Varietal Differences in the Phenolic Content and Superoxide Radical Scavenging Potential of Wines from Different Sources

Michikatsu Sato,[†] Narasimhan Ramarathnam,*[,‡] Yumiko Suzuki,[†] Tetsuro Ohkubo,[†] Masao Takeuchi,[‡] and Hirotomo Ochi[‡]

Central Research Laboratories, Mercian Corporation, 9-1 Johnan 4-Chome, Fujisawa 251, Japan, and Japan Institute for the Control of Aging, Nikken Foods Company, Ltd., 723-1 Haruoka, Fukuroi-city, Shizuoka 437-01, Japan

Thirty-one wine samples differing in their origin of production and vintages were analyzed for total phenolic content, total and free sulfur dioxide contents, and superoxide radical scavenging potentials. The polyphenol content of red wine ranged from 735.9 to 2858 ppm, and that of white wine was in the range 259.4−720.5 ppm. Total sufur dioxide content ranged from 21.9 to 270.7 ppm, and had no correlation to the color of the wine. Superoxide radical scavenging activity values ranged from 39.3 to 215.9 units/mL for the white wine, and those of red varieties were ∼5−10 times higher. No correlation was observed between the free and total sulfur dioxide contents in the different wine samples tested and their superoxide radical scavenging activity values. A direct correlation between the color of the wine ($r = 0.7517$), its phenolic content ($r = 0.9908$), and the ability of the wine constituents to scavenge superoxide radical was, however, established by a simple regression analysis.

Keywords: *Wine; phenolics; radical scavenger; varietal difference*

INTRODUCTION

Oxygen, a vital component for the survival of the human species, is present in the atmosphere as a stable triplet biradical ($^3O_2$) in the ground state. Once inhaled, it undergoes a gradual reduction process and ultimately gets metabolized into water. In this process, however, a small amount of reactive intermediates, such as superoxide radical ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) are formed (Sies, 1993). These reactive species, collectively termed reactive oxygen species (ROS), can easily initiate the peroxidation of the membranal lipids, leading to the accumulation of lipid peroxides. The peroxidation products by themselves and their secondary oxidation products, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), are highly reactive; they react with biological substrates such as protein, amines, and deoxyribonucleic acid (DNA; Kehrer, 1993). Such processes play host to several degerative diseases, and may make a significant contribution to the risk of human aging and cancer (Marnett *et al*., 1985).

Most living species have efficient defense systems to protect themselves against the oxidative stress induced by ROS. The capacity of such protective systems, however, gradually decreases with aging, resulting in disturbances to the normal redox equilibrium established in healthy systems (Cutler, 1984). Therefore, to replenish this aging-induced loss, there is a need to provide the body with a constant supply of phytochemicals through the regular intake of proper diet.

Studies have suggested that diet has a marked impact on cancer (Doll and Peto, 1981), and that this impact may be due to protective agents in foods, many of which are phytochemicals (Ames, 1983). Imbalances in fatty acid metabolism have also been implicated as being responsible for many chronic diseases, such as arthritis, cancer, and atherogenesis (Ames, 1983; Aruoma and Halliwell, 1991; Caragay, 1992; Kinsella *et al*., 1990; Steinberg, 1988, 1992). Plant foods and products, including beverages, may exert beneficial effects by reducing fat intake and by providing a better balance of fatty acids. Flavonoid substances are widely distributed in plants. Though the function of flavonoids in plants seems to be more evolutionary in nature, their metal-chelating capability together with their radical scavenging property have enabled flavonoids to be thought of as natural plant antioxidants (Bors and Saran, 1987). By providing a wide spectrum of phytochemicals such as flavonoids, plant products may moderate lipid peroxidation and retard the progress of many chronic diseases (Kinsella *et al*., 1993).

Polyphenol oligomers, namely procyanidins, are known to be widely distributed in the plant kingdom. They have been successfully applied in the treatment of several types of vascular disorders because of their positive biochemical effects on blood vessels. The vasorelaxing activity of wine and other grape products, such as grape juices and grape skin extracts, have already been well documented (Fitzpatrick *et al*., 1993) and is believed to be induced by quercetin and tannic acid that are present in the grape skin. Procyanidins were also proved to be effective free radical scavengers and inhibitors of oxidative enzymes such as xanthine oxidase (Ricardo da Silva *et al*., 1991; Facino *et al*., 1994).

