ANTIOXIDANTS & REDOX SIGNALING
Volume 6, Number 5, 2004


# News & Views

# Long-Lasting Antioxidant Activity in a 600-Year-Old Fermented Fruit Juice

ALBERTO A.E. BERTELLI,[1] ROBERTO MORELLI,[2] ROBERTO LO SCALZO,[2] and FRANCA FERRARA[3]

## WINE-BASED MEDICAL THERAPEUTICS

MODERN MEDICINE has recently acknowledged the therapeutic value of an ancient remedy: wine.

Wine, together with olive oil, fruit, vegetables, cereals, and fish, is one of the staples of the so-called Mediterranean diet, which was found to be associated with low cardiovascular mortality more than 50 years ago (16). More recently, epidemiological studies have shown that moderate consumption of wine, but not of alcohol, is associated with a reduction in cardiovascular mortality (15, 21, 24, 27, 28, 38, 46). A meta-analysis of 13 studies in nearly 300,000 subjects has confirmed that moderate wine consumption has cardioprotective properties and reduces also the risk of cerebrovascular disease (18).

The constituents of wine differ considerably from those of other alcoholic beverages (9), and many experimental studies have been carried out to identify its active principles.

Antioxidants are considered to be responsible for the protective effects of the Mediterranean diet, together with its high content in polyunsaturated fats (16, 48).

Wine, especially red wine, contains considerable quantities of polyphenols (up to 5 g/L), which are well-known antioxidants and free radical scavengers. *In vitro* studies have shown that these polyphenols are able to inhibit human low-density lipoprotein oxidation and could therefore contribute toward the prevention of thrombosis and atherosclerosis (20, 46).

This hypothesis is supported by the recent finding of an inverse relationship between oxidized low-density lipoprotein antibodies and daily wine consumption in a cross-sectional study in 551 elderly Italian subjects (17). Moreover, the scavenging properties of polyphenols can inhibit the interaction between endothelium-derived nitric oxide (NO) and superoxide anion. This increases NO levels in the endothelium and may contribute toward prevention of endothelial dysfunction, which occurs in the early stages of atherogenesis and promotes thrombogenesis (46).

The positive effects of wine on endothelial dysfunction are supported by a study in 42 healthy volunteers allocated either to a Mediterranean diet or to a high-fat diet for 1 month and then given wine (240 ml daily) for another month. Wine was able to correct the reduction in brachial artery diameter observed in the subjects on the high-fat diet; it also enhanced the positive effects of the Mediterranean diet and corrected the negative effects of a high-fat diet on total plasma antioxidant capacity (29).

Resveratrol is one of the most important typical polyphenols of wine. In the hearts of mice subjected to ischemia and reperfusion, it reduced myocardial infarct size and the number of apoptotic cardiomyocytes, and improved postischemic ventricular function to a significantly greater extent than no treatment or ethanol. Moreover, the lower malonaldehyde content of the hearts of resveratrol-treated mice showed that oxidative stress was lower in these animals than in controls (42). These properties were no longer evident in inducible NO synthase knockout mice or in the heart of wild-type mice given an inducible NO synthase inhibitor (26). Thus, NO upregulation and reduction of oxidative stress appear to be the key cardioprotective mechanisms.

Another important mechanism that has recently been shown for polyphenols contained in olive oil and red wine, including resveratrol, is the transcriptional inhibition of vascular cell adhesion molecule-1 (VCAM-1); this finding indicates that the Mediterranean diet prevents endothelial leukocyte adhesion, an early step in atherogenesis (10, 19). The results of these studies also suggest that a Mediterranean diet may modify familial predisposition to cardiovascular disease by regulating the expression on proinflammatory/ proatherogenic genes.

Resveratrol also possesses other biological properties of interest: antiinflammatory activity, expressed as reduction in the production of tumor necrosis factor-α (TNF-α), interleukin-1β, interleukin-8, and granulocyte macrophage colony stimulating factor, antiinfective activity, including

[1]Department of Human Morphology, University of Milan, Milano, Italy.
[2]Institute of Sciences on Molecular Technology, National Research Institute CNR, Milano, Italy.
[3]Institute of Pharmacology, Toxicology and Chemotherapy, University of Milan, Milano, Italy.

stimulation of phagocytosis, and anticancer activity, including cytostatic activity, stimulation of apoptosis, and antimutagenic effects (4, 6, 7, 14).

