ELSEVIER

PII: S0308-8146(97)00100-3

Food Chemistry, Vol. 61, No. 1/2, pp. 71–75, 1998

0308-8146/98 $19.00 + 0.00

Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation

Anne S. Meyer,* Marina Heinonen & Edwin N. Frankel

Department of Food Science and Technology, University of California, Davis, CA 95616, USA

(Received 7 March 1997; accepted 14 April 1997)

Flavonoids and phenolic acids are currently believed to exert cardioprotective effects in humans via their ability to inhibit oxidation of low-density lipoprotein (LDL). The influence of chemical structure on antioxidant activity of catechin, quercetin, cyanidin, caffeic acid, and ellagic acid was evaluated by measuring inhibition of copper-catalysed human LDL oxidation *in vitro*. The five plant phenols investigated all possess a similar *o*-dihydroxy moiety. The order of antioxidant activity was catechin > cyanidin ≈ caffeic acid > quercetin > ellagic acid. The observed differences in activities are discussed in terms of structural dissimilarities of the compounds. Potential synergistic or antagonistic effects between catechin, cyanidin, caffeic acid, quercetin, and ellagic acid were investigated by measuring the antioxidant activities on LDL of 20 different combinations of two/three of these phenols. All the antioxidant effects were additive except for combinations including ellagic acid with catechin, where ellagic acid exerted a significant antagonistic effect. It is proposed that the mechanism behind this antagonistic interaction is due to hydrogen bonding between carbonyls in ellagic acid and *o*-dihydroxyl groups in catechin.

INTRODUCTION

Flavonoids and phenolic acids are ubiquitous bioactive compounds found in plant foods and beverages (Herrmann, 1989; Ho, 1992; Macheix *et al*., 1990). Flavonoids can be grouped in several structural classes including anthocyanins, flavones, flavan-3-ols (catechins), flavonols, and tannins (Goodwin and Mercer, 1992). These flavonoid compounds share the same basic structure consisting of two aromatic rings joined in a chroman structure by a three-carbon unit: C_6-C_3-C_6 (Goodwin and Mercer, 1992; Macheix *et al*., 1990). Phenolic acids in plant foods and beverages comprise mainly derivatives of hydroxybenzoic and hydroxycinnamic acids (Herrmann, 1989; Ho, 1992; Macheix *et al*., 1990). The diversity in structure and reactivity between different flavonoids and phenolic acids are principally due to variations in the patterns of hydroxylation and methylation of the aromatic rings (Macheix *et al*., 1990).

Both flavonoids and phenolic acids are closely associated with the sensory attributes of fresh and processed plant foods (Ho, 1992; Macheix *et al*., 1990). Recently, however, these phenolic compounds have received increased attention because of their potential antioxidant activities that may exert cardioprotective effects in humans (Kinsella *et al*., 1993). Thus, it was shown that intake of flavonoids, especially quercetin found in onions, tea, and apples, was inversely related to coronary heart disease mortality (Hertog *et al*., 1993, 1995; Knekt *et al*., 1996). Further, it has been suggested that the high amounts of antioxidative flavonoids and phenolic acids consumed with red wine may explain the French Paradox, i.e. the reduced coronary heart disease mortality in certain regions of France (Frankel *et al*., 1993; Kanner *et al*., 1994; Kinsella *et al*., 1993).

Evidence is growing that oxidative modification of low-density lipoprotein (LDL) is conducive to atherogenic reactions, and hence increases coronary heart disease morbidity and mortality (Esterbauer *et al*., 1992; Steinberg, 1992). Antioxidants that inhibit LDL oxidation may thus reduce atherogenesis. Currently, there is no direct evidence that flavonoids and phenolic acids reduce LDL oxidation *in vivo*, but several studies have shown that these phenolic compounds have the capacity to reduce LDL oxidation *in vitro*: micromolar concentrations of phenolic compounds in wine and grape juice

*To whom correspondence should be addressed at: Department of Biotechnology, Building 221, Technical University of Denmark, 2800 Lynbgy, Denmark.