Phytochemicals, especially those in wine products, may also be critical factors in reducing the mortality from coronary heart diseases for certain segments of the French population. A comparative study initiated by the WHO has shown marked differences in mortality and morbidity from coronary heart diseases among, especially, French and U.S. populations (NRC, 1989). Despite a similar intake of a saturated fatty acid diet

* Author to whom correspondence should be addressed (telephone +81-538-49-0125; fax +81-538-49-1267).

[†] Central Research Laboratories.

[‡] Japan Institute for the Control of Aging.

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and comparable levels of plasma cholesterol content, the French subjects were less susceptible to coronary heart diseases than the U.S. subjects.

Using multivariant analyses, Renaud and de Lorgeril (1992) postulated that consumption of wine was the only dietary factor responsible for this discrepancy, commonly referred to as the French paradox. Further work on this observation demonstrated that the nonalcoholic portion of the red wine, phenolic substances in particular, were able to inhibit the oxidation of human low-density lipoproteins (Frankel *et al*., 1993; Kanner *et al*., 1994). In small doses, alcohol can also have beneficial effects on thrombosis (Renaud *et al*., 1992).

In view of this background, the present investigation was initiated to study the differences among phenolic contents of red and white wines from different regions of production and vintages. An attempt was also made to establish a correlation between the phenolic content of wine and its potential to scavenge superoxide radicals.

MATERIALS AND METHODS

Wine. Thirty-one samples of wine, differing in their vintages, variety, and region of production, were imported directly from the producers in several countries. The samples were stored, with intact seals, in the dark at 4 ± 1 °C until the assays were completed.

Chemicals. L-Ascorbic acid (vitamin C) and sodium carbonate (Na_2CO_3) were purchased from Nacalai Tesque Company Ltd., Kyoto, Japan. Folin−Ciocalteu reagent, hypoxanthine, and diethylenetriaminepentaacetic acid (DETAPAC) were purchased from Sigma Chemical Company, St. Louis, MO. Xanthine oxidase, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), and superoxide dismutase (SOD) were purchased from LABOTEC Company Ltd., Kyoto, Japan.

Total Phenolic Content. The total phenolic contents of the wine samples were determined with the Folin−Ciocalteu reagent (Singleton and Rossi, 1965). Each wine sample (0.5 mL) was diluted in a test tube with glass distilled water to 5.0 mL. Folin−Ciocalteu reagent (5.0 mL) was added, and the contents of the test tube were mixed thoroughly. After an interval of 3.0 min, 5.0 mL of 10% Na_2CO_3 solution was added, and the mixture was allowed to stand for 1 h with intermittent mixing. The blue color that developed was measured on an Hitachi 200-10 model spectrophotometer. The concentration of the total phenolic content of the wine sample was determined by comparison with the optical density values of different concentrations of a standard phenolic compound, gallic acid. This analysis for each wine sample was carried out in triplicate, and the values for total phenolic contents were expressed in terms of gallic acid equivalent (GAE).

Sulfur Dioxide Content. The free and bound sulfur dioxide (SO_2) contents of the wine samples were measured by the aspiration−titration method of Rankine (Rankine and Pocock, 1970).

Superoxide Radical Scavenging Activity (SOSA). The SOSA values of the wine samples were determined by the hypoxanthine−xanthine oxidase superoxide generating system. The assay was carried out on an electron-spin resonance (ESR) spectrometer (model JES-FR80, JEOL Ltd., Tokyo, Japan) according to the method of Mitsuta *et al*. (1990). Fifty microliters of hypoxanthine were placed in a test tube, and 40 μL of DETAPAC (5.5 μM), 50 μL of distilled water, 10 μL of DMPO, and 50 μL of xanthine oxidase were added successively. The ESR measurement was made after 40 s of incubation at 20 °C. The relative intensity (RI) ratio of the superoxide signal intensity to the manganese (Mn) signal intensity was taken as the control value. The SOSA values were determined by measuring the reduction in RI values when 50-μL wine samples were used in place of distilled water. The reduction in RI was compared with the reductions noted when various concentrations of superoxide dismutase (SOD) enzyme were used. The assay for each of the wine samples was determined in triplicate. One milligram of pure SOD had an SOSA value equivalent to 3500 units.

