extracts did not change significantly (Table 4).

In another study, the proanthocyanidin composition of grape skins was compared with that of grape seeds (25). Grape seeds had higher amounts of flavan-3-ols than skins, and these substances were present in a relatively water-insoluble form in the seeds. Procyanidin B_2 was the major dimer of grape seeds and B_1 was the major dimer of the skins (26). Soluble flavan-3-ols were suggested to be carried through into the must during the wine-making process when fermentation was carried out in the presence of the solid parts of the grape. In general, the wine's hydroxycinnamates come from juice, the flavonoids from grape skins or seeds, and hydroxybenzoates from degradation during aging (27).

Flavonols ranged from 0 to 26.8 mg/L (67 mg/kg of grapes) (Table 3). These phenolic compounds were absent in the extract of Niabell, but constituted a significant portion of the total phenols in the extracts of the white grapes, Sauvignon Blanc (60.6%), Thompson seedless (70.1%), and Chardonnay (75.0%).

In the present study no apparent relation was observed between the gross phenolic composition of grape extracts and their antioxidant activity in the liposome oxidation system. On the one hand, the grape extracts (Calzin, Petite Sirah, Niabell, Concord, Merlot, Cabernet Sauvignon, Cabernet Franc) showing a wide range of antioxidant activity contained more than 90% of anthocyanins (Table 3). On the other hand, the extracts of Sauvignon Blanc and Chardonnay, which showed more antioxidant activity, contained a relatively high content of flavonols (75.0 and 60.6%, respectively), and no anthocyanins. The extracts of Emperor and Red Globe, which also had high antioxidant activity, contained 15.4 and 10.4% flavonols, and 62.6 and 78.7% anthocyanins, respectively. Since the HPLC analysis used in this study could only separate the phenolic compounds into classes, it is possible that a better relationship could be derived from the analyses of individual components in each class of phenolic compounds in

TABLE 4
Phenolic Content and Antioxidant Activity of Grape Extracts of Intact vs. Crushed Seeds[a]

Grape extracts	Total phenol	Inhibition of CD (%)	Inhibition of Hx (%)
CS intact seeds	565 ± 1.5 b	12.9 ± 2.9 a	20.4 ± 0.4 b,c
CS crushed seeds	1780 ± 63 a	13.6 ± 1.9 a	16.7 ± 0.6 c
PS intact seeds	1115 ± 44 b	17.5 ± 0.4 a	26.5 ± 0.7 a
PS crushed seeds	1741 ± 37 a	11.6 ± 2.2 a	24.5 ± 1.8 a,b

[a]See Tables 1 and 3 for abbreviations. Inhibition of CD and Hx determined at 10 μM total phenols as GAE in mg/L. Values are mean ± SD ($n = 2$). Values followed by the same letter are not significantly different at $P < 0.05$.

grapes. Indeed, studies in our laboratory showed that individual pure anthocyanins have high and varying antioxidant activities in inhibiting liposome oxidation (28). Phenolic compounds in grapes also may have potential synergistic effects, especially between flavonols and anthocyanins. Phenolic compounds may act as primary antioxidants by reacting with either free radical intermediates and/or with metal initiators of oxidation by chelation. Antioxidant mechanisms for flavonoids in foods and biological systems may involve free radical scavenging, metal chelation, and oxygen-free radical scavenging (29–34).

This study confirms the potential antioxidant activities of the grape phenolics in liposome systems. The different activities of the grape extracts can be ascribed to their different phenolic compositions. Further studies are needed, however, with individual phenolic compounds of grapes to elucidate the different antioxidant mechanisms, and possible synergism. This is the subject of our present research.

ACKNOWLEDGMENTS

This research was supported by Research Grant Award No. IS-2260-93RC from BARD-The United States-Israel Binational Agricultural Research and Development Fund. We are grateful to M.A. Walker and E. Hall for their help in selecting the grape varieties for this study.

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[Received December 9, 1996; accepted May 30, 1997]