The chemical composition of the various types of wine can differ considerably. Major differences have been ascertained between red and white wine, the latter being poor in resveratrol and in polyphenols, in general.

Nevertheless, an ethanol-free white wine extract was able to exert cardioprotective effects: it increased aortic and coronary blood flow, as well as left ventricular developed pressure, and reduced myocardial infarct size, as well as malondialdehyde, in rat hearts significantly versus controls and other ethanol-free white wine extracts. Interestingly, the cardioprotective white wine extract exerted the most pronounced antioxidant activity (13). The monophenols tyrosol and caffeic acid, which are present in extra virgin olive oil, appear to be the main compounds that contribute to the antioxidant activity of white wine. These compounds also exert other effects, such as the inhibition of the release of inflammatory cytokines, including TNF-α, interleukin-1β, and interleukin-6, at concentrations that are found in the bloodstream after modest wine or olive oil intake (3, 5, 22).

Also grape extracts and seeds have proved to be cardioprotective in the same heart model used for the studies with red and white wine (12, 35, 43). Grapes also contain melanin, which is an antioxidant that possesses antiinflammatory and immunomodulating properties, including the ability to inhibit cytokine (TNF-α, interleukin-1β, and interleukin-6) production (2).

## WINE AS AN ARCHAEOLOGICAL ARTEFACT

Technological advances enable investigators to carry out in-depth investigations on the composition of very small traces of substances on archaeological artefacts. The detection of large quantities of tartaric acid identifies the trace as the remnant of grape juice or wine; the detection of polyphenols and of fermentation derivatives is then required for the differential diagnosis (31). This can easily be achieved with high-pressure liquid chromatography (HPLC) and gas chromatography (GC).

Archaeochemists have thus found traces of wine on a number of artefacts, including a 7,000-year-old Neolithic jar, which pushes the history of wine making back by 2,000 years (31).

A number of authors have provided us with literature on wine-making techniques and usage in Greek and Roman times, when wine played an important role in medicine: it was the basic ingredient of dozens of "composite" remedies including also other ingredients, such as fruit juice, spices, roots, and honey (49). The historian Pliny the Elder (37) tells us that wine was always diluted with something else, such as water, which could also be seawater "to enliven the wine's smoothness." Columella (11) describes the procedure of simmering grape syrup slowly in a lead pot or lead-lined copper kettle; this resulted in the formation of "sugar of lead" or lead acetate, a potent fungicide that preserved the fluid well. In the last century, Hoffman followed the recipes of Columella and ended up with wines that contained 15–30 mg/L lead acetate—50–100 times the currently legal limit (32, 33). Indeed, Pliny describes a pandemic disease that resembles saturnine gout; historians claim that the disease contributed to the fall of the Roman empire (32, 37).

The medicinal use of wine continued during the Middle Ages, when its virtues were extolled not only by the great school of medicine at Salerno (Italy), but even by textbooks of Arabic medicine. At the same time, the Chinese used plant extracts containing high concentrations of polyphenols, such as resveratrol (49).

## THE DISCOVERY AT FAVIGNANA

In September 2000, the corroded relict of a ship was found offshore along the west coast of Sicily, at Favignana, near Marsala, by Sicily's regional archaelogical team, led by Sebastiano Tusa (44) (Fig. 1). Its cargo consisted of a number of unusually small amphorae (27.6 cm high including the stopper) made of pewter and of notable artistic value (Fig. 2). Subsequent archaeological investigations established that the vessels dated back to 1300–1400 AD.

The amphorae were tightly closed with screw stoppers, and the contents of some of them were still intact and liquid. The small size and artistic beauty of the vessels induced us to speculate that they contained a special liquid, possibly a medicinal remedy, and to ask the archaeologists to give us a sample so we could analyze it.

## WHAT WAS THE MEDIEVAL FLUID FOUND IN THE FAVIGNANA AMPHORAE?

The medieval fluid was submitted to HPLC (see Appendix, note 1). Following detection of tartaric acid, the experimental plan was laid for the detection of organic acids and phenols usually present in compounds belonging to the vegetable kingdom (see Appendix, note 2). The results are shown in Table 1. The finding of tartaric acid indicated that the medieval fluid was a fruit juice, made at least partly from grapes. The concentration of ascorbic acid (1.6 mg/ml) indicated that fruit juice rich in vitamin C, such as orange juice, had been added, as the content of ascorbic acid in grapes is low (36).