RESULTS AND DISCUSSION

Total Phenolic Content. Of the wine samples tested for polyphenolic contents, 23 were red wines, 7 were white wines, and 1 was a rosé type. The polyphenolic contents of the wine samples tested in this investigation ranged from 259.4 ppm on the lower end, in the case of *Jerez* white wine imported from Spain, to 2858 ppm detected in the Chilean red wine (Table 1). Figure 1 is an illustration of the content of total phenolics in the different wine samples in decreasing order. The polyphenolic contents of all the red wines were in the higher order, ranging from 1115.5 to 2858 ppm (Figure 1). Only one of the red wine samples, *Douro-Pourto* imported from Portugal, had a lower value of 735.9 ppm. The two red wines imported from Chile showed the highest phenolic contents of 2858 and 2837 ppm. The phenolic content of white wines ranged from 259.4 ppm for the *Jerez* variety of white wine from Spain to 720.5 ppm for a sample of *Madeira* imported from Portugal. Only one rosé wine sample was tested for phenolic content, that of Japan, and the value was 340.3 ppm.

Sulfur Dioxide Content. The total sulfur dioxide (SO_2) content of the 31 wine samples tested ranged from 21.9 ppm for the red wine variety *Margaux* imported from France to 270.7 ppm for the white wine of Japan (Table 1). Unlike the content of total phenolics, a direct correlation between the color of the wine and its total SO_2 content was not observed. In general, of the seven white wines tested, *Sauternes* of France and *Katsunuma* and another variety of white wine of Japan showed the highest content of SO_2; that is, 270.7, 270.1, and 210.7 ppm, respectively. The sample of rosé wine had a total SO_2 content of 171.4 ppm. Values of the other four tested white wines were 31.3 and 60.5 ppm (*Tokaji*, Hungary), 49.4 ppm (*Jerez*, Spain), and 47.4 ppm (*Madeira*, Portugal).

Superoxide Radical Scavenging Activity (SOSA) Values. All 31 samples of wine samples used in the present investigation were tested for their SOSA values. The ability to scavenge superoxide radical by the wine constituents ranged from 39.3 to 1189.1 units/mL (Table 1). An attempt was also made to establish the correlation between the color of wine, its total phenolic content, and SOSA values. As illustrated in Figure 2A, the color of the wine samples, expressed in terms of their optical density (OD) values at 520 nm, had a pronounced influence on their superoxide radical scavenging activities.

Also, by simple regression analysis, the phenolic content of each of the tested wine samples was shown to be positively correlated ($r = 0.9908$) to its SOSA values (Figure 2B). An illustration of the decreasing order of the SOSA values is shown in Figure 3. All of the red wines showed higher SOSA values, ranging from 286.5 units/mL for a sample of *Douro-Porto* imported from Portugal to 1189.1 units/mL for a sample of *Napa valley* red wine imported from the United States (Figure 3).

The white wines tested in this investigation had SOSA values at the lower end of the spectrum (Figure 3). The SOSA values for two white wines of Japanese origin were 87.5 and 39.3 units/mL. The white wine *Madeira* of Portugal, that had a higher phenolic content (720.5 ppm, Table 1) than the average value for white wine, also showed a higher SOSA value of 215.9 units/

Table 1. Contents of Total Phenolics, Free and Total Sulfur Dioxide (SO_2), SOSA Values, and Optical Density (OD_{520}) of Wine Samples[a]