were thus demonstrated previously to inhibit oxidation of human LDL *in vitro* (Frankel *et al.*, 1993, 1995; Lanningham-Foster *et al.*, 1995). However, the phenolic profiles of wines were too complex to determine the significance of individual compounds and especially of specific combinations of phenols on LDL antioxidant activity (Frankel *et al.*, 1995). Further, several purified flavonoids including flavan-3-ols (catechins and procyanidins), anthocyanins, flavonols, and a number of phenolic acids were demonstrated individually to exert significant antioxidant activity in various *in vitro* LDL assays (De Whalley *et al.*, 1990; Laranjinha *et al.*, 1994; Nardini *et al.*, 1995; Teissedre *et al.*, 1996; Vinson *et al.*, 1995). Ellagic acid was found to inhibit *in vitro* lipid oxidation in an erythrocyte membrane ghost system and in a rat liver microsome assay (Osawa *et al.*, 1987), but was a weak inhibitor of LDL oxidation (Laranjinha *et al.*, 1994; Teissedre *et al.*, 1996).

In analogy to antioxidants for edible oils (Hudson and Lewis, 1983) phenolic compounds possessing multiple hydroxyl groups, especially 3',4' *o*-dihydroxy groups, are generally the most efficient antioxidants towards LDL oxidation (Laranjinha *et al.*, 1994; Nardini *et al.*, 1995; Rice-Evans *et al.*, 1996; Vinson *et al.*, 1995). Little is known, however, about how variations in the remaining molecular structure of different plant phenols possessing an identical 3',4' *o*-dihydroxy group affects antioxidant activity towards LDL. Furthermore, only limited knowledge is available about any interactions between flavonoids and phenolic acids in inhibiting LDL oxidation. Information on antioxidant interactions of different plant phenols would provide a better understanding of the antioxidant effects of phytochemicals in complex mixtures such as wines and grape juices.

The objectives of the present work were: (1) to investigate potential differences in antioxidant action toward LDL of catechin, quercetin, cyanidin, caffeic acid, and ellagic acid, possessing the same 3',4' *o*-dihydroxy structures (Fig. 1); and (2) to elucidate any interactive antioxidant effects in mixtures of these different phenols.

MATERIALS AND METHODS

Materials

Catechin, caffeic acid, ellagic acid, and dimethyl- sulphoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO). Quercetin and cyanidin (as cyanidin chloride) were from Extrasynthese (Genay, France).

Isolation of human LDL

Blood was collected from three normolipidemic males and plasma LDL was prepared by sequential ultracentrifugation (Orr *et al.*, 1991) as described previously (Frankel *et al.*, 1993).

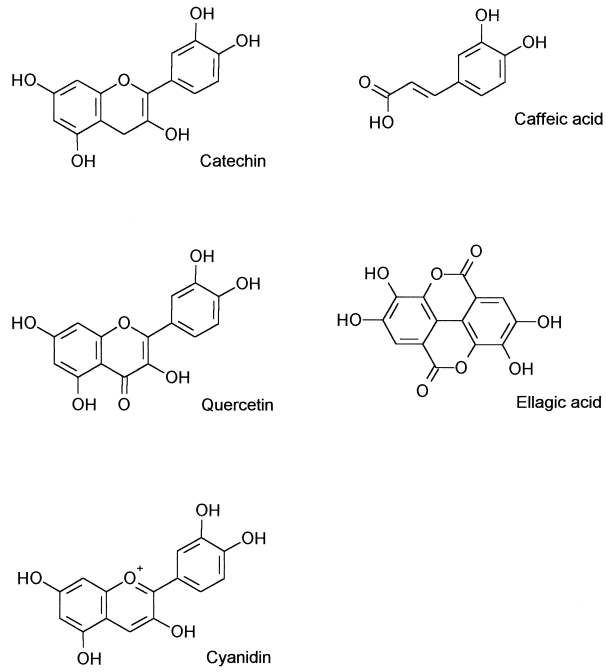

Fig. 1. Structures of 3',4' *o*-dihydroxy phenols used in this study.