The medieval fluid was then submitted to GC focused on the detection of primary and secondary compounds resulting from the fermentation of grapes (see Appendix, note 3). This investigation detected a low concentration of ethanol [3.01 g/100 ml, three to four times lower than the concentration in modern wine (40)] and compounds that are the typical result of secondary fermentation of grapes: acetaldehyde, methanol, 2-methyl-1-butanol and 3-methyl-1-butanol (39) (Table 2).

The medieval fluid was subsequently subjected to atomic absorption spectrophotometry to establish its content in sodium and lead (see Appendix, note 4). The Na+ concentration was low (4,608 ppm). As sea infiltration was a possible explanation for the low concentration of ethanol, a comparison was made with threefold-diluted seawater samples collected near the site of wreckage. The concentrations results were similar:


**FIG. 1. Site of wreckage in the Mediterranean Sea.**

medieval fluid 4,608 versus 4,900 ppm, suggesting that seawater had seeped into the vessel through microcracks, diluting the fluid. However, the intentional addition of salty water to the juice "to enliven the wine's smoothness" as described by Pliny (37) cannot be ruled out.

The concentration of lead was found to be high (6 mg/L, equivalent to 20 times the current legal limit of 0.3 mg/L) (33). The medieval fluid was contained in amphorae made of pewter, an alloy that contains lead, so its high concentration may be due to leaching from the vessel for centuries. However, it is known that Roman food and wine were contaminated by lead following the custom to simmer grape syrup in lead pots, and lead poisoning is believed to have contributed to the fall of the Roman Empire (11, 32). Thus, the high concentration may have been due to lead contamination during its production, to which leaching contributed even further. Either way, the finding of a fluid for oral consumption in a vessel partly made of lead suggests that lead poisoning had still not been recognized in the Middle Ages. Indeed, lead-induced gout, an inflammatory disorder involving the joints, was widespread in the upper classes in England three centuries later (1).

TABLE 1. HPLC ANALYSIS OF MEDIEVAL FLUID

| | *Retention Time (min)* | *Concentration (mg/ml)* |
|---|---|---|
| λ = 210 nm | | |
| Chinic acid | 5.3 | 64.0 |
| Tartaric acid | 5.6 | 1.2 |
| Malic acid | 7.2 | 0.6 |
| Lactic acid | 8.7 | 44.1 |
| Acetic acid | 9.1 | 78.5 |
| Succinic acid | 15.4 | 60.6 |
| λ = 254 nm | | |
| Ascorbic acid | 8.9 | 1.6 |

TABLE 2. GC ANALYSIS OF MEDIEVAL FLUID: SECONDARY FERMENTATION COMPOUNDS

| *Substance* | *Concentration (mg/L)* |
|---|---|
| Acetaldehyde | 65 |
| Methanol | 20 |
| 2-Methyl-1-butanol | 15 |
| 3-Methyl-1-butanol | 69 |

**FIG. 2. Photograph of sealed pewter amphora shortly after retrieval from the shipwreck.**

The next investigation was aimed at the quantification of phenols expressed by the Folin-Ciocalteau index (45), using wines as reference fluids (see Appendix, note 5). The polyphenol content of the medieval fluid was low as compared with the red wine and white Marsala wine: the Folin–Ciocalteau polyphenol index was only 9 mg of gallic acid/100 g versus 285 mg/100 g in the red wine and 32 mg/100 g in the white Marsala wine.

In summary, the analysis of the medieval fluid with modern technology established that it was a fermented white grape juice, to which citrus juice and salty water may have been added; the fluid was contaminated with lead.

## THE ANTIOXIDANT ACTIVITY OF THE MEDIEVAL FLUID

The antioxidant activity of the medieval fluid was investigated by radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) detection and electron paramagnetic resonance (EPR) and compared with that of modern products of grape fermentation, *i.e.,* a modern threefold-diluted red wine and white Marsala wine.

Radical DPPH detection, a spectrophotometric test that evaluates antioxidant activity, was performed according to the method of Brand-Williams *et al.* (8) with a few modifications (see Appendix, note 6).

Antioxidant activity was also measured against the hydroxyl (ΠOH) free radical generated in a Fenton system by using an EPR spin-trapping method (41) (see Appendix, note 7).

According to DPPH, red wine exerted the highest antioxidant activity, whereas the antioxidant activities of the medieval fluid and white Marsala wine were almost identical (Fig. 3).