color, region, country	vintage	main variety	phenolics (ppm)[b]	free SO_2 (ppm)	total SO_2 (ppm)	SOSA (units/mL)	OD_{520}
red wines							
Coonawarra, Australia	1991	Cabernet Sauvignon	2138.0	15.5	23.2	787.0	5.250
Chile	1987	Cabernet Sauvignon	2858.0	20.6	69.6	1122.0	5.095
Chile	1988	Cabernet Sauvignon	2837.0	18.1	86.4	1109.0	5.140
Pauillac, France	1988	Cabernet Sauvignon	2492.5	36.3	62.5	999.2	4.550
Margaux, France	1988	Cabernet Sauvignon	2690.5	15.5	21.9	952.0	3.692
Côte de Beaune, France	1989	Pinot noir	2110.5	18.1	37.3	766.0	1.789
Côtes-du-Rhône, France	1989	Syrah	1756.0	18.1	44.4	582.9	3.427
Touraine, France	1989	Cabernet Sauvignon	2261.0	27.2	85.7	815.7	2.554
Bordeaux, France	1990	Cabernet Sauvignon	2081.5	34.3	109.9	792.8	2.337
Beaujolais, France	1992	Gamay	1301.0	37.3	47.4	441.6	1.641
Egri, Hungary	—[c]	blended	1225.0	16.1	80.7	409.0	2.949
Umbria, Italy	1982	Sangiovese	2282.5	15.1	111.9	870.7	2.662
Piemonte, Italy	1982	Nebbiolo	2793.0	13.1	41.3	1161.0	2.866
Piemonte, Italy	1985	Nebbiolo	2415.5	22.2	152.3	951.4	2.407
Piemonte, Italy	1987	Nebbiolo	2227.5	10.3	29.7	890.0	1.506
Nagano, Japan	1989	Merlot	2072.5	18.1	84.7	744.8	2.400
Japan	1989	Cabernet Sauvignon	2151.0	18.1	47.4	789.9	3.175
Japan	—[c]	blended	1810.0	34.3	187.5	751.0	2.400
Douro-Porto, Portugal	1976	blended	735.9	11.1	30.2	286.5	1.217
Douro-Porto, Portugal	—[c]	blended	1115.5	10.1	56.0	369.1	3.478
Russia	—[c]	blended	2169.5	10.3	144.4	842.0	—[d]
Rioja, Spain	1985	Tempranillo	1802.0	15.1	103.9	838.0	2.168
Napa valley, USA	1989	Cabernet Sauvignon	2727.0	20.2	42.3	1189.1	4.775
white wines							
Sauternes, France	—[c]	Sémillon	357.1	33.3	270.7	41.6	—[d]
Tokaji, Hungary	—[c]	Furmint	312.5	7.6	60.5	43.4	—[d]
Tokaji, Hungary	—[c]	Furmint	373.2	6.0	31.3	60.9	0.154
Katsunuma, Japan	1987	Koshu	555.1	5.5	270.1	87.5	—[d]
Japan	—[c]	blended	295.1	32.8	210.7	39.3	—[d]
Madeira, Portugal	—[c]	Riesling	720.5	8.1	47.4	215.9	1.306
Jerez, Spain	—[c]	Palomino	259.4	7.6	49.4	46.6	0.032
rosé wine							
Japan	—[c]	blended	340.3	40.3	171.4	50.6	0.280

[a] Results are averages of three determinations. [b] Gallic acid equivalent. [c] Not available. [d] Not determined.

