ELSEVIER

Food Chemistry 73 (2001) 11–16

Food Chemistry

www.elsevier.com/locate/foodchem

Changes in phenolic composition of wines submitted to in vitro dissolution tests

M.V. Martínez-Ortega, M.C. García-Parrilla, A.M. Troncoso *

Área de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Sevilla, C/ P. García González s/n, E- 41012, Seville, Spain

Received 30 May 2000; received in revised form 18 September 2000; accepted 18 September 2000

Abstract

Wine is an important source of dietary antioxidants, due to its content of phenolic compounds. These compounds may be degraded in the gastrointestinal tract before absorption. A hydroalcoholic standard solution containing 14 phenolic compounds and 25 wines (red, white and Sherry wines) were submitted to a dissolution test employing gastric and intestinal fluids according to The United States Pharmacopeia conditions. Concentration of each phenol was determined, before and after the treatments, by high-performance liquid chromatography with a photodiode array detector. In the standard solution, phenolic acids were rather stable in gastric and intestinal media. Flavonoids were affected most. When wines were submitted to this test, gallic acid, caffeic acid, catechin and quercetin increased in concentrations while resveratrol (*trans*-, *cis*- and their glucosides), caffeoyltartaric acid and ethyl gallate resisted gastric and intestinal media.

Keywords: Wine phenols; HPLC; Gastrointestinal fluids; Bioavailability; Dissolution test

1. Introduction

Most phenolic compounds present in wine show beneficial physiological properties, e.g. cardioprotective, anticarcinogenic and antiinflammatory activities based on their ideal chemical structure for free radical-scavenging activities, so they are supposed to protect human health against oxidative damage (Rice-Evans, Miller, & Paganga, 1997). Phenolic compounds may act as cardioprotective agents or at least they possess in vitro vasorelaxing effects and inhibit platelet aggregation (Andriambeloson et al., 1998; Schramm, Pearson, & German, 1997). This can be explained by their antioxidant power. (Soleas, Tomlinson, Diamandis, & Goldberg, 1997). Several studies showed the inhibition of low density lipoprotein (LDL) oxidation (Frankel, Waterhouse, & Teissedre, 1995; Teissedre, Frankel, Waterhouse, Peleg, & German, 1996) and lipid peroxidation in vitro (Kanner, Frankel, Granit, German, & Kinsella, 1994).

Whether or not the intake of large amounts of fruits and vegetables in the diet supplies the same amounts of phenolic compounds as wine is a contentious question. Several methods have been developed in order to assess these compounds in different foods and in a full diet to confirm epidemiological studies (Hollman, Hertog, & Katan, 1996). Recent worldwide research has focused on the determination of phenolic compounds in wines. (Goldberg, Tsang, Karumanchiri, & Diamandis, 1996; Waterhouse & Teissedre, 1997) and some compounds in particular, e.g. resveratrol (Martínez-Ortega, García-Parrilla, & Troncoso, 2000). Naturally they are present in fruit as polymeric complexes, which need to be degraded adequately before being absorbed. The advantage of wine with regards to other foods is that alcoholic fermentation produces partial or total rupture of these aggregates and flavonoids are in a stable solution (Goldberg, 1995). It has been postulated that ethanol would contribute indirectly to the antioxidant capacity of wine by increasing the bioavailability of phenolics. This could be due to the fact that ethanol can inhibit the chemical interactions between salivary proteins and red wine tannins (Serafini, Maiani, & Ferro-Luzzi, 1997).

There is now increasing evidence about absorption of phenolics and some of them have been detected in human plasma and other biological fluids (Bourne & Rice-Evans, 1999; Pietta & Simonetti, 1999; Terao, 1999).

* Corresponding author. Tel. : +34-54-556760; fax: +34-54-233765.

