

Antioxidant properties of commercial grape juices and vinegars

Alberto Dávalos, Begoña Bartolomé, Carmen Gómez-Cordovés *

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva, 3, 28006 Madrid, Spain

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Abstract

Antioxidant activity of commercial red ($n = 5$) and white ($n = 5$) grape juices and wine vinegars ($n = 5$) were determined by the oxygen radical absorbance capacity (ORAC) assay using fluorescein (FL) as fluorescent probe. The ORAC-FL values varied from 14.6 to 25.0 μmol of trolox equivalents/ml for red grape juices, from 3.5 to 11.1 μmol of trolox equivalents/ml for white grape juices, and from 4.5 to 11.5 μmol of trolox equivalents/ml for wine vinegars. Differences in the antioxidant activities among grape juice, wine, and vinegar were attributed to their different phenolic contents and compositions and to other non-phenolic antioxidants present in the samples. These data confirm grape juice and wine vinegar as good dietary sources of antioxidants.

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1. Introduction

Polyphenols are found in food (vegetables, fruits, chocolate, tea, coffee, wine, grape juice, vinegar) at different concentrations (Scalbert & Williamson, 2000). Epidemiological evidence indicates an inverse relationship between the intake of food rich in phenolic compounds (i.e. flavonoids) and the reduction of certain chronic diseases and coronary heart disease mortality (Hertog, Kromhout, & Aravanis, 1995; Keli, Hertog, Feskens, & Kromhout, 1996; Steinmetz & Potter, 1996). Although polyphenols have been shown to exhibit different biological activities, such as antioxidant, to decrease platelet aggregation and endothelial adhesion, to mediate nitric oxide production, to suppress cancer cell growth, and to reduce oxidative stress (Andriantsitohaina, 1999; Facino et al., 1999; Leikert et al., 2002; Rice-Evans, Miller, & Paganga, 1996; Soles, Grass, Josephy, Goldberg, & Diamandis, 2002),

the exact nature of their protective effect remains to be established.

Vitis vinifera fruits show a high concentration and a great variety of phenolic compounds (Macheix, Fleuriet, & Billot, 1990). Thus, grape juice is a rich source of flavonoids and other phenolics in the human diet (Rice-Evans et al., 1996). Positive health benefits of the consumption of grape juice, such as improvement of the endothelial function, increase of the serum antioxidant capacity, protection of LDLs against oxidation, decrease of native plasma protein oxidation, and reduction of platelet aggregation, have also been reported (Chou et al., 2001; Day, Kemp, Bolton, Hartog, & Stansbie, 1997; Keevil, Osman, Reed, & Folts, 2000; O'Byrne, Devaraj, Grundy, & Jialal, 2002; Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999). The fermented fruit grape products – wine (alcoholic) and vinegar (alcoholic and acetic fermentations) – are also rich in polyphenols. Evidence of a negative association between coronary heart disease (CHD) mortality and wine consumption has suggested possible protective effects of wine. Although it is known that moderate consumption of ethanol reduces the incidence of CHD, certain studies (Leger, Cochrane, & Moore, 1979; Renaud & De

* Corresponding author. Tel.: +34 91 562 29 00; fax: +34 91 564 48 53.

E-mail address: cgcordoves@ifi.csic.es (C. Gómez-Cordovés).

Lorgeril, 1992) indicate that red wine beneficial effects can be attributed to its content of phenolic compounds, which come from the must and can be modified through the wine-making process. Although much less studied, wine vinegar also exhibits potential health benefits, such as the anti-hypertensive effects observed in rats (Kondo, Tayama, Tsukamoto, Ikeda, & Yamori, 2001; Nishikawa et al., 2001; Sugiyama, Saitoh, Takahara, Satoh, & Hashimoto, 2003).

With increasing interest in the function and diversity of antioxidants in foods, several *in vitro* rapid methods for measuring antioxidant activity of food, beverages and biological samples have been developed (Prior & Cao, 1999). Among them, the oxygen radical absorbance capacity (ORAC) assay has gained much attention because it deals with peroxy radicals, the most abundant radicals in biological systems. The ORAC assay considers both the inhibition time and the inhibition degree (as the reaction goes to completion) and directly estimates the chain-breaking antioxidant activity (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). Recently, the ORAC assay has been improved by using fluorescein (ORAC-FL) instead of β -phycoerythrin as the fluorescent probe (Ou, Hampsch-Woodill, & Prior, 2001).