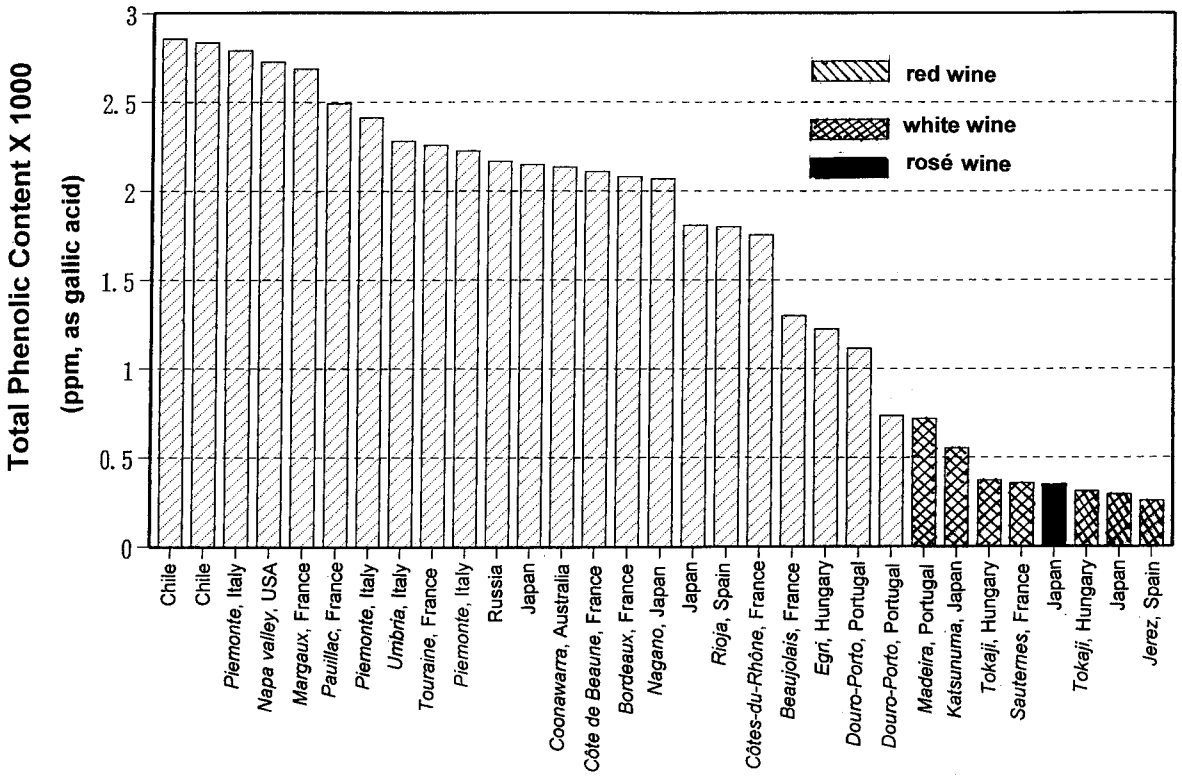

Figure 1. Content of total phenolic components in different samples of wine.

mL. The rosé wine used in the present investigation had an SOSA value of 50.6 units/mL. The other white wines imported from Hungary, France, and Spain had SOSA values in the range of 41.6 to 60.9 units/mL.

Sulfur dioxide is added in wine processing for the inhibition of microbial growth. During the fermentation process, it gets strongly bound to acetaldehyde. As a reducing agent, the effect of sulfur dioxide on the

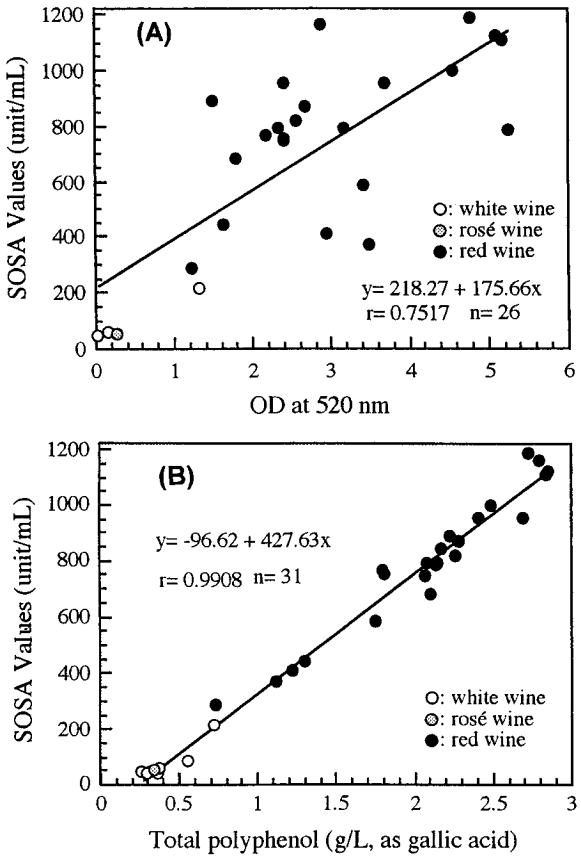

Figure 2. Relationship between (A) color of wine (OD at 520 nm) and (B) total phenolic contents (gallic acid equivalent) on the SOSA values of different wine samples.

superoxide radical has never been investigated before. In the present investigation, the values of the free and total SO_2 contents in red and white wines and their SOSA values were compared to establish the relationship between them. However, neither the free SO_2 levels nor the total SO_2 levels had any effect on the SOSA values of the tested wine samples (Table 1).