Inhibition of human LDL oxidation

Antioxidant activities of individual phenolic compounds and their mixtures were assessed by measuring inhibition of copper-catalysed oxidation of human LDL *in vitro* by monitoring hexanal production by static headspace gas chromatograpy (Frankel *et al.*, 1992). Measurement of hexanal was shown earlier to be a sensitive method for monitoring oxidative susceptibility of LDL (Frankel *et al.*, 1992). To evaluate antioxidant activity, individual compounds were dissolved and systematically combined in DMSO and tested in random order at concentrations of 2.5, 5.0 and 7.5 μM in the LDL assay. In all experiments the concentrations of phenolic compounds were calibrated to add equal sample sizes of 10 μl to 0.25 ml LDL in phosphate-buffered saline (Frankel *et al.*, 1992). The results obtained after triplicate analyses were expressed as percent relative inhibition (In (%)): In (%) = $[(C\text{-}S)/C] * 100$, where C was the amount of hexanal formed in the control, and S was the amount of hexanal formed in the sample (Frankel *et al.*, 1995).

Differences in antioxidant activities were tested by one-way analysis of variance using Minitab Statistical Software from Addison-Wesley (Reading, MA). Potential synergistic or antagonistic effects were evaluated by comparing the total inhibition obtained by the sum of the individual compounds' effects to the degree of inhibition obtained by a combination of compounds tested at the same total concentration. Interaction between antioxidants was tested statistically by regular, two-sided hypothesis testing at the 5% significance level (Montgomery, 1991).

RESULTS

Antioxidant activities of individual phenolic compounds

Antioxidant activities were evaluated by measuring the inhibition of hexanal formation during copper-catalysed human LDL oxidation *in vitro*. The antioxidant effects of the five phenolic compounds, catechin, quercetin, cyanidin, caffeic acid and ellagic acid (Fig. 1) were investigated individually at three different levels. As expected, large differences in antioxidant activity were observed between the five compounds. Catechin, quercetin, cyanidin and caffeic acid showed high antioxidant activity, but ellagic acid had no effect on LDL oxidation at the levels investigated (Table 1). Further, catechin, quercetin, cyanidin, and caffeic acid exerted positive, linear dose–response effects, i.e. the higher the addition level, the higher the antioxidant activity (Table 1). However, the increase in antioxidant activity with increased concentrations varied markedly between the different compounds. At 2.5 μM, the inhibition of LDL oxidation varied from 3.3 to 85.5% with catechin exerting the highest antioxidant activity, and ellagic acid exerting the lowest, or no effect (Table 1). At 2.5 μM, cyanidin and caffeic acid exerted similar antioxidant activities of 53.4 and 49.0% inhibition, respectively, while quercetin exerted only 29.4% inhibition (Table 1). At 5.0 and 7.5 μM, the trends were the same with catechin exerting the highest and quercetin consistently the lowest activity, ellagic acid showing low or no antioxidant activity (Table 1). At 7.5 μM, however, the antioxidant activity of catechin (96.5%) was not statistically different from the activities exerted by cyanidin (96.7%) and caffeic acid (97.5%) (Table 1). In summary, the antioxidant protection of LDL by the different phenols decreased in the order at 2.5 μM: catechin $>$ cyanidin $\approx$ caffeic acid $>$ quercetin $\gg$ ellagic acid; at 5.0 and 7.5 μM, the order was: catechin $\approx$ cyanidin $\approx$ caffeic acid $>$ quercetin $\gg$ ellagic acid.