Antioxidant activities of certain grape-derived beverages, such as red and white wines, have been extensively assessed, but there is not much information regarding antioxidant properties of grape juice on wine vinegar. The present work was performed to determine the range of variability of the oxygen radical scavenging activity of commercial grape juices and vinegars by the ORAC-FL method.

2. Materials and methods

2.1. Chemicals and apparatus

Fluorescein (FL) disodium salt was purchased from Sigma (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH) were obtained from Aldrich (Milwaukee, WI). A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with a 485-P excitation and a 520-P emission filter was used. The equipment was controlled by Fluostar Galaxy software (version 4.11-0). Measurements were carried out using black 96-well microplates (96F untreated microwell, Nunc™, Denmark).

2.2. Samples

Five red grape juice (RGJ), five white grape juice (WGJ) and five wine vinegar (WV) samples were pur-

chased from local markets. As indicated by the manufactures, RGJ #1, 3 and 5, and WGJ #6, 7 and 9, proceeded from concentrated grape juice. RGJ #4, and WGJ #8 and 10 proceed directly from squeezed grapes. RGJ #2 proceed from organic red grapes. WV #11 was fermented from a Cabernet Sauvignon red wine, #12 and 15 from sherry wines, #13 from white wine and #14 from oak-aged white wine. Samples #11, 12, 13 and 15 were aged in oak-barrels. No antioxidant additives were reported in any sample, except for WGJ #10 that contained E-220, E-300 and E-330. Both grape juices and wine vinegars were diluted in 75 mM phosphate buffer (pH 7.4) for analysis. Total polyphenol (TP) content was determined according to the Folin-Ciocalteu method (Singleton & Rossi, 1965), using gallic acid as standard. The results were expressed as mg of gallic acid equivalents per litre of sample.

2.3. ORAC-fluorescein assay

The ORAC-fluorescein (ORAC-FL) assay was based on that proposed by Ou et al. (2001) and further modified by Dávalos, Gómez-Cordovés, and Bartolomé (2004). Briefly, the reaction was performed in 75 mM phosphate buffer (pH 7.4) and the final assay mixture (200 μ l) contains fluorescein (120 μ l, 70 nM final concentration) as oxidizable substrate, AAPH (60 μ l, 12 mM final concentration) as oxygen radical generator, and antioxidant (20 μ l, either trolox [1–8 μ M, final concentration] or sample [at different concentrations]). The reaction was performed at 37 °C and fluorescence was recorded every minute for 80 min. A blank (control) using phosphate buffer instead of the antioxidant was carried out in each experiment. The fluorescein stock solution (1.17 mM) was made in 75 mM phosphate buffer (pH 7.4) and stored under dark conditions at 4 °C for 4 weeks. AAPH and trolox solutions in 75 mM phosphate buffer (pH 7.4) were prepared daily. All reaction mixtures were prepared in duplicate and at least three independent runs were performed for each sample. Fluorescent measurements were normalized to the curve of the blank (no antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as:

$$\text{AUC} = 1 + \sum_{i=1}^{i=80} f_i/f_0,$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i . The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. Regression equations between net AUC and antioxidant concentration were calculated for all the samples. ORAC-FL values were expressed as trolox equivalents by using the standard curve calculated for each experiment. Final

ORAC-FL values were expressed as μmol of trolox equivalents/ml of grape juice or wine vinegar.

2.4. Statistical analysis

A statistical programme (SPSS for Windows, release 11.0.1, SPSS, Inc., Chicago, IL, 2001) was used for data processing.

3. Results and discussion

Fig. 1 depicts the kinetic behaviour of the fluorescein/AAPH system in the absence and in the presence of different concentrations of red grape juice (RGJ), white grape juice (WGJ) and wine vinegar (WV). Antioxidants

present in both RGJ and WGJ completely neutralise the peroxy radicals generated in the system, which delays the decay of the fluorescence curve until a certain time, proportional to the antioxidant concentration (Fig. 1(a) and (b)). This kinetic pattern was also noted for wine and certain pure phenolic compounds (Dávalos et al., 2004). However, for the WV samples, a slight decrease in fluorescence, was accomplished due to the delay of the fluorescence decay. It was checked that acetic acid at the concentrations found in wine vinegar, did not influence the fluorescence intensity of fluorescein, either in the absence or in the presence of AAPH (results not shown). Consequently, the differences in the ORAC-FL kinetic behaviour observed for grape juice and wine vinegar were attributed to the different natures of the antioxidant substances present in the two types of samples.