Red wines have been proved to inhibit the Cu^{2+}-catalyzed oxidation of human low-density lipoproteins very effectively (Frankel et al., 1993). In the present study, a positive correlation between the phenolic content of wine and the superoxide radical scavenging potentials has been demonstrated. In view of the evidence presented, we postulate that consumption of red wine in moderate amounts may indeed be one of the several ideal ways to regulate free-radical-reaction-mediated disorders, such as coronary heart disease, atherogenesis, cancer, and aging. Further work to isolate, fractionate, and characterize the active phenolic components in red wine are in progress, and the results will soon be reported elsewhere.

CONCLUSIONS

In vivo lipid peroxidation has been implicated as the primary cause of many heart-related diseases, atherosclerosis, cancer, and aging. Phytochemicals, especially plant phenolics and flavonoids, are becoming the new class of potent antioxidants to address these health-related problems. These constituents can react with active oxygen radicals, such as hydroxyl radicals (Hussain *et al.*, 1987), superoxide anion radicals (Afanaslev *et al.*, 1989), and lipid peroxy radicals (Torel *et al.*, 1986), and inhibit lipid oxidation at the early stages. Phenolic constituents also inhibit cyclooxygenase and lipoxygenase of platelets and macrophages, and hence reduce thrombotic tendencies *in vivo* (Moroney *et al.*, 1988).

In this investigation, phenolic components of red wine were demonstrated to possess superoxide radical scavenging potentials. If red wine is consumed regularly, at a moderate level of 150−300 mL/day, one can get a supply of natural antioxidants in their crude state that is equivalent to 50−100 mg of pure SOD enzyme. This

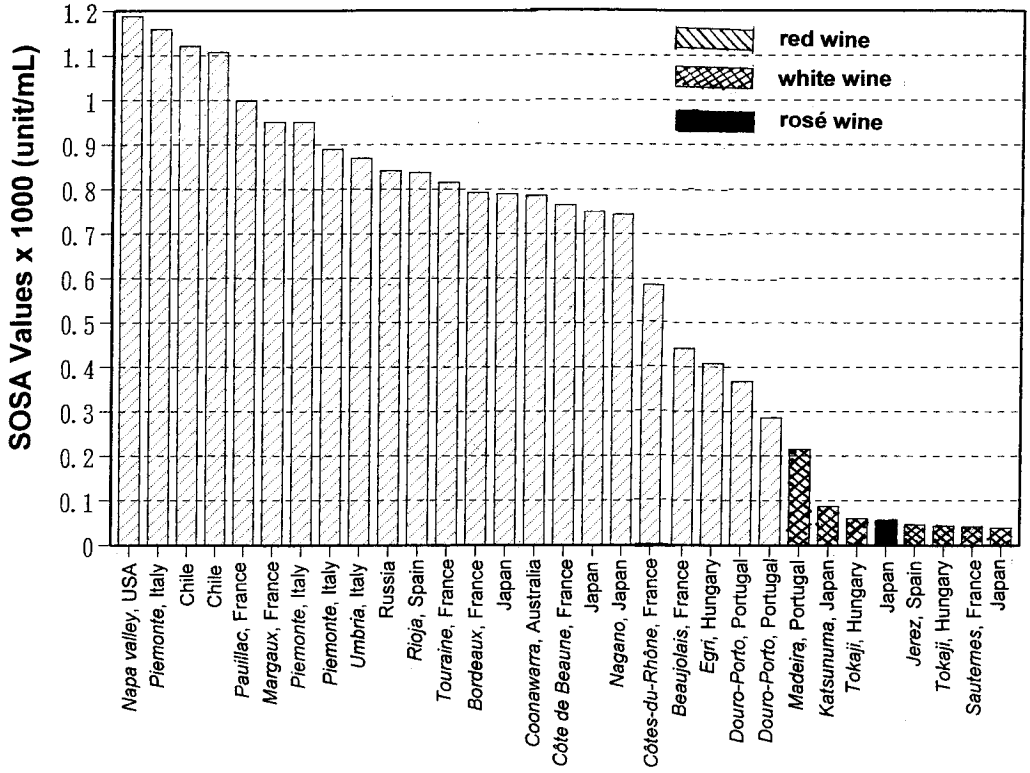

Figure 3. Superoxide radical scavenging activity (SOSA) values of different wine samples.

supply could perhaps provide additional protection in the inhibition of *in vivo* oxidation of cellular components. However, further studies are essential to provide evidence for the antioxidant role of phenolic components of wine *in vivo*.