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00270-3

Studies performed in rats and humans, fed flavonols and catechins, have confirmed that they are partly absorbed by the intestine (Pietta & Simonetti, 1999). Moreover, catechin was detected in human plasma after the intake of a diet containing red wine, fruits and vegetables (Carando, Teissedre, & Cabanis, 1998). Products of the metabolism of catechin such as 3′-O-methylcatechin and catechin sulfated conjugates have also been determined in human plasma after the ingestion of red wine (Donovan, Bell, Kasimkarakas, German, & Walzem, 1999).

Formerly, it was postulated that glycosylated forms of flavonoids were not absorbable from the digestive tract (Pietta & Simonetti, 1999). In a human study with ileostomy subjects, it was found that the absorption of orally administered quercetin was 24%, while the absorption of quercetin glycosides from onions was 52% (Hollman, de Vries, van Leeuwen, Mengelers, & Katan 1995). In addition, rutin and other glycosides, such as phloretin, have been measured in the plasma of non-supplemented healthy volunteers (Paganga & Rice-Evans, 1997).

There is a need to elucidate the extent to which phenolic compounds are absorbed and whether they are degraded or not in the intestinal tract. The purpose of the present work is to ascertain if gastrointestinal conditions could affect the original phenolic composition of wines and evaluate their stability or possible transformation to other compounds. As digestion is an initial step involving changes in pH and activity of proteolytic enzymes, it seems quite interesting to evaluate possible changes of phenolics. Indeed, such in vitro assays have been applied to compare the bioavailability of minerals from rich dietary sources such as tea (Powell, Burden, & Thompson, 1998) and as an initial step prior to in vivo assays (Hollman et al., 1995). When testing in vitro bioavailability of medicines, the USP (USP 23) proposes the dissolution test (using gastrointestinal fluids) as a necessary prior step to evaluate in vivo bioavailability. Among all the methods employed to determine phenolic composition, reverse-phase gradient high-performance liquid chromatography (HPLC) with diode array detection, has been proclaimed as a very adequate technique in recent papers (Roggero, 1997; Soleas, Goldberg, Ng, Karumanchiri, Tsang, & Diamandis, 1997).

2. Materials and methods

2.1. Samples

Twenty-five wines from Spain were analysed, including 12 young red wines from different varieties (vintages from 1994 to 1997), six young white wines (vintage 1996 and 1997) and seven Sherry wines (two "fino", two "manzanilla" and three "oloroso" wines). All of them were purchased in the market.

2.2. Standards

Standards of phenolic compounds were from Merck (gallic acid, *p*-hydroxy benzoic acid, ferulic acid), Sigma (vanillic acid, syringaldehyde, rutin, *trans*-resveratrol) and Fluka [tyrosol, (+)-catechin, caffeic acid, (−)-epicatechin, *p*-coumaric acid, quercetin dihydrate, kaempferol].

Phenolic compounds were dissolved in a hydroalcoholic solution (10% v/v) at concentrations usually found in wines. *Cis*-resveratrol was obtained by UV light exposure from the trans isomer (Roggero & García-Parrilla, 1995) using a UV lamp "Atom" model A/70, No. 614, series 02.

2.3. Test solutions

Gastric and intestinal fluids were prepared as follows (USP 23). Gastric fluid (simulated): dissolve 2.0 g of sodium chloride and 3.2 g of pepsin in 7.0 ml of hydrochloric acid and sufficient water to make 1000 ml. This test solution has a pH of about 1.2.

Intestinal fluid (simulated): dissolve 6.8 g of monobasic potassium phosphate in 250 ml of water, mix, and add 190 ml of 0.2 N sodium hydroxide and 400 ml of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with 0.2 N sodium hydroxide to a pH of 7.5 ±0.1. Dilute with water to 1000 ml.

Enzymes employed in test solutions were pepsin, from hog stomach (Fluka 77163), and pancreatin, from porcine pancreas (Sigma P-1500).