The concentration interval, leading to a linear relationship between the net area under the curve (AUC) and the antioxidant concentration, was determined for all the samples (Table 1). Within this interval, any antioxidant concentration gave the same oxygen radical absorbance capacity (ORAC-FL) value (Table 1). As expected, the ORAC-FL values were higher for red (14.6–25.0 μmol of trolox equivalents/ml) than for white (3.5–11.1 μmol of trolox equivalents/ml) grape juices. The wine vinegars studied exhibited ORAC-FL values (4.5–11.5 μmol of trolox equivalents/ml) similar to white grape juices. Analysis of variance (ANOVA) showed differences ($p < 0.05$) among groups but the 95% confi-

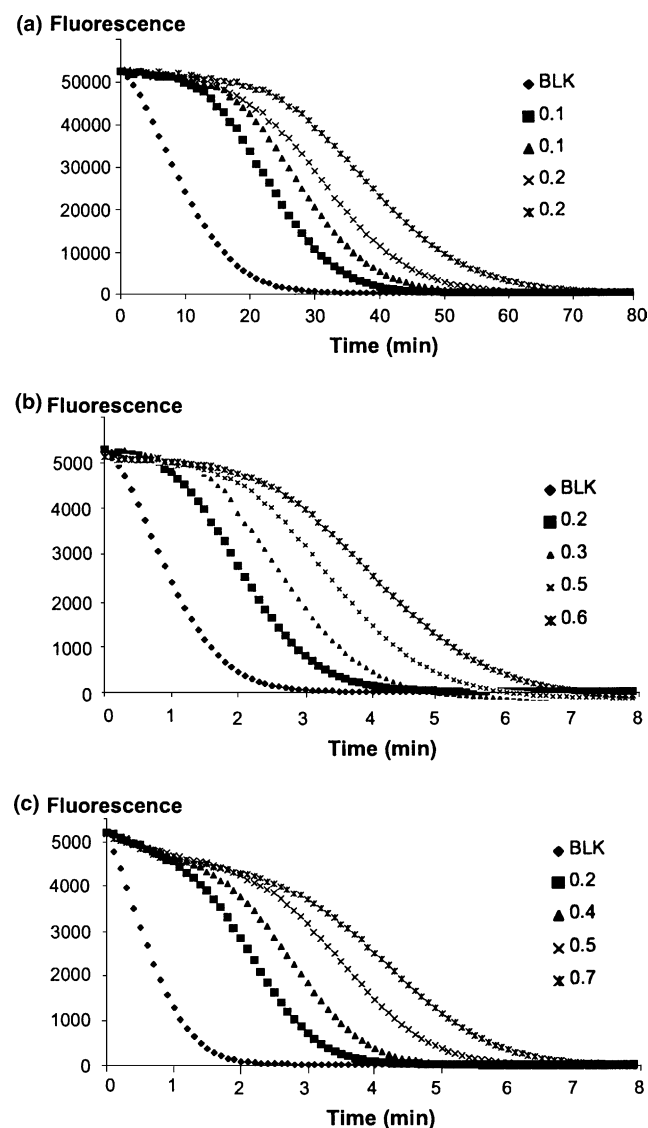


Fig. 1. Time course of the reaction of fluorescein with AAPH in the absence (BLK) and in the presence of a red grape juice (a), a white grape juice (b), and a wine vinegar (c).