The similarity of the antioxidant activities of the medieval fluid and the white Marsala wine was confirmed by EPR already after twofold concentration of the medieval fluid (scavenging activity: red wine, 100.0; white Marsala wine, 81.7; medieval fluid, 48.5; medieval fluid concentrated 2:1, 82.8).

## OTHER BIOLOGICAL PROPERTIES OF THE MEDIEVAL FLUID

The immunomodulatory and antiinflammatory activities of the medieval fluid were studied, because substances contained in fermented grape juice, such as resveratrol, melanin, tyrosol, caffeic acid, and others, possess these properties (2–4, 6, 12–14, 22, 35, 43).

The immunomodulatory activity of the medieval fluid was studied by measuring the capacity of U937 human monocytoid cells obtained from the American Tissue Culture Collection (Rockville, MD, U.S.A.) to phagocytize *Bacillus subtilis* after incubation with the medieval fluid in the presence and absence of lead versus incubation with a control solution containing 3% alcohol and salt. The method has already been described in detail elsewhere (50).

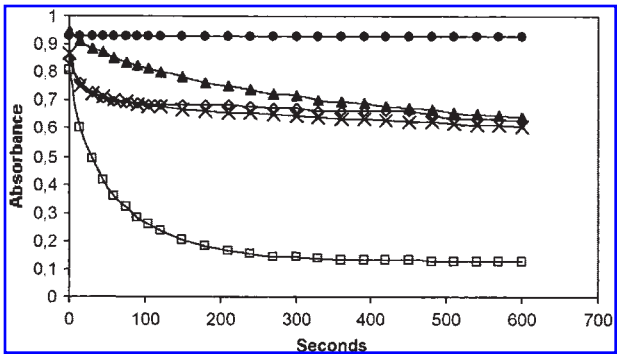


**FIG. 3. Results of antioxidant assay using the stable synthetic free radical DPPH.** Antioxidant activity was measured by evaluating absorbance decrease at 517 nm in the presence of potential scavenger products, and comparing results with a well-known scavenger molecule, Trolox. A reference solution was made by adding 0.1 ml of a 1 m*M* ethanolic Trolox solution to the reaction mixture (final concentration 0.04 m*M*). Threefold-diluted red wine (□) produced a clear reduction, reaching a plateau at ~0.2 absorbance units 200 s after the reaction. Plots of medieval fluid (×) and white wine (▲) were close to the plot of 0.013 m*M* Trolox (◆), especially at the end of the reaction time, but white wine initially had slower kinetics of DPPH degradation than medieval fluid. ●, reference.

The antiinflammatory activity of the medieval fluid was studied measuring the levels of inducible TNF-α, which has proved to play an important role in inflammatory diseases, such as rheumatoid arthritis and ankylosing spondylitis, so that its inhibition may be considered a clinically meaningful index of antiinflammatory properties (23, 34). TNF-α levels were measured in the supranatants collected from the samples of fluid from the phagocytosis experiment. The method has already been described in detail elsewhere (5).

The medieval fluid reduced the release of TNF-α by human monocytoids independently of lead (medieval fluid − Pb, 2,618 ± 0.18 pg/ml; medieval fluid + Pb, 2,381 ± 0.31 pg/ml; versus 3% alcohol + salt control, 17,739 ± 5,485 pg/ml), but increased their capacity to phagocytize *Bacillus subtilis* only after lead removal (phagocytosis frequency: medieval fluid − Pb = 62 ± 31%, medieval fluid + Pb = 43 ± 26%; versus 3% alcohol + salt control = 46 ± 14%). The results confirming nonspecific immunity enhancement are consistent with previous data obtained with resveratrol, tyrosol, and melanin, and with Roman medical practice (Galen used wine to disinfect the wounds of gladiators) (2–4, 14, 22, 49).

## WHAT HAVE WE LEARNED FROM THE ANALYSIS OF A 600-YEAR-OLD FRUIT JUICE?

To our knowledge, this is the first time that the antioxidant activity and other biological properties of a medieval fruit juice have been studied with modern techniques.

The conclusions that can be drawn from this observational report are limited by the small sample size due to the small amount of medieval fluid made available to us. However, all differences were important from a biological point of view (Fig. 3).

The very long-term persistence of antioxidant activity of fermented fruit juice stresses the importance of fruit in maintaining the oxidant/antioxidant balance in the organism and its role as a staple of the Mediterranean diet.

