Commercial Grape Juices Inhibit the in Vitro Oxidation of Human Low-Density Lipoproteins

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This study was aimed at determining the antioxidant activity of commercial grape juices in inhibiting the copper-catalyzed oxidation of human low-density lipoproteins (LDL) in vitro and at relating this activity to the phenolic composition of the juices. This work also evaluated the effect of vitamin C on this antioxidant activity. When standardized to a total phenolic concentration of 10 μM gallic acid equivalents (GAE), samples of grape juices inhibited LDL oxidation from 62 to 75%. White grape juices inhibited LDL oxidation on the average by 72%, Concord purple grape juice by 67%, and grape juice blends (mixture of white and Concord grape juice) by 63%. Vitamin C had no significant effect on the antioxidant activity of the grape juices tested. The antioxidant activity of Concord juice samples was related to their anthocyanin levels, while that of the white grape juices was related to their levels of flavan-3-ols and hydroxycinnamates, as determined by HPLC. On the basis of the same total phenolic concentration, the antioxidant activity of grape juices toward LDL oxidation was comparable to that of several California red wine. However, based on their undiluted total phenolic concentration, the Concord and blends of grape juices had comparable activity to that of the red wines, while the white grape juices were less active.

Keywords: *LDL oxidation; phenolic compounds; antioxidants; grape juice; flavonoids; hexanal; HPLC*

INTRODUCTION

The oxidation of human low-density lipoproteins (LDL) is now recognized to be a key event in the initiation and progression of atherosclerosis (Steinberg et al., 1989; Steinberg, 1992). The atherogenicity of oxidized LDL is due to its high concentration of polyunsaturated fatty acids (PUFAs), which are highly susceptible to free radical oxidation (Esterbauer et al., 1992; Steinberg, 1992). Dietary antioxidants have been suggested to play a potentially important protective role against atherosclerosis and coronary heart disease by inhibiting the oxidation of LDL (Esterbauer et al., 1991; Steinberg, 1992b; Kearney and Frei, 1994).

The phenolic compounds in red wine and grape extracts were shown to be very effective in inhibiting the oxidation of human LDL in vitro (Frankel et al., 1993, 1995; Kanner et al., 1994; Teissedre et al., 1996; Meyer et al., 1997). Very little has been reported on the antioxidant activity of phenolic compounds present in commercial grape juices. In one study, one sample of pure whole-pressed Concord grape juice was shown to retard the development of conjugated dienes and thiobarbituric acid-reactive substances (TBARS), after 2000−8000-fold dilution, in copper-oxidized human LDL (Lanningham-Foster et al., 1995). In another study, a grape skin extract was reported to have an IC_{50} of 0.951 as compared to 0.187 for pure catechin, based on total phenol and formation of TBARS in the copper-catalyzed oxidation of a mixture of human LDL and VLDL (Vinson et al., 1995). However, the validity of TBARS for measuring lipid peroxidation has been questioned in the literature because it lacks specificity, and this test has not been recommended for human biological materials (Janero, 1990; Halliwell and Gutteridge, 1990).

Grapes constitute one of the major sources of phenolic compounds among different fruits (Macheix et al., 1990). Obviously, the phenols present in grape juice mainly originate from grapes. Grape phenols include flavonoids such as anthocyanins, flavan-3-ols (catechins), flavonols, and polyphenolic tannins in addition to the nonflavonoid hydroxycinnamic acids, hydroxybenzoic acids, and stilbenes (Singleton, 1982; Bourzeix et al., 1986). The flavonoids, the free hydroxycinnamates, and the hydroxybenzoic acids originate mainly from grape skins and seeds, while the hydroxycinnamates, especially those esterified to tartaric acid, and some monomeric benzoic acids generally come from the pulp (Singleton, 1982; Bourzeix et al., 1986; Singleton et al., 1978; de Sinom et al., 1992; Prieur et al., 1994). As mentioned above, many of these compounds were shown previously to inhibit human LDL oxidation in vitro. Furthermore, several flavonoids, notably catechins and procyanidins extracted from grape seeds, were demonstrated to act