Table 1

Total polyphenol content and ORAC-FL value for grape juices and wine vinegars

| Sample | Total polyphenols ^a | ORAC-FL value ^b | Concentration range ^c ($\mu\text{l/ml}$) |
|---------------------------|--------------------------------|----------------------------|---|
| <i>Red grape juices</i> | | | |
| #1 | 1177 \pm 20 | 25.0 \pm 1.9 | 0.07–0.25 |
| #2 | 1114 \pm 13 | 22.9 \pm 1.8 | 0.10–0.25 |
| #3 | 812 \pm 23 | 20.6 \pm 1.3 | 0.13–0.33 |
| #4 | 876 \pm 24 | 18.9 \pm 0.4 | 0.14–0.33 |
| #5 | 705 \pm 10 | 14.6 \pm 0.6 | 0.14–0.33 |
| <i>White grape juices</i> | | | |
| #6 | 474 \pm 4 | 11.1 \pm 1.0 | 0.20–0.67 |
| #7 | 436 \pm 2 | 9.8 \pm 1.1 | 0.20–0.67 |
| #8 | 229 \pm 2 | 5.1 \pm 0.28 | 0.25–0.67 |
| #9 | 352 \pm 2 | 8.6 \pm 0.16 | 0.33–1.00 |
| #10 | 151 \pm 7 | 3.5 \pm 0.12 | 0.50–2.00 |
| <i>Wine vinegars</i> | | | |
| #11 | 867 \pm 7 | 11.5 \pm 1.1 | 0.20–0.59 |
| #12 | 860 \pm 14 | 11.0 \pm 0.8 | 0.20–0.67 |
| #13 | 306 \pm 4 | 4.7 \pm 0.38 | 0.33–1.43 |
| #14 | 360 \pm 3 | 4.5 \pm 0.45 | 0.25–1.00 |
| #15 | 637 \pm 16 | 6.9 \pm 0.31 | 0.50–1.00 |

Results are the means \pm SD ($n = 3$).

^a Expressed as mg of gallic acid equivalents/l.

^b Expressed as μmol of trolox-equivalents/ml.

^c Concentration range in the final assay mixture.

dence intervals overlapped for WGJ and WVs (Fig. 2(a)). A previous study showed higher antioxidant activities for red wines than for rose or white wines, ORAC-FL values ranging from 3.18 (white wine) to 63.8 (oak-aged red wine) μmol of trolox equivalents/ml of wine (Dávalos et al., 2004). Bottle-aged red wines exhibited ~ 9 - and ~ 3 -fold higher ORAC-FL values than white and rose wines, respectively (Dávalos et al., 2004).

As expected, RGJs had higher TP contents than did WGJs and WVs. Data are in agreement with those previously reported by other authors (Alonso, Guillén, & Barroso, 2003; Lugasi & Hóvári, 2003). Analysis of variance (ANOVA) showed significant differences ($p < 0.05$) among groups; the 95% confidence intervals for WVs overlapped those for RGJ and WGJs (Fig. 2(b)).

It is known that antioxidant activity of grape-derived products is influenced, not only by their content of polyphenols, but also by their phenolic compositions, all of which are influenced by vintage, grape variety, wine-making techniques, and ageing conditions. To investi-

gate the contribution of phenolic constituents to the antioxidant activity of grape juice and vinegar, linear regression between ORAC-FL values and total polyphenol (TP) content was calculated (Table 2). Results were compared to those from wine reported previously (Dávalos et al., 2004) (Table 2). Good positive linear correlation ($p < 0.01$) between the antioxidant activity (ORAC-FL value) and the TP content was observed in all cases (Table 2). The slope of the linear regression model (a) decreased in the order: red wine > white grape

Table 2
Linear regression of the ORAC-FL value vs total polyphenols

| Sample | $y = ax + b$ | | R^2 |
|------------------------------------|--------------|---------|--------|
| | a | b | |
| White grape juices ($n = 5$) | 0.0234 | -0.0548 | 0.9913 |
| Red grape juices ($n = 5$) | 0.0183 | 3.2859 | 0.8531 |
| Red wines ($n = 6$) ^a | 0.0324 | -10.645 | 0.9582 |
| Wine vinegars ($n = 5$) | 0.0123 | 0.2837 | 0.9487 |

^a Calculated from Singleton and Rossi (1965).