An open issue is what was responsible for the persistent antioxidant activity. Both red and white wines notoriously possess antioxidant activity thanks to their content of poly- and monophenols (13, 15), which were scanty in the medieval fluid. However, it did contain a fairly high concentration of another antioxidant, namely, ascorbic acid (1.6 mg/ml), which therefore appears to have contributed to the positive outcome of the tests.

Interestingly, modern technology established that the composition of the medieval fluid corresponded to recipes for "composite" medicinal remedies used in Roman times: wine (the fermented grape juice may have been wine, although this cannot be demonstrated with certainty) diluted with seawater and citrus fruit juice. Thus, the hypothesis that the small pewter vessel contained a medicinal remedy appears to have been correct.

The sea bottom environment reproduces the conditions of the ideal wine cellar: total darkness, which prevents light from giving wine a cardboard-type flavor, and stable temperature only a few degrees above freezing, which slows down the aging process (25, 47). Indeed, century-old champagne has been retrieved from shipwrecks in excellent condition, when the bottle was properly sealed as the medieval pewter vessel was (30, 47).

What did the sommelier have to say about the medieval fermented grape juice after 600 years? "Dull white color with a pale golden hue; aroma of leather-dried grapes and aromatic vine; acid, astringent and salty taste devoid of bitterness. The taste is strong and unusually persistent . . ." Tasting was not allowed to continue in view of the high lead content of the fluid.

In conclusion, the opportunity to analyze a 600-year-old fruit juice has provided evidence for the first time that the antioxidant, antiinflammatory, and immunomodulatory properties of fermented fruit can persist for centuries, provided that it is properly stored.

## ACKNOWLEDGMENTS

We thank the Laboratorio Unione Italiana Vini di Verona for additional analysis of the medieval fluid, Prof. Sebastiano Tusa (Centro del Restauro di Palermo)—the discoverer of the wrecked ship—for having supplied the samples of medieval fluid, Jennifer Hartwig, M.D., for editorial assistance, the President of the International Association of Sommeliers, Mr. Vaccarini, for his tasting notes, and the Regional Institute for Vine and Wine of the Region of Sicily for supplying the photograph of the amphora.

## ABBREVIATIONS

DMPO, 5,5-dimethylpyrrolidine-*N*-oxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EPR, electron paramagnetic resonance; GC, gas chromatography; HPLC, high-pressure liquid chromatography; NO, nitric oxide; ·OH, hydroxyl radical; PBS, phosphate buffer solution; TNF-α, tumor necrosis factor-α.

## APPENDIX

1. Inertsil ODS-3 4.6 × 250 mm column with 5 μm particle size and $H_3PO_4$ 0.02 *M* mobile phase at a flow rate of 0.7 ml/min at 20°C; repeat analysis: Inertsil PH 4.6 × 250 mm column with 5 μm particle size and $H_2O/CH_3CN/CH_3COOH$ (80:17:3) mobile phase at a flow rate of 0.5 ml/min at 50°C; extraction protocol: centrifugation and fivefold dilution with mobile phase.
2. Compounds were detected by an ultraviolet wave detector at 210 (organic acids, including acetic, succinic, lactic, malic, and ascorbic acids), 254 (ascorbic acid), 280 (simple phenols), and 320 (cinnamates) nm.
3. Headspace method, using an automatic headspace sampler, conditioning 2 ml of each sample in a closed 3-ml vial at 80°C for 1 hr; 500 μl of headspace was injected into the gas chromatograph; a 30-m Carbowax 20M column was used with He as carrier at 0.8 ml/min, programmed at 40°C for 5 min, then 30°C/min and 150°C for 5 min; the injector was at 230°C, FID was at 250°C; retention time of ethanol was 5.2 min.
4. PerkinElmer atomic spectrophotometer model M2100, sodium assay on the substrates (0.4 ml) dissolved in 3.6 ml of $HNO_3$ 0.1 *M* and $La(NO_3)3{\cdot}6H_2O$ 5 m*M* after calibration

with standards; lead was determined by graphite furnace atomic absorption spectrometry.