2.4. Apparatus

Samples were submitted to gastric and intestinal fluids in a USP Turugrau apparatus automatized dissolution test using the paddle method at 37°C and 100 rpm (USP 23).

The chromatographic equipment was a Waters 600E System Controller connected to a Photodiode Array Detector Waters 996. Data treatment was performed in a Waters Millennium 2.0 data station. The column was a Merck LiChroCART®250×4 mm) Superspher® 100 RP-18, Ref. 116056, protected by a guard cartridge LiChroCART® 4-4, Ref. 50957, E. Merck. Filters used were Anotop® (Anopore®); 0.2-μm, Whatman (Cat. No. 6809 1022).

2.5. Digestion

Standard phenols and wines were each submitted to gastric and intestinal fluids. A 125 ml aliquot of each wine or standard solution, was submitted to an equal volume of gastric fluid for 30 min. Another 125-ml aliquot of wine or standard solution was submitted to an equal volume of intestinal fluid for 2 h. Final concentrations of phenols in simulated media were then

twice diluted with regard to the original sample. This process was performed in triplicate in the standard solution and in duplicate for the real samples.

2.6. Chromatographic analysis

Levels of all the phenolic compounds described above, were determined in all wine samples before and after gastric and intestinal treatment by HPLC. Chromatographic conditions were previously described (Roggero, Archier, & Coen, 1991) with a gradient in which three eluents are used: A (acetic acid — water (1/99); B (acetic acid — water — (6/94) and C (acetic acid — water — acetonitrile (5/65/30). The method employed possesses sufficient selectivity and sensitivity to allow direct analysis of wines without prior sample preparation, except filtration before injection.

The flow was 0.5 ml/min and the temperature was set at 22°C. The volume injected was 50 μl. The compounds in the samples were identified both by retention time and by spectra matching (Table 1). Quantitative assays were achieved using external calibration curves for all standard phenols by dissolution of the standard solution.

Phenolic compounds, which are not available in the market as standards, have been identified by their retention time and spectra according to the literature. These compounds have been assayed by assuming that their molar absorptivity is the same as that of the corresponding free standard molecule. In this way, caffeoyltartaric acid has been quantified using the same UV response as caffeic acid, ethyl gallate the same as gallic acid and *trans*-resveratrol glycoside the same as *trans*-resveratrol (Roggero, 1997). *Cis*-resveratrol and its glycoside were quantified by the ϵ basis (Roggero & García-Parrilla, 1995).

Table 1
Retention time (t_R), maximum absorbance (λ max) and peak number of the phenolic compounds assayed

Phenolic compound	t_R (min)	Peak number	λ max
Gallic acid	16.5	1	280
Caffeoyltartaric acid	24.5	2	330
p-Hydroxybenzoic acid	31.7	3	280
Tyrosol	33.2	4	280
Catechin	38.9	5	280
Vanillic acid	46.8	6	260
Caffeic acid	50.1	7	330
Ethyl gallate	58.5	8	280
Epicatechin	67.5	9	280
p-Coumaric acid	71.9	10	313
Syringaldehyde	78.6	11	313
Ferulic acid	86.7	12	330
gl-t-Resveratrol	94.8	13	307
Rutin	106.6	14	355
gl-c-Resveratrol	108.2	15	280
trans-Resveratrol glucoside	112.3	16	307
cis-Resveratrol glucoside	118.5	17	280
Quercetin	124.6	18	355
Kaempferol	135.7	19	355

Figs. 1 and 2 show the chromatograms obtained for a red wine (1) before and (2) after gastric and (3) intestinal treatments.

3. Results and discussion

3.1. Standard solution

Concentrations of the different phenolic compounds after gastric or intestinal treatment are shown in Table 2. Some phenolic compounds are rather stable while the levels of other compounds clearly decrease. Phenols whose concentrations are not affected after gastric treatment are: gallic acid, *p*-hydroxybenzoic acid, tyrosol, vanillic acid, syringaldehyde, caffeic acid, *trans*-resveratrol and kaempferol.