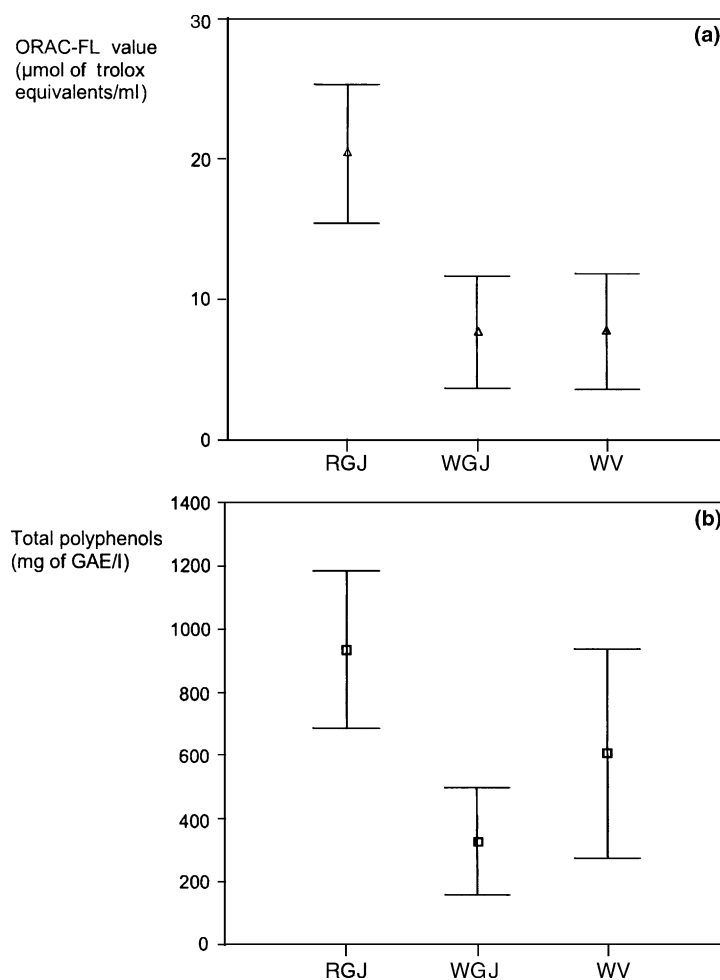


Fig. 2. ANOVA analysis of the ORAC-FL value (a) and the total polyphenol content (b) of red grape juice (RGJ), white grape juice (WGJ) and wine vinegar (WV). Error bars represent the 95% confidence intervals for means.

juice > red grape juice > wine vinegar (Table 2). This indicated that the “antioxidant activity provided by a unit of polyphenol” (ORAC-FL value/PT content) was higher for red wine and lower for wine vinegar. Therefore, red wine contains phenolic structures with higher antioxidant activity than those contained in the other samples, or/and the other samples contain higher proportions of non-phenolic antioxidants. Acetic fermentation of wine may decrease the content of those wine phenolics with high antioxidant activity or/and may lead to new vinegar phenolic compounds with lower antioxidant activity than those originally present in wine. It is also important to note that fruit juices comprise other antioxidant components, such as vitamins, which may act synergistically with phenolics or may significantly increase the total antioxidant activity of the final product (Lugasi & Hóvári, 2003).

Red wine has also been observed to exhibit higher antioxidant activity than grape juice and wine vinegar by other methodologies. Antioxidant activity, measured by the total antioxidant status (TAS) test (which measures the ability of antioxidants to scavenge the ABTS^{•+} radical), was found to be ~5.4- and 7.1-fold higher for commercial red wines (as a mean) than for red grape juices (as a mean) (Lugasi & Hóvári, 2003) and red wine vinegars (as a mean) (Alonso et al., 2003), respectively. Commercial red wines (as a mean) showed ~3.0-fold higher antioxidant activity than did wine vinegar assayed by the FRAP and TRAP methodologies, and ~3.5-fold when assayed by the TEAC 1 methodology (Pellegrini et al., 2003). The same order of variation was found by the ORAC-FL assay in our study: ~2.7, ~5.5 and ~5.4-fold higher antioxidant activities for red wine (as a mean) than for red grape juice (as a mean), white grape juice (as a mean) and wine vinegar (as a mean), respectively.

The oxygen radical absorbance capacity assay (ORAC), based on the chemical damage caused to the fluorescent (fluorescein, FL) substrate by the peroxy radicals produced in situ, has been quite widely used to assess the free radical antioxidant activity of pure compounds, fruit and vegetable extracts, wines, and biological fluids (Ou et al., 2001; Ou et al., 2002; Prior et al., 2003). To our knowledge, this is the first report of the ORAC-FL kinetic behaviour and ORAC-FL values for grape juice and wine vinegar, which allows comparison with other beverages and foods studied by the same methodology. The ORAC methodology can also be used to assess the influences of geography, variety and technological factors on the antioxidant activities of grape juice and wine vinegar.

In summary, in this paper the ORAC-FL methodology has been successfully applied to complex beverages (grape juice) and seasoning ingredients (vinegar) rich in polyphenols. Variability ranges of the antioxidant activity of commercial samples are reported. This data could

be used as an “additional quality parameter” for promoting the consumption of these grape products as sources of polyphenols and of other health-promoting substances.