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as free radical oxygen scavengers in aqueous in vitro model systems (Ricardo da Silva et al., 1991; Uchida et al., 1987). However, the extraction method, including crushing and pressing of grapes for juice production, significantly affects the phenolic composition of the resulting juice. Also, during commercial grape juice production in the United States (where *Vitis lubruscana* hybrids are processed) a hot break process is used, where the grapes are heated to 60 °C prior to pressing, and subjected to exogenous pectinolytic enzymes to increase juice yields (McLellan et al., 1993). The phenolic profiles of commercial grape juices are therefore not necessarily similar to nor the same as those of fresh grapes or those reported for different grape parts (skins, seeds). There are thus no reports on how antioxidant activity of commercial grape juices are related to their phenolic composition.

It is estimated that fruit juices account for 7% of the total beverage consumption in the United States (Clydesdale et al., 1994). Fruit and grape juices may thus be an important source of phenolic compounds and may provide potential nutritional benefits as dietary antioxidants.

This study was aimed at (1) determining how effective commercial grape juices are at protecting human LDL from oxidation in vitro; (2) relating the antioxidant activity of selected juices to their phenolic composition; and (3) establishing whether vitamin C fortification influences their antioxidant activity.

MATERIALS AND METHODS

Grape Juice Samples. Commercial grape juices, with and without vitamin C fortification, were obtained from Welch's Co. (Westfield, NY). The vitamin C content of these juices was determined by fluorescent detection prior to analysis for total phenolic content (AOAC, 1995). For comparative purposes, Concord grapes harvested from the experimental vineyards of the Univesity of California at Davis were extracted with 60% aqueous methanol for 1 min and for 24 h, as decribed previously (Meyer et al., 1997).

Determination of Total Phenols. The concentration of total phenols in grape juices and extracts was measured by the Folin−Ciocalteu assay (Singleton and Rossi, 1965), using gallic acid as the standard. Results were expressed as milligram per liter gallic acid equivalents (GAE).

Inhibition of LDL Oxidation. The grape juices were diluted to a final total phenol concentration of 10 μM GAE, and the antioxidant activity was determined by measuring the amount of hexanal formed from the in vitro copper-catalyzed oxidation of human LDL by static headspace gas chromatography (Frankel et al., 1992). Duplicate samples of 0.25 mL human LDL (prepared by standard sequential density ultracentrifugation followed by dialysis) in phosphate saline buffer and standard solutions of hexanal were measured into special headspace 6-mL bottles sealed with silicone rubber Teflon caps and incubated in a water bath shaker for 2 h at 37 °C in the presence of 80 μM $CuSO_4$. Hexanal produced by the oxidation of PUFA in LDL was identified by comparing the peak retention time to that of a standard hexanal solution. The results of replicate analyses were expressed as % inhibition = [(control − sample)/control] × 100. The relative percent inhibition of the undiluted juices was calculated by multiplying the percent inhibition values obtained at 10 μM total phenol concentration by the dilution factor used in the LDL oxidation assay and by taking the highest value as 100%. Values for relative percent inhibition were compared to those of different commercial red wines reported previously (Frankel et al., 1995).

Phenolic Composition by High-Performance Liquid Chromatography (HPLC). Four samples of grape juice and two extracts of Concord grapes were analyzed by HPLC as described by Lamuela-Raventos and Waterhouse (1994). On the basis of spectral identification, the phenolic compounds were quantified by classifying them into five groups and by calibrating with individual authentic compounds as follows: benzoic acids as gallic acid equivalents (GAE), peak 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 (RUE), peak area 365 nm (Meyer et al., 1997).

Statistical Analysis. Differences in antioxidant activity were tested by one-way analysis of variance (ANOVA). Means were considered significantly different at $p \leq 0.05$. Correlation coefficients of relative percent inhibition of LDL oxidation versus concentration of total phenols were determined by linear regression analysis. All statistical analyses were performed using Minitab Statistical Software (Addison-Wesley, Reading, MA).