When the standard solution was submitted to intestinal fluid, most of the phenolic concentrations were diminished to a greater extent than after gastric conditions, proving that they are more stable in acidic conditions. In the case of gallic acid, its absortivity is highly influenced by the pH. The following compounds are not affected: tyrosol, *p*-hydroxybenzoic acid, vanillic acid, syringaldehyde, caffeic acid, *p*-coumaric acid, ferulic acid and trans-resveratrol. Concentrations of all flavonoids tested (catechin, epicatechin, rutin, quercetin and kaempferol) clearly decreased (Table 2). Thus, the intestinal medium produces considerable changes.

3.2. Wines

It must be pointed out that not all standards assayed were found in the samples analysed, some of them were not detected in any of the wines (i.e. tyrosol, vanillic acid and syringaldehyde). Moreover, other phenolic compounds have been detected in a small number of samples, so no further conclusions could be drawn. This is the case with *p*-hydroxybenzoic acid, coumaric acid, ferulic acid, rutin and kaempferol. Data are presented in Table 3, where samples have been classified according to the type of wine.

Gallic acid concentration increases for red and sherry wines in both treatments: gastric and intestinal (Table 3). It only decreases slightly for some white wine samples after intestinal treatment. This could be due to the presence of complex chemical structures such as galloylproanthocyanidins in wines, especially in red wines, which can be broken to liberate gallic acid monomers (da Silva, Rigaud, Cheynier, Cheminat, & Moutounet, 1991).

Ethyl gallate has been detected in a few wine samples, as its concentration is stable after gastric and intestinal treatments (Table 3) as could be expected by its ester structure.