LITERATURE CITED

Afanaslev, I. B.; Dorozhko, A. I.; Bordskii, A. V. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.* **1989**, *38*, 1763−1769.

Ames, B. Dietary carcinogenesis and anticarcinogenesis. *Science* **1983**, *221*, 1256−1260.

Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

Aruoma, O. I.; Halliwell, B. In *Free Radicals and Food Additives*; Taylor and Frances: London, 1991.

Bors, W.; Saran, M. Radical scavenging by flavonoid antioxidants. *Free Radicals Res. Commun.* **1987**, *2*, 289−294.

Caragay, A. B. Cancer preventive foods and ingredients. *Food Technol.* **1992**, *46*, 65−68.

Cutler, R. G. Antioxidants, aging, and longevity. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1984; pp 371−428.

Doll, R.; Peto, R. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1191−1308.

Facino, R. M.; Carini, M.; Aldini, G.; Bombardelli, E.; Morazzoni, P.; Morelli, R. Free radicals scavenging action and anti-enzyme activities of procyanidines from *Vitis vinifera*. *Drug Res.* **1994**, *44*, 592−601.

Fitzpatrick, D. F.; Hirschfield, S. L.; Coffey, R. G. Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am. J. Physiol.* **1993**, *265*, H774−H778.

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.

Hussain, S. R.; Cillard, J.; Cillard, P. Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* **1987**, *26*, 2489−2491.

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.

Kehrer, J. P. Free radicals as mediators of tissue injury and disease. *CRC Crit. Rev. Toxicol.* **1993**, *23*, 21−48.

Kinsella, J. E.; Lokesh, B.; Stone, R. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: Possible mechanisms. *Am. J. Clin. Nutr.* **1990**, *52*, 1−28.

Kinsella, J. E.; Frankel, E; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, 85−90.

Marnett, L. J.; Hurd, H. K.; Hollstein, M. C.; Levin, D. E.; Esterbauer, H.; Ames, B. N. Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA 104. *Mutat. Res.* **1985**, *148*, 25−34.

Mitsuta, K.; Mizuta, Y.; Kohno, M.; Hiramatsu, M.; Mori, A. The application of ESR spin-trapping technique to the evaluation of SOD-*like* activity of biological substances. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 187−191.

Moroney, M.-A.; Alcaraz, M. J.; Forder, R. A. Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J. Pharm. Pharmacol.* **1988**, *40*, 787−792.

NRC. *Diet and Health*; National Research Council; National Academy: Washington, DC, 1989.

Rankine, B. C.; Pocock, K. F. Alkalimetric determination of sulfur dioxide in wine. *Aust. Wine Brew. Spirit Rev.* **1970**, *88*, 40−44.

Renaud, S. C.; de Lorgeril, M. Wine, alcohol, platelets and the French Paradox for coronary heart disease. *Lancet* **1992,** *339*, 1523−1526.

Renaud, S. C.; Beswick, A. D.; Fehily, A. M.; Sharp. D. S.; Elwood, P. C. Alcohol and platelet aggregation: The caerphilly prospective heart disease study. *Am. J. Clin. Nutr.* **1992**, *55*, 1012−1017.

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.

Sies, H. Strategies of antioxidant defense. *Eur. J. Biochem.* **1993**, *215*, 213−219.

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

Steinberg, D. Antioxidants in the prevention of human atherosclerosis. *Circulation* **1988**, *85*, 2338−2344.

Steinberg, D. Metabolism of lipoproteins and their role in pathogenesis of atherosclerosis. *Atherosclerosis Rev.* **1992**, *18*, 1−16.

Torel, J.; Cillard, J.; Cillard, P. Antioxidant activity of flavonoids and reactivity with peroxy radicals. *Phytochemistry* **1986**, *25*, 383−385.

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