Table 1. Total Phenol and Inhibition of LDL Oxidation of Commercial Grape Juices[a]

grape juices	total phenol GAE (mg/L)	% inhibition at 10 μM GAE	dilution factor
	White Grape		
1W	327	75 ± 3.5c	4
2W	389	71 ± 2.8c	5
3W	254	70 ± 4.2c	3
av	323 ± 68a	72 ± 2.6c	
	100% Concord Grape		
1C	1654	70 ± 0.7c	19
2C	1971	64 ± 5.0bc	23
3C	1742	68 ± 4.2bc	20
av	1789 ± 164c	67 ± 3.0bc	
	Blends of Grapes		
1B	1407	62 ± 0.7bc	17
2B	1541	65 ± 4.2bc	18
av	1474 ± 95b	64 ± 2.1bc	
catechin		94 ± 0.7c	
	Concord Grape Extracts		
1 min	711	48 ± 2.3ab	8.2
24 h	786	48 ± 3.6ab	9

[a] Average values ± standard deviations followed by different letters are significantly different at $p < 0.05$.

RESULTS

Antioxidant Activities. The antioxidant activities of the commercial grape juices were compared at the same total phenol concentration of 10 μM, on the basis of our previous work with pure phenolic compounds that were tested at different concentrations (Teissedre et al., 1996). The white grape juices inhibited the copper-induced LDL oxidation by 70−75%, the Concord grape juices by 64−70%, and the grape juice blends by 62 and 65% as compared to 94% for pure catechin (Table 1). The aqueous methanol extracts of Concord grapes had lower antioxidant activities than the commercial grape juices with 48% inhibition of LDL oxidation at 10 μM total phenol concentration. When tested at 20 μM total phenol these Concord grape extracts inhibited LDL oxidation by 89−90% (data not shown). The extraction time had no effect on their relative antioxidant activities. Under the conditions of the LDL copper-oxidation assay used, added vitamin C had no effect on the antioxidant activities of seven of the samples of the grape juices tested; in one sample, vitamin C decreased the antioxidant activity (Figure 1).

Total Phenols and Phenolic Composition. The concentration of total phenols varied from 254 to 389 mg/L GAE, averaging 323 mg/L GAE in the white grape juices; from 1654 to 1971 mg/L GAE, averaging 1789

Table 2. Classes of Phenolic Compounds Identified in Commercial Grape Juices by HPLC[a]

grape juice samples	total phenols by HPLC (mg/L)	benzoic acids, GAE (mg/L)	cinnamates, CFAE mg/L	anthocyanins, ME (mg/L)	flavan-3-ols, CCE (mg/L)	flanonols, RUE (mg/L)
1W	58	3.2 (5%)	38.0 (66%)	0 (0.0%)	10.7 (19%)	5.7 (10%)
2W	75	4.0 (5%)	22.5 (30%)	0 (0.0%)	40.3 (53%)	8.6 (11%)
1C	784	24.1 (3%)	203.9 (26%)	443.7 (57%)	51.6 (7%)	60.4 (8%)
3C	604	34.3 (6%)	165.1 (27%)	318.2 (53%)	33.1 (5%)	52.9 (9%)
Concord grape extracts						
1 min	817	1.1 (0.1%)	18.7 (2%)	765 (94%)	7.9 (1%)	24.6 (3%)
24 h	662	5.2 (1%)	10.3 (2%)	593 (90%)	32.5 (5%)	21.1 (3%)

[a] Values 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.

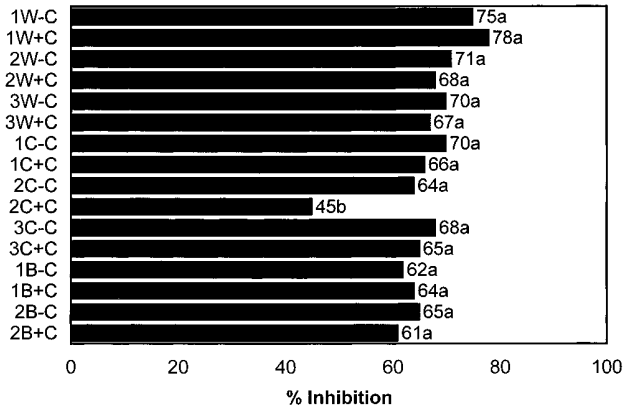

Figure 1. Effect of vitamin C on inhibition of LDL oxidation by grape juices. Percent inhibition values followed by different letters are significantly different at $p < 0.05$. Unfortified samples contained less than 1 mg /L or undetectable amounts of vitamin C; to the fortified samples 190 mg of vitamin C/L was added.