5. Method: the following were poured into a 25-ml flask: 0.1 ml of water (blank) or 0.1 ml of 0.1% gallic acid solution in water (reference) or 0.1-ml sample of medieval fluid or one of two different kinds of wine, a threefold diluted red wine and white Marsala wine; 1.25 ml of Folin–Ciocalteau reagent; 2.50 ml ml $Na_2CO_3$-saturated solution; these solutions were set to 25-ml volume with water, kept 2 h in the dark, and their absorbance was read against blank at 730 nm. Phenol index was expressed as mg of gallic acid/100 ml of solution.
6. A final reaction volume of 2.6 ml was used. The assayed substrates (medieval fluid or modern wines) were added (0.1 ml) to 96% spectrophotometric grade ethanol (1.9 ml) and 0.1 ml of $H_2O$, then were mixed with the DPPH solution (0.5 ml, 0.5 m*M*) to the final concentration of 0.1 m*M*. The blank solution was made by adding the same volume (0.1 ml) of pure ethanol solution to the DPPH solution; a reference solution was made by adding 0.1 m*M* ethanol Trolox solution (final concentration 0.04 m*M*) to the reaction mixture. The kinetics of DPPH bleaching was calculated by reading the absorbance at 517 nm every 30 s for 10 min, immediately after mixing the solutions. Each assay was replicated five times.
7. The hydroxyl radical (·OH) produced in the Fenton reaction mixture was trapped with 5,5-dimethylpyrrolidine-*N*-oxide (DMPO). The resultant adduct DMPO·OH, consisting of four vertical signals, was detected by an X-band EPR spectrometer Varian E-line Century series. For ·OH measurement, the mixture without the scavenging compounds (blank) contained: 0.7 of phosphate buffer solution, pH 7.4 (PBS); 0.2 ml of Fe-EDTA 10–12 m*M*; 0.2 ml of 50 m*M* DMPO 50 m*M* in PBS; 0.2 ml of $H_2O_2$ 10 m*M*. The mixture with the test scavenging compound contained: 0.5 ml of PBS; 0.2 ml of scavenger (wine or diluted wine); 0.2 ml of Fe-EDTA 10–12 m*M*; 0.2 ml of 50 m*M* DMPO 50 m*M* in PBS; 0.2 ml of $H_2O_2$ 10 m*M*. These solutions were accurately mixed in a glass tube assay and successively placed in the EPR probe, a capillary tube of 100-mm length and 1.3-mm internal diameter. EPR spectra were recorded after exactly 2 min. The instrumental parameters were: frequency, 9.26 GHz; power, 5 mW; field set, 3,390 gauss; scan time, 64 s; time constant, 0.5 s; gain, 16,000; modulation, 1 gauss. The scavenger activity percent of the test compound for $^{\Pi}$OH was expressed by the following formula: $I = 100 - [(h_x/h_0)] \times 100$ where $I$ was scavenger activity, $h_0$ and $h_x$ were the relative heights of the signal 2 (mm) of the DMPO$^{-}$ $^{\Pi}$OH adduct spectra in a reaction mixture without and with the scavenger compound, respectively. To avoid interference of ethanol in the EPR analysis, the wines were deprived of alcohol at low temperature in a centrifugal evaporator; the initial volume was restored with water. Each assay was repeated 10 times.