Antioxidant interactions

The selected polyhydroxy phenols were studied systematically in combinations of two or three compounds (Tables 2 and 3). When 2.5 μM catechin was paired with the other compounds at the same level to give a total phenol concentration of 5.0 μM the antioxidant activities ranged from 41.8 to 85.8% (Table 2). None of these activities exceeded those expected by adding the antioxidant activities of the individual compounds at 2.5 μM (Table 2), nor that of catechin at 5.0 μM (87.8% inhibition) (Table 1). Rather, the antioxidant activities obtained were generally lower than expected (Table 2). This result could be due to the high antioxidant activity of catechin exerted at 2.5 and 5.0 μM (85.5 and 87% inhibition) (Table 1). When catechin was paired with ellagic acid, however, the inhibition decreased to 41.8% (Table 2). This significant lower activity can be defined as an antagonistic effect of ellagic acid (Table 2). In a 1:1 mixture of cyanidin and caffeic acid, the inhibition obtained was also significantly lower than the sum of the individual effects (Table 2). However, this antagonistic effect may be insignificant as the observed 90.4% inhibition was higher than that obtained for each individual compound at 5 μM (84.9 and 85.1%, respectively).

Table 1. Inhibition of human LDL oxidation by phenolic compounds[a]

Concentrations (μM)	Catechin	Quercetin	Cyanidin	Caffeic acid	Ellagic acid
2.5	85.5 ± 0.2^{b}	29.4 ± 5.3^{d}	53.4 ± 2.5^{c}	49.0 ± 2.3^{c}	3.3 ± 4.0^{e}
5.0	87.8 ± 2.0^{b}	50.6 ± 0.1^{c}	84.9 ± 2.9^{b}	85.1 ± 1.2^{b}	-14.7 ± 4.4^{e}
7.5	96.5 ± 0.7^{a}	86.0 ± 1.1^{b}	96.7 ± 0.7^{a}	97.5 ± 0.2^{a}	13.7 ± 7.1^{e}

[a]Data are given as average percent inhibition of triplicate analyses $\pm$ S.D. Numbers followed by the same letter are not significantly different at $p < 0.05$.

Table 2. Antioxidant interactions on inhibition of LDL oxidation of two-compound combinations[a]

Combination	Expected inhibition[b] (%)	Observed inhibition (%)	Test statistic t_0	Interaction[c] ($p < 0.05$)
Catechin + quercetin	$\geq 100.0 \pm 5.5$	77.3 ± 0.1	7.14	Antagonistic
Catechin + cyanidin	$\geq 100.0 \pm 2.7$	85.8 ± 0.6	8.87	Antagonistic
Catechin + caffeic acid	$\geq 100.0 \pm 2.6$	84.3 ± 0.8	10.06	Antagonistic
Catechin + ellagic acid	88.9 ± 4.3	41.8 ± 3.7	14.49	Antagonistic
Quercetin + cyanidin	82.8 ± 7.7	75.4 ± 0.8	1.64	None
Quercetin + caffeic acid	78.4 ± 7.6	75.8 ± 2.7	0.57	None
Quercetin + ellagic acid	32.7 ± 9.3	27.6 ± 5.2	0.83	None
Cyanidin + caffeic acid	$\geq 100.0 \pm 4.8$	90.4 ± 0.7	3.41	Antagonistic
Cyanidin + ellagic acid	56.7 ± 6.5	48.3 ± 7.8	1.43	None
Caffeic acid + ellagic acid	52.4 ± 6.4	50.2 ± 2.8	0.55	None

[a]Data are given as mean values of triplicate analyses $\pm$ SD. Concentrations of individual phenols were 2.5 μM resulting in a total addition level of 5 μM.
[b]Calculated by summation of individual antioxidant effects obtained at 2.5 μM addition level (Table 1).
[c]The obtained test statistic was compared with t_{crit} ($p < 0.05$) = $t_{0.025,4}$ = 2.78.