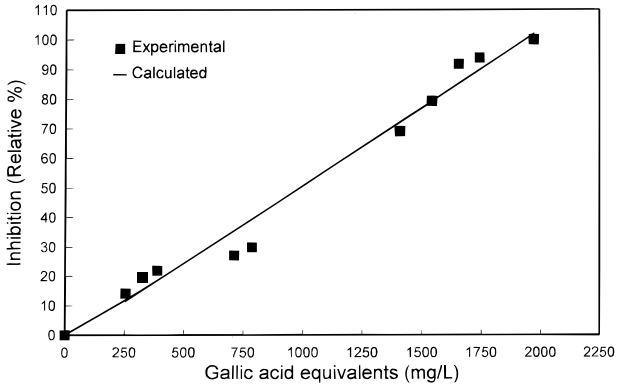

Figure 2. Relative percent inhibition of LDL oxidation by grape juices vs total phenol content as gallic acid equivalents. Correlation coefficient, $r = 0.99$. Regression equation, calculated $Y = 0.053X - 1.96$.

mg/L, in the Concord grape juices; and from 1407 to 1541 mg/L GAE, averaging 1474 mg/L in the grape juice blends (Table 1). In the samples fortified with vitamin C (190 mg/L), the total phenol concentrations increased by an average of 105 mg/L GAE in the white grape juices and 180 m/L GAE in the juices of Concord grapes and blends of grapes. The samples of Concord grapes prepared by a 1 min extraction with aqueous methanol had a total phenol concentration of 711 mg/L GAE, which increased to 786 mg/L GAE in the sample prepared by a 24 h extraction.

To relate the antioxidant activity of grape juices to phenolic components, the phenolic composition of two white and two Concord grape juices was analyzed by HPLC (Table 2). Two Concord grape extracts were also included for comparison with the commercial juice samples. The five main groups of phenolic compounds identified included benzoic acids, hydroxy cinnamates, anthocyanins, flavan-3-ols, and flavonols and were expressed respectively as equivalents of gallic acid, caffeic acid, malvin, catechin, and rutin.

Anthocyanins are responsible for the red and purple color of fruits and were found only in the samples of Concord grape juices and extracts. The contents of anthocyanins represented 53−57% of the commercial Concord grape juices and 90−94% of the Concord grape extracts. Malvidin, one of the important components of red grapes, was previously found to be a more active antioxidant in protecting LDL against oxidation than the corresponding glucoside malvin (Satué-Garcia et al., 1997). The white grape juices contained higher levels of hydroxycinnamates (30 and 66%), flavan-3-ols (19 and 53%), and flavonols (10 and 11%) as compared to the Concord grape juices and extracts (Table 2). Components of these three classes of phenolic compounds, including catechin, caffeic acid, and rutin, were shown previously to be powerful phenolic antioxidants in the LDL oxidation assay (Teissedre et al., 1996).

The total phenol content as determined by HPLC was higher in the two commercial Concord grape juice samples than the two white grape juice samples (Table 2). While the extraction time did not affect the antioxidant activity of extracts of Concord grapes (Table 1), their phenolic composition differed markedly (Table 2). With 24 h extraction time, both hydroxybenzoates and flavan-3-ols were obtained in higher yields, but the amounts of hydroxycinnamates, anthocyanins, and flavonols were lower as compared to the sample prepared with 1 min extraction time.

When the antioxidant activities of the grape juice samples were compared on the basis of their total phenol contents, the calculated relative LDL antioxidant activity at 10 μM GAE gave a correlation coefficient r of 0.99 (Figure 2). When the highest inhibition value obtained with one of the Concord grape juice was set to 100%, the calculated average relative inhibition values were 18.5% for the white grape juices, 74.3% for the grape juice blends, 95.2% for the Concord grape juices, and 28.5% for the Concord grape extracts. In a previous study of 14 California red wines, the average relative inhibition of LDL oxidation was 74% for Merlot and 70% for Cabernet Sauvignon relative to 100% for Petite Sirah (Frankel et al., 1995).