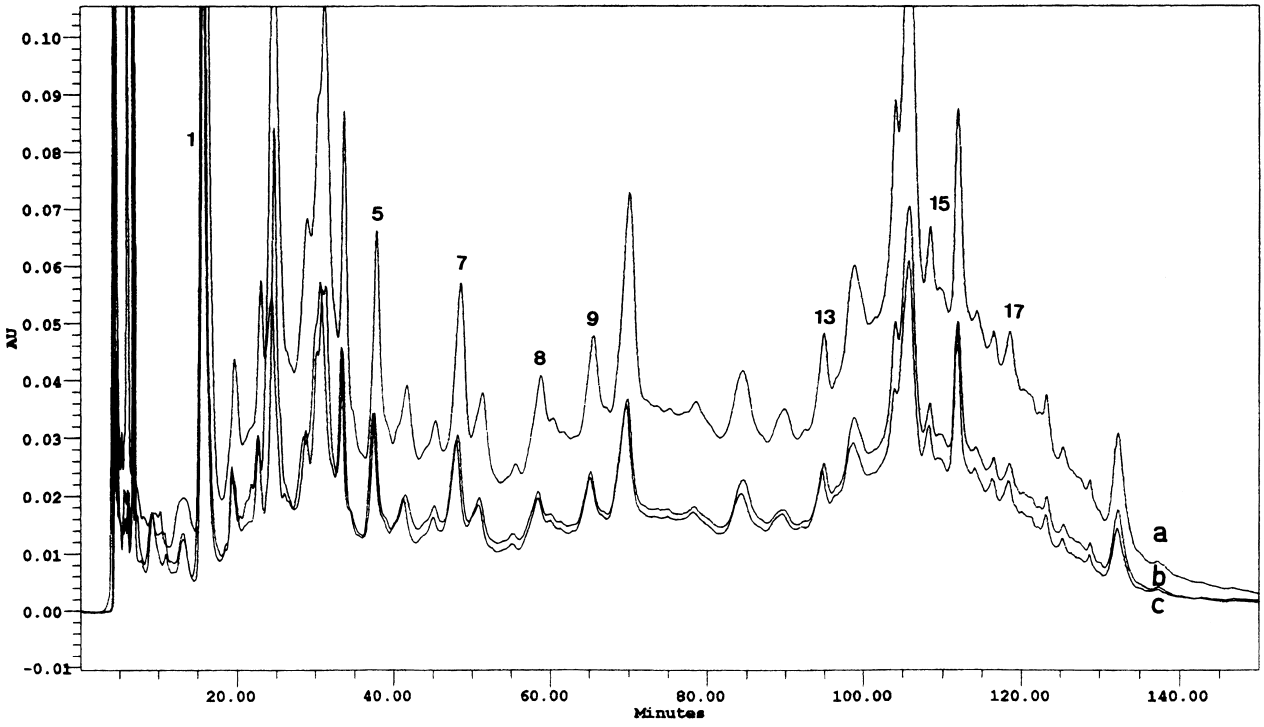

Fig. 1. Chromatograms (280 nm) corresponding to a wine sample before treatment (a) after gastric assay (b) and after intestinal assay (c). (Peak numbers correspond to phenolic compounds described in Table 1.)

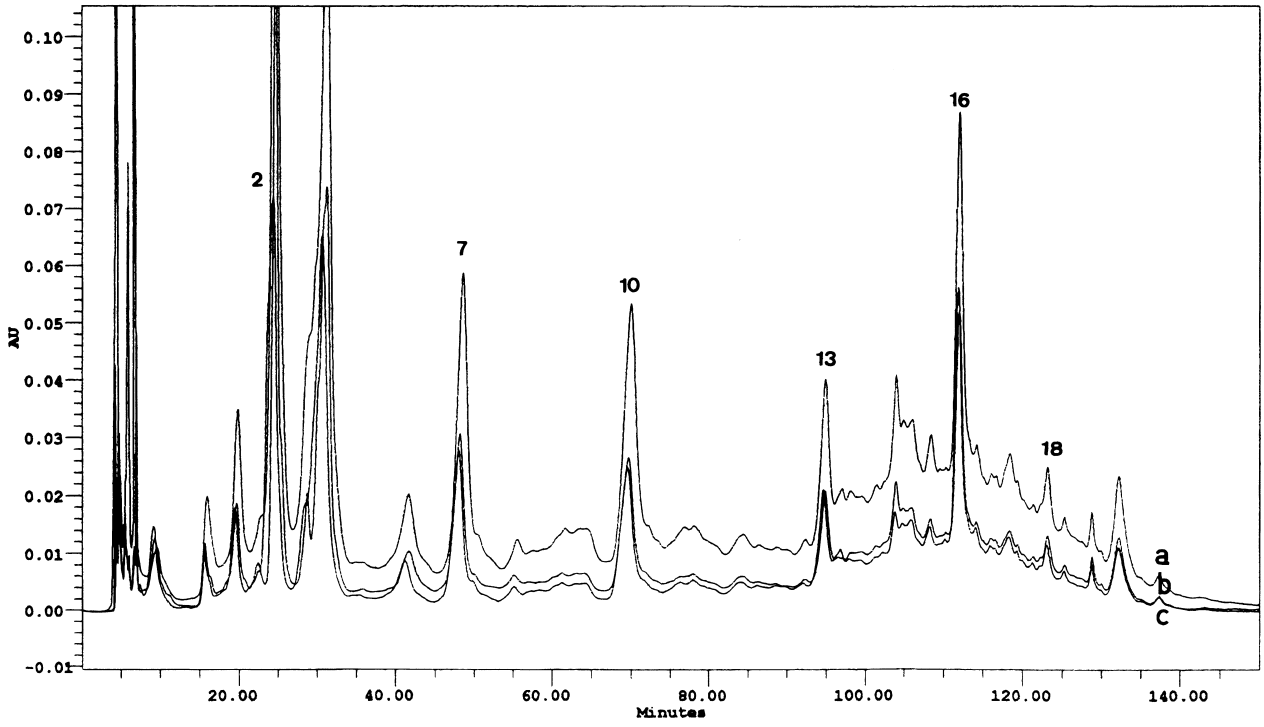

Fig. 2. Chromatograms (313 nm) corresponding the same wine sample before treatment (a) after gastric assay (b) and after intestinal assay (c). (Peak numbers correspond to phenolic compounds described in Table 1.)