## REFERENCES


1. Appelboom T and Bennett JC. Gout of the rich and famous. *J Rheumatol* 13: 618–622, 1986.
2. Avramidis N, Kourounaklis A, Hadjipetrou L, and Senchuk V. Anti-inflammatory and immunomodulating properties of grape melanin. Inhibitory effects on paw edema and adjuvant induced disease. *Drug Res* 48: 764–771, 1998.
3. Bertelli A, Migliori M, Bertelli AAE, Origlia N, Filippi C, Panichi V, Falchi M, and Giovannini L. Effect of some white wine phenols in preventing inflammatory cytokine release. *Drugs Exp Clin Res* 28: 11–15, 2002.
4. Bertelli AAE, Ferrara F, Diana G, Fulgenzi A, Corsi M, Ponti W, Ferrero ME, and Bertelli A. Resveratrol, a natural stilbene in grapes and wine, enhances intraphagocytosis in human promonocytes: a co-factor in antiinflammatory and anticancer chemopreventive activity. *Int J Tissue React* 21: 93–104, 1999.
5. Bertelli AAE, Migliori M, Panichi V, Longoni B, Origlia N, Ferretti A, Cuttano MG, and Giovannini L. Oxidative stress and inflammatory reaction modulation by white wine. *Ann N Y Acad Sci* 957: 295–301, 2002.
6. Bhat KP and Pezzuto JM. Cancer chemopreventive activity of resveratrol. *Ann N Y Acad Sci* 957: 210–229, 2002.
7. Bhat KPL, Kosmeder JW 2nd, and Pezzuto JM. Biological effects of resveratrol. *Antioxid Redox Signal* 3: 1041–1064, 2001.
8. Brand-Williams W, Cuvelier ME, and Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* 28: 25–30, 1995.
9. Burns J, Crozier A, and Lean ME. Alcohol consumption and mortality: is wine different from other alcoholic beverages? *Nutr Metab Cardiovasc Dis* 11: 249–258, 2001.
10. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, and De Caterina R. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation. Antiatherogenic properties of Mediterranean diet phytochemicals. *Arterioscler Thromb Vasc Biol* 23: 622–629, 2003.
11. Columella. De re rustica. 12: 20.
12. Cui J, Juhasz B, Tosaki A, Maulik N, and Das DK. Cardioprotection with grapes. *J Cardiovasc Pharmacol* 40: 762–769, 2002.
13. Cui J, Tosaki A, Bertelli AA, Bertelli A, Maulik N, and Das DK. Cardioprotection with white wine. *Drugs Exp Clin Res* 28: 1–10, 2002.
14. Culpitt SV, Rogers DF, Fenwick PS, Shah P, De Matos C, Russell REK, Barnes PJ, and Donnelly LE. Inhibition by red wine extract, resveratrol, of cytokine release by alveolar macrophages in COPD. *Thorax* 58: 942–946, 2003.
15. Das DK, Sato M, Ray PS, Maulik G, Engelman RM, Bertelli AA, and Bertelli A. Cardioprotection of red wine: role of polyphenolic antioxidants. *Drugs Exp Clin Res* 25: 115–120, 1999.
16. De La Torre-Boronat MC. Scientific basis for the health benefits of the Mediterranean diet. *Drugs Exp Clin Res* 25: 155–161, 1999.
17. Di Bari M, Zacchei S, Kritchevsky SB, Anichini M, Cesaretti S, Chiarlone M, Masotti G, and Marchionni N. Anti-oxidized LDL antibodies and wine consumption: a population-based epidemiological study in Dicomano, Italy. *Ann Epidemiol* 13: 189–195, 2003.
18. Di Castelnuovo A, Rotondo S, Iacovielle I, Donati M, and de Gaetano G. Wine consumption and vascular risk: a meta-analysis. *Proceedings of the XXV Congres Mondial de la Vigne et du Vin*, Section IV, Wine and Health, Paris, France, June 19–23, 2000, pp. 9–16.