DISCUSSION

The potential cardio-protective effects of wine, especially red wine, was proposed to be related to the antioxidant activity of nonalcoholic phenolic components (Frankel et al., 1993). California red and white wines inhibited the copper-catalyzed oxidation of human LDL

in vitro from 27 to 65% when tested at 10 μM total phenolic concentration (Frankel et al., 1995). In the present study, commercial grape juices showed comparable inhibitory effects on LDL oxidation as the red wines when tested at the 10 μM phenolic concentration. In the Concord grape juices, antioxidant activity appears to be associated with the levels of anthocyanins, whereas in the white grape juice samples antioxidant activity was related to the levels of hydroxycinnamates (caffeic acid) and flavan-3-ols (catechin). In the grape juice samples, antioxidant activity thus appears to be distributed among all phenolic constituents, which is consistent with the data on wine and grape extracts (Frankel et al., 1995; Meyer et al., 1997). The extracts of fresh Concord grapes had lower antioxidant activity and total phenolic contents than the commercial Concord grape juices. The Concord juices also contained higher levels of benzoic acids, cinnamates, flavan-3-ols, and flavonols than the aqueous methanol extracts of Concord grapes (Table 2).

Although vitamin C was not found to affect antioxidant activity, it increased the apparent total phenolic content. However, vitamin C may have been readily decomposed in the presence of the copper concentration used in the LDL oxidation assay. The increase in total phenolic content observed in the grape juice samples fortified with vitamin C may be due the high reactivity of vitamin C with the Folin−Ciocalteu reagent (Singleton and Rossi, 1969). Although vitamin C may have a stabilizing effect on simple phenolic compounds, it has a destabilizing effect on anthocyanins (Markakis, 1982). The degradation of anthocyanins by vitamin C is attributed to the formation of hydrogen peroxide in the presence of metals (Jackman and Smith, 1992).

Although the in vitro evidence indicates that phenolic compounds are potent inhibitors of LDL oxidation, it is not yet clear whether these compounds are absorbed in humans in a metabolically active form. Recent studies claimed antioxidant activity of red wine in vivo (Kondo et al., 1994; Maxwell et al., 1994; Fuhrman et al., 1995). However, these studies used nonspecific methods to determine antioxidant activity. Grape juices contain more glycosylated phenolic compounds than wines (Singleton, 1982, 1987). A recent study with healthy ileostomy subjects showed that quercetin glucosides are more readily absorbed than the quercetin aglycone (Hollman et al., 1995). Better methods are needed for evaluating antioxidant activity in vivo to support the potential health effects of phenolic compounds in wines and grape juices.

LITERATURE CITED

AOAC (Association of Official Agricultural Chemists). Vitamins and other nutrients. In *AOAC Official Methods of Analysis*; Plenum Press: New York, 1995; pp 17−18.

Bourzeix, M.; Weyland, D.; Heredia, N. A. A study of catechins and procyanidins of grape clusters, the wine and other byproducts of the vine. (translated from French). *Bull. Off. Int. Vigne Vin* **1986**, *59*, 1171−1254.

Clydesdale, F. M.; Kolasa, K. M.; Ikeda, J. P. All you want to know about fruit juice. *Nutr. Today* **1994**, *29*, 14−28.

de Simon, B. F.; Hernandez, T.; Estrella, I.; Gomez-Cordoves, C. Variation in phenol content in grapes during ripening: low-molecular-weight phenols. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 351−354.

Esterbauer, H.; Puhl, H.; Dieber-Rotheneder, M.; Waeg, G.; Rabl. H. Effects of antioxidants on oxidative modification of LDL. *Ann. Med.* **1991**, *23*, 573−581.

Esterbauer, H.; Gebicki, J.; Puhl, H.; Jürgens, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.* **1992**, *13*, 341−390.