Table 2
Concentration of phenols (mg/l) in the standard hydroalcoholic solution (Standard), when diluted before treatments (Initial), after gastric treatment (Gastric) and after intestinal treatment (Intestinal)

Phenol	Standard	Initial	Gastric	Intestinal
Gallic acid	14.0	7.0	7.0±0.1	3.5±0.1
p-Hydroxybenzoic acid	11.6	5.3	5.1± 0.6	5.0±0.4
Tyrosol	7.0	3.5	3.5±0.2	3.4±0.2
Vanillic acid	8.0	4.0	4.0±0.1	3.5±0.1
Syringaldehyde	5.8	2.9	2.9±0.2	2.9±0.2
Caffeic acid	11.0	5.5	5.4±0.4	5.0±0.4
p-Coumaric acid	44.0	22.0	20.9±0.7	20.3±1.0
Ferulic acid	24.0	12.0	11.4±0.1	11.4±0.7
trans-Resveratrol	0.4	0.2	0.2±0.1	0.2±0.1
Catechin	7.2	3.6	2.9±0.2	2.8±0.3
Epicatechin	17.2	8.6	7.6±1.0	7.5±0.4
Rutin	31.4	15.7	9.6±0.8	7.9±1.3
Quercetin	12.0	6.0	5.3±0.8	2.3±0.8
Kaempferol	2.6	1.3	1.2±0.2	0.5±0.5

Table 3
Concentrations (mg/l) of phenolic compounds in wines assayed, before (Initial) and after gastric (Gastric) and intestinal (Intestinal) treatments

Phenol	Wine	Samples	$\bar{x}$ Initial	$\bar{x}$ Gastric	$\bar{x}$ Intestinal
Gallic acid	White	3	1.2±0.3	1.7±0.5	0.9±0.3
	Sherry	7	1.8±0.9	2.6±1.1	2.7±1.1
	Red	11	9.1±1.9	9.8±2.3	10.5±1.9
Ethyl gallate	Red	5	0.4±0.1	0.4±0.2	0.4±0.2
Caffeic acid	White	3	0.3±0.1	0.3±0.1	0.4±0.2
	Sherry	3	0.4±0.2	0.3±0.1	1.2±0.1
	Red	11	1.5±1.0	1.5±1.0	1.8±1.0
Caffeoyl-tartaric acid	White	6	5.4±2.5	5.6±2.7	5.7±2.3
	Sherry	6	3.5±1.6	3.8±1.6	3.8±2.0
	Red	11	5.7±1.8	5.6±1.8	5.7±1.7
gl-t-Resveratrol	Red	6	0.3±0.0	0.3±0.0	0.3±0.0
gl-c-Resveratrol	Red	6	1.4±0.1	1.4±0.1	1.4±0.2
trans-Resveratrol	Red	7	0.5±0.2	0.5±0.3	0.7±0.3
cis-Resveratrol	White	1	0.1	0.2	0.2
	Red	5	0.6±0.2	1.0±0.2	0.8±0.4
Catechin	White	2	2.4±0.3	2.1±0.2	2.6±0.6
	Red	12	12.3±2.8	13.7±4.0	13.2±3.1
Epicatechin	Red	10	8.5±3.2	7.6±1.9	7.6±2.2
Quercetin	Red	6	2.3±1.1	3.0±1.4	3.2±1.9

Caffeic acid appears to be stable when wine is analysed after gastric and intestinal treatments (Table 3) as was previously observed in the standard solution (Table 2). However, for some Sherry wine samples analysis of variance proves that the increase in its concentration was significant as a consequence of intestinal treatment ($P < 0.05$).