Table 3. Antioxidant interactions on inhibition of LDL oxidation of three-compound combinations[a]

Combination	Expected inhibition[b] (%)	Observed inhibition (%)	Test statistic t_0	Interaction[c] ($p < 0.05$)
Catechin + quercetin + cyanidin	$\geq 100.0 \pm 8.0$	95.7 ± 0.3	0.94	None
Catechin + quercetin + caffeic acid	$\geq 100.0 \pm 7.8$	95.3 ± 0.2	1.04	None
Catechin + quercetin + ellagic acid	$\geq 100.0 \pm 9.5$	73.7 ± 1.5	4.73	Antagonistic
Catechin + cyanidin + caffeic acid	$\geq 100.0 \pm 5.1$	98.0 ± 0.5	0.68	None
Catechin + cyanidin + ellagic acid	$\geq 100.0 \pm 6.7$	85.5 ± 0.2	3.72	Antagonistic
Catechin + caffeic acid + ellagic acid	$\geq 100.0 \pm 6.6$	88.2 ± 0.4	3.09	Antagonistic
Quercetin + cyanidin + caffeic acid	100.0 ± 10.1	97.5 ± 0.2	0.44	None
Quercetin + cyanidin + ellagic acid	86.1 ± 11.8	76.2 ± 1.0	1.46	None
Quercetin + caffeic acid + ellagic acid	81.8 ± 11.6	82.4 ± 0.1	−0.10	None
Cyanidin + caffeic acid + ellagic acid	$\geq 100.0 \pm 8.8$	96.9 ± 0.3	0.60	None

[a]Data are given as mean values of triplicate analyses ± SD. Concentrations of individual phenols were 2.5 μM, resulting in a total addition level of 7.5 μM.
[b,c] See footnotes *b* and *c*, Table 2.

No synergistic interactions were found when combinations of three phenolic compounds were tested. However, ellagic acid again exerted an antagonistic interaction in any combinations including catechin. Thus, the inhibitory activities on LDL oxidation of the combinations (catechin + quercetin + ellagic acid), (catechin + cyanidin + ellagic acid), and (catechin + caffeic acid + ellagic acid) were all significantly lower than the sum of individual effects (Table 3). This finding is consistent with the antagonistic effect of the combination of catechin and ellagic acid.

In general, with both the two- and three-compound combinations the antioxidant effects of the hydroxyphenols tested were additive. However, in combinations with ellagic acid and catechin, ellagic acid exerted a significant antagonistic effect on the antioxidant activity of catechin.

DISCUSSION

Pure flavonoids and phenolic acids exerted considerable antioxidant potency towards copper-catalysed human LDL oxidation *in vitro*, with catechin being the most potent antioxidant compound. These results are in general agreement with previous findings (Nardini *et al.*, 1995; Teissedre *et al.*, 1996). However, in contrast to an earlier report (Teissedre *et al.*, 1996), we found that cyanidin had a higher antioxidant activity than quercetin. Furthermore, in the present study ellagic acid did not affect LDL oxidation at the levels tested. To explain the observed variability in inhibition of LDL oxidation between the different compounds, differences in their structural features can be considered. Thus, catechin, cyanidin, and quercetin are all 3,5,7,3',4' pentahydroxy phenols having identical arrangements of hydroxylgroups in their A and B rings (Fig. 1). Therefore, the differences in antioxidant activities can be attributed to variations in the structure of the middle, C, ring. Variabilities in the state of oxidation of the C_3 unit are well known to confer differences in reactivity between flavonoids (Goodwin and Mercer, 1992; Ho, 1992). The present study showed that the presence of the double bond, in addition to the 4-oxo group in the C ring of quercetin, conferred a lower antioxidant activity towards LDL compared with catechin, having a saturated, heterocyclic C ring. Furthermore, in cyanidin, the presence of the unsaturated bonds in the C ring lowered the antioxidant activity slightly as compared with catechin.

Rice-Evans *et al.* (1996) recently proposed a hierachy of antioxidant activities of polyphenols based on radical scavenging of the $ABTS^{\cdot+}$ cation in an artificial, aqueous test system using the hydrophilic tocopherol analogue trolox as a reference compound. Obviously, the latter system is very different from the LDL assay used in our study. Nevertheless, with the radical cation system, quercetin exerted the highest antioxidant activity (4.7 'trolox equivalents'), with cyanidin slightly lower (4.4 'trolox equivalents'), and catechin significantly lower (2.4 'trolox equivalents'). This comparison demonstrates that the ranking of antioxidant activity of polyphenols is strongly dependent on