Frankel, E. N.; German, J. B.; Davis, P. A. Headspace gas chromatography to determine human low-density lipoprotein oxidation. *Lipids* **1992**, *27*, 1047−1051.

Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

Frankel, E. N.; Waterhouse, A. L.; Teissedre; P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

Fuhrman; B.; Lavy; A.; Aviram; M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 549−554.

Halliwell; B.; Gutteridge; J. M. C. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol.* **1990**, *186B*, 1−85.

Hollman, P. C. H.; de Vries, J. H. M.; van Leeuwen, S. D.; Mengelers, M. J. B.; Katan, M. B. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am. J. Clin. Nutr.* **1995**, *62*, 1276−1282.

Jackman, R. L.; Smith, J. L. Anthocyanins and betalains. In *Natural Food Colorants*; Goodwin, T. W., Ed.; Academic Press: New York, 1992; pp 183−231.

Janero, D. R. Malonaldehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and tissue injury. *Free Radical Biol. Med.* **1990**, *9*, 515−540.

Kanner, J.; Frankel, E. N.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* **1994**, *42*, 64−69.

Kearney, J. F.; Frei, B. Antioxidant protection of low-density lipoprotein and its role in the prevention of atherosclerosis vascular disease. In *Natural Antioxidants in Human Health and Disease;* Frei, B., Ed.; Academic Press: San Diego, CA, 1994; pp 303−351.

Kondo, K.; Matsumoto, A.; Kurata, H.; Tanahashi, H.; Koda, H.; Amachi, T.; Itakura, H. Inhibition of oxidation of low-density lipoprotein with red wine. *Lancet* **1994**, *344*, 1152.

Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1−5.

Lanningham-Foster, L.; Chen, C.; Chance, D. S.; Loo, G. Grape extract inhibits lipid peroxidation of human low-density lipoprotein. *Biol. Pharm. Bull.* **1995**, *18*, 1347−1351.

Macheix, J.-J.; Fleuriet, A.; Billot, J. The main phenolics of fruits. In *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; pp 1−98.

Markakis, P. Stability of anthocyanins in foods. In *Anthocyanins as Food Colorants*; Markakis, P., Ed.; Academic Press: London, U.K., 1982; pp 163−180.

Maxwell, S.; Cruickshank, A.; Thorpe, G. Red wine and antioxidant activity in serum. *Lancet* **1994**, *334*, 103−194.

McLellan, M. R.; Acree, T. Grape Juice. In *Fruit Juice Processing Technology*; Nagy, S., Chen, C. S., Shaw, P. E., Eds.; AgScience Inc.: Auburndale, FL, 1993; p 318−333.

Meyer, A. S.; Yi, O−S.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638−1643.

Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.

Ricardo da Silva, J. M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991**, *39*, 1549−1552.

Satué-Garcia, M. T.; Heinonen, M.; Frankel, E. N. Anthocyanins as antioxidants on human LDL and lecithin-liposome systems. *J. Agric. Food Chem.* **1997**, *45*, 3362−3367.

Singleton, V. L. Grape and wine phenolics: background and prospects. In *Proceedings of University of California Davis, Grape Wine Centennial Symposium*; Webb, A. D., Ed.; Department of Viticulture and Enology, UC Davis: Davis, CA, 1982; pp 215−227.

Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69−77.

Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

Singleton, V. L.; Timberlake, C. F.; Lea, A. G. H. The phenolic cinnamates of white grapes and wine. *J. Sci. Food Agric.* **1978**, *29*, 403−410.

Steinberg, D. Metabolism of lipoproteins and their role in pathogenesis of artherosclerosis. *Atherosclerosis Rev.* **1992a**, *18*, 1−6.

Steinberg, D. Antioxidants in the prevention of human atherosclerosis. *Circulation* **1992b**, *85*, 2337−2344.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol. Modification of low-density lipoproteins that increase its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915−924.

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in Vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

Uchida, S.; Edamatsu, R.; Hiramatsu, M.; Mori, A.; Nonaka, G. Y.; Nishioka, I.; Niwa, M.; Ozaki, M. Condensed tannins scavenge active oxygen free radicals. *Med. Sci. Res.* **1987**, *15*, 831−832.

Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2800−2802.

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