19. Ferrero ME, Bertelli AAE, Fulgenzi A, Pellegatta F, Corsi MM, Bonfrate M, Ferrara F, De Caterina R, Giovannini L, and Bertelli A. Activity in vitro of resveratrol on granulocyte and monocyte adhesion to endothelium. *Am J Clin Nutr* 68: 1208–1214, 1998.
20. Frankel EN, Kanner J, German JB, Parks E, and Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* 341: 454–457, 1993.
21. Friedman LA and Kimball AW. Coronary heart disease mortality and alcohol consumption in Framingham. *Am J Epidemiol* 124: 481–489, 1986.
22. Giovannini L, Migliori M, Filippi C, Origlia N, Panichi V, Falchi M, Bertelli AAE, and Bertelli A. Inhibitory activity of the white wine compounds, tyrosol and caffeic acid, on lipopolysaccharide-induced tumor necrosis factor-α release in human peripheral blood mononuclear cells. *Int J Tissue React* 24: 53–56, 2002.
23. Gorman JD, Sack KE, and Davis JC. Treatment of ankylosing spondylitis by inhibition of tumor necrosis factor alpha. *N Engl J Med* 346: 1349–1356, 2002.
24. Gronbaek M, Becker U, Johansen D, Gottschau A, Schnohr P, Hein HO, Jensen G, and Serensen TIA. Type of alcohol consumed and mortality from all causes, coronary heart disease and cancer. *Ann Intern Med* 133: 411–419, 2000.
25. Hicks S. Wine bottles should be constantly cool. *Los Altos Town Crier* April 11, 2001, 54 (15).
26. Imamura G, Bertelli AA, Bertelli A, Otani H, Maulik N, and Das DK. Pharmacological preconditioning with resveratrol: an insight with iNOS knockout mice. *Am J Physiol Heart Circ Physiol* 282: H1996–H2003, 2002.
27. Klatsky AL, Armstrong MA, and Friedman GD. Alcohol and mortality. *Ann Intern Med* 117: 646–654, 1992.
28. Klatsky AL, Friedman GD, Armstrong MA, and Kipp H. Wine, liquor, beer and mortality. *Am J Epidemiol* 158: 585–595, 2003.
29. Leighton F, Cuevas A, Guasch V, Perez DD, Strobel P, San Martin A, Urzua U, Diez MS, Foncea R, Castillo O, Mizon C, Espinoza MA, Urguiaga I, Rowzowski J, Maiz A, and Germain A. Plasma polyphenols and antioxidants, oxidative DNA damage and endothelial function in a diet and wine intervention study in humans. *Drugs Exp Clin Res* 25: 133–141, 1999.
30. Mansson PH. Sunken treasure from the "other" Titanic. Wine Spectator Oct 31, 1987.
31. McGovern PE. Vin extraordinaire. *The Sciences* Nov/Dec, 27–31, 1996.
32. Nriagu JO. Saturnine gout among Roman aristocrats. Did lead poisoning contribute to the fall of the Empire? *N Engl J Med* 308: 660–663, 1983.
33. Office International de la Vigne et du Vin (O.I.V.) Résolution Oeno 1/96.
34. Paleolog E. The therapeutic potential of TNF-alpha blockade in rheumatoid arthritis. *Expert Opin Investig Drugs* 12: 1087–1095, 2003.
35. Pataki T, Bak I, Kovacs P, Bagchi D, Das DK, and Tosaki A. Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. *Am J Clin Nutr* 75: 894–899, 2002.
36. Pellati R. *Tutti i Cibi dalla "A" alla "Z" [All Kinds of Food from "A" to "Z"]*. Milano: Edizioni Oscar Mondadori, 1999, pp. 101–102, 500–501.
37. Pliny. Natural history. 14: 29.
38. Renaud SC, Gueguen R, Siest G, and Salamon R. Wine, beer and mortality in middle-aged men from Eastern France. *Arch Intern Med* 159: 1865–1870, 1999.
39. Ribéreau-Gayon P, Dubourdieu D, Donéche B, and Lonvaud A. Vol. I, Microbiologia del vino. Vinificazioni. In: *Trattato di Enologia.* Milano: Edagricole Srl, 2003, pp. 55, 57, 76–78, 256, 394.
40. Robinson J (Ed). *The Oxford Companion to Wine.* Oxford and New York: Oxford University Press, 1994, p. 1068.
41. Sanchez-Moreno C. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci Tech Int* 8: 121–137, 2002.
42. Sato M, Ray PS, Maulik G, Maulik N, Engelman RM, Bertelli AA, Bertelli A, and Das DK. Myocardial protection with red wine extract. *J Cardiovasc Pharmacol* 35: 263–268, 2000.
43. Sato M, Bagchi D, Tosaki A, and Das DK. Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia/reperfusion-induced activation of JNK-1 and C-JUN. *Free Radic Biol Med* 31:729–737, 2001.
44. Shah M and Harrington S. Undersea archaeologists discover historic vessels of wine. *Wine Spectator* Nov 30, 15, 2000.
45. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16: 144–158, 1965.
46. Stoclet JC. Bonum vinum laetificat cor hominum. *Med Sci Monit* 7: 842–847, 2001.
47. Suckling J. Underwater Cellar. *Wine Spectator* Sept 15, 1987.
48. Ulbricht TLV and Southgate DAT. Coronary heart disease: seven dietary factors. *Lancet* 338: 985–992, 1991.
49. Vintani PG. Fragments of tradition: revisiting the virtues of wine. *Drugs Exp Clin Res* 25: 163–165, 1999.
50. Zelig BJ. *Manual of Macrophage Methodology.* New York and Basel, Marcel Dekker Inc., 1981, pp. 271–273.

Address reprint requests to:
*Alberto A.E. Bertelli*
*Department of Human Morphology*
*University of Milan*
*Via Mangiagalli 31*
*20133 Milano, Italy*

*E-Mail:* studiojh@usa.net

Received for publication May 11, 2004; accepted June 13, 2004.

**This article has been cited by:**

1. Prof. Mahin D. Maines . 2005. The Heme Oxygenase System: Update 2005The Heme Oxygenase System: Update 2005. *Antioxidants & Redox Signaling* **7**:11-12, 1761-1766. [Citation] [PDF] [PDF Plus]