Caffeoyltartaric acid was relatively stable in artificial media (Table 3) and the slight changes observed are not significant.

Both of the resveratrol isomeric forms and their glycosides have been identified and quantified in sample wines. Though they are present in low concentrations, *trans*- and *cis*-resveratrol and their glycosides resist gastric and intestinal treatments (Table 3). This result is very interesting since resveratrol has been shown to be a chemopreventive and cardioprotective agent in recent research (Jang et al., 1997).

Catechin levels increased in most red wine samples after gastric treatment but not significantly (Table 3). White wines, containing catechin in lower amounts, show less marked changes as was observed for the standard solution. In the case of red wines, catechin increases, but not significantly.

Epicatechin concentrations in red wines decrease after gastric treatment. Similar changes were observed when submitted to intestinal treatment (Table 3). Epicatechin shows identical evolution, in the standard solution (Table 2), to real wines.

Quercetin levels were only determined in red wines; they increased after both treatments. Catechin and quercetin seem to be more stable in the real wine medium than standard solution (Table 3). Quercetin is expected to suffer autoxidation at pH = 7.5 and wine may act as a buffer, limiting this effect.

4. Conclusions

When a dissolution test is applied to a phenol standard hydroalcoholic solution, flavonoid compounds are affected to a greater extent than simple phenolic acids, except for gallic acid, which diminished by nearly 50% due to the pH under intestinal conditions. Hydroxycinnamates and resveratrol concentrations are stable after gastric and intestinal conditions.

In general, phenols seem to be more stable to gastrointestinal conditions in wine samples than in hydroalcoholic phenolic solution. Gallic acid increases in the majority of the samples, as do catechin and quercetin.

Resveratrol and related compounds proved to be resistant to gastrointestinal treatment.

Acknowledgements

The dissolution test was performed in the laboratory of the Department of Tecnología Farmacéutica, with the advice of Dr. M. R. Jiménez-Castellanos; we thank her for effective assistance. Also, the authors gratefully acknowledge the Fundación Farmacéutica Avenzoar for financial support of this work by the concession of a Research Grant.

References

Andriambeloson, E., Magnier, C., Haan-Archipoff, G., Lobstein, A., Anton, R., Beretz, A., Stoclet, J. C., & Andriantsitohaina, R.

(1998). Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. *Journal of Nutrition, 128*, 2324–2333.

Bourne, L. C., & Rice-Evans, C. A. (1999). Detecting and measuring bioavailability of phenolics and flavonoids in humans: pharmacokinetics of urinary excretion of dietary ferulic acid. In L. Packer, *Methods in enzimology* (pp. 91–106). Berkeley, California: Academic Press.

Carando, S., Teissedre, P. L., & Cabanis, J. C. (1998). Comparison of (+)-catechin determination in human plasma by high-performance liquid chromatography with two types of detection: fluorescence and ultraviolet. *Journal of Chromatography, B, 707*, 195–201.

da Silva, J. M. R., Rigaud, J., Cheynier, V., Cheminat, A., & Moutounet, M. (1991). Procyanidin dimers and trimers from grape seeds. *Phytochemistry, 30*(4), 1259–1264.

Donovan, J. L., Bell, J. R., Kasimkarakas, S., German, J. B., & Walzem, R. L. (1999). Catechin is present as metabolites in human plasma after consumption of red wine. *Journal of Nutrition, 129*, 1662–1668.

Frankel, E. N., Waterhouse, A. L., & Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry, 43*, 890–894.

Goldberg, D. M. (1995). Does wine work? *Clinical Chemistry, 41*, 14–16.

Goldberg, D. M., Tsang, E., Karumanchiri, A., & Diamandis, E. P. (1996). Method to assay the concentrations of phenolic constituents of biological interest in wines. *Analytical Chemistry, 68*, 1688–1694.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H. S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., & Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science, 275*, 218–220.