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References

- Alonso, A. M., Guillén, D. A., & Barroso, C. G. (2003). Development of an electrochemical method for the determination of antioxidant activity. Application to grape-derived products. *European Food Research and Technology*, *216*, 445–448.
- Andriantsitohaina, R. (1999). Regulation of vascular tone by plant polyphenols: role of nitric oxide. *General Physiology and Biophysics*, *18*(suppl. 1), 3–5.
- Chou, E. J., Keevil, J. G., Aeschliman, S., Wiebe, D. A., Folts, J. D., & Stein, J. H. (2001). Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. *American Journal of Cardiology*, *88*, 553–555.
- Day, A. P., Kemp, H. J., Bolton, C., Hartog, M., & Stansbie, D. (1997). Effect of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation. *Annals of Nutrition & Metabolism*, *41*, 353–357.
- Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of oxygen radical absorbance capacity (ORAC-Fluorescein) assay. *Journal of Agricultural and Food Chemistry*, *52*, 48–54.
- Facino, R. M., Carini, M., Aldini, G., Berti, F., Rossoni, G., Bombardelli, E., et al. (1999). Diet enriched with procyanidines enhances antioxidant activity and reduces myocardial post-ischemic damage in rats. *Life Science*, *64*, 627–642.
- Hertog, M. G. L., Kromhout, D., Aravanis, C., et al. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in Seven Countries Study. *Archives of Internal Medicine*, *155*, 381–386.
- Keli, S. O., Hertog, M. G. L., Feskens, E. J. M., & Kromhout, D. (1996). Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Archives of Internal Medicine*, *154*, 637–642.
- Keevil, J. G., Osman, H. E., Reed, J. D., & Folts, J. D. (2000). Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *Journal of Nutrition*, *130*, 53–56.
- Kondo, S., Tayama, K., Tsukamoto, Y., Ikeda, K., & Yamori, Y. (2001). Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. *Bioscience Biotechnology and Biochemistry*, *65*, 2690–2694.
- Leger, A. S., Cochrane, A. L., & Moore, F. (1979). Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet*, *313*, 1017–1020.
- Leikert, J. F., Räthel, T. R., Wohlfart, P., Cheynier, V., Vollmar, A. M., & Dirsch, V. D. (2002). Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation*, *106*, 1614–1617.
- Lugasi, A., & Hóvári, J. (2003). Antioxidant properties of commercial alcoholic and nonalcoholic beverages. *Nahrung/food*, *47*, 79–86.

- Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. Florida: CRC Press.
- Nishikawa, Y., Takana, Y., Nagai, Y., Mori, T., Kawada, T., & Ishihara, N. (2001). Antihypertensive effects of kurosu extract, a traditional Viniegra produced from unpolished rice, in the SHR rats. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *48*, 73–75.
- O'Byrne, D. J., Devaraj, S., Grundy, S. M., & Jialal, I. (2002). Comparison of the antioxidant effects of concord grape juice flavonoids and alpha-tocopherol on markers of oxidative stress in healthy adults. *American Journal of Clinical Nutrition*, *76*, 1367–1374.
- Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, *49*, 4619–4626.
- Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J., & Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, *50*, 3122–3128.
- Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., et al. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition*, *133*, 2812–2819.
- Prior, R. L., & Cao, G. (1999). In vivo total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology and Medicine*, *27*, 1173–1181.
- Prior, R. L., Hoang, H. A., Gu, L., Wu, X., Bacchiocca, M., Howard, L., et al. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC_{FL})) of plasma and other biological samples. *Journal of Agricultural and Food Chemistry*, *51*, 3273–3279.
- Renaud, S., & De Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, *339*, 1523–1526.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*, 933–956.
- Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, *130*, 2073S–2085S.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics and phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.
- Soleas, G. J., Grass, L., Josephy, P. D., Goldberg, D. M., & Diamandis, E. P. (2002). A comparison of the anticarcinogenic properties of four red wine polyphenols. *Clinical Biochemistry*, *35*, 119–124.
- Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S., & Folts, J. D. (1999). Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation*, *100*, 1050–1055.
- Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association*, *96*, 1027–2039.
- Sugiyama, A., Saitoh, M., Takahara, A., Satoh, Y., & Hashimoto, K. (2003). Acute cardiovascular effects of a new beverage made of wine Viniegra and grape juice, assessed using an in vivo rat. *Nutrition Research*, *23*, 1291–1296.