Hollman, P. C. H., de Vries, J. H. M., van Leeuwen, S. D., Mengelers, M. J. B., & Katan, M. B. (1995). Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Americam Journal of Clinical Nutrition, 62*, 1276–1282.

Hollman, P. C. H., Hertog, M. G. L., & Katan, M. B. (1996). Analysis and health effects of flavonoids. *Food Chemistry, 57*, 43–46.

Kanner, J., Frankel, E. N., Granit, R., German, B., & Kinsella, J. E. (1994). Natural antioxidants in grapes and wines. *Journal of Agricultural and Food Chemistry, 42*, 64–69.

Martínez-Ortega, M. V., García-Parrilla, M. C., & Troncoso, A. M. (2000). Resveratrol content in wines and musts from the south of Spain. *Nahrung, 4*, 253–256.

Paganga, G., & Rice-Evans, C. A. (1997). The identification of flavonoids as glycosides in human plasma. *FEBS Letters, 401*, 78–82.

Pietta, P., & Simonetti, P. (1999). Dietary flavonoids and interaction with physiologic antioxidants. In L. Packer, M. Hiramatsu, & T. Yoshikawa, *Antioxidant food supplements in human health* (pp. 283–308). San Diego, California: Academic Press.

Powell, J. J., Burden, T. J., & Thompson, R. P. H. (1998). In vitro mineral availability from digested tea: a rich dietary source of manganese. *Analyst, 123*, 1721–1724.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Roggero, J. P., Archier, P., & Coen, S. (1991). Wine phenolics analysis via direct injection:enhancement of the method. *Journal of Liquid Chromatography, 14*, 533–538.

Roggero, J. P., & García-Parrilla, M. C. (1995). Effects of ultraviolet irradiation on resveratrol and changes in resveratrol and various of its derivatives in the skins of ripening grapes. *Sciences des Aliments, 15*, 411–422.

Roggero, J. P. (1997). Wine phenolics analysis via direct injection. Wine nutritional and therapeutic benefits. *American Laboratory News, 2*, 6–11.

Schramm, D. D., Pearson, D. A., & German, J. B. (1997). Endothelial cell basal PGI_2 release is stimulated by wine in vitro: one mechanism that may mediate the vasoprotective effects of wine. *Journal Nutritional Biochemistry, 8*, 647–651.

Serafini, M., Maiani, G., & Ferro-Luzzi, A. (1997). Effect of ethanol on red wine tannin-protein (BSA) interactions. *Journal of Agricultural and Food Chemistry, 45*, 3148–3151.

Soleas, G. J., Goldberg, D. M., Ng, E., Karumanchiri, A., Tsang, E., & Diamandis, E. P. (1997). Comparative evaluation of four methods for assay of cis- and trans-resveratrol. *American Journal of Enology and Viticulture, 48*, 169–176.

Soleas, G. J., Tomlinson, G., Diamandis, E. P., & Goldberg, D. M. (1997). Relative contributions of polyphenolic constituents to the antioxidant status of wines: development of a predictive model. *Journal of Agricultural and Food Chemistry, 45*, 3995–4003.

Teissedre, P. L., Frankel, E. N., Waterhouse, A. L., Peleg, H., & German, B. (1996). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agriculture, 70*, 61.

Terao, J. (1999). Dietary flavonoids as plasma antioxidants on lipid peroxidation: significance of metabolic conversion. In L. Packer, M. Hiramatsu, & T. Yoshikawa, *Antioxidant food supplements in human health* (pp. 255–268). San Diego, California: Academic Press.

United States Pharmacopeia (USP 23), The National Formulary (NF 18) (1995). United States Pharmacopeial Convention.

Waterhouse, A. L. & Teissedre, P. L. (1997). Levels of phenolics in California varietal wines. *Wine: nutritional and therapeutic benefits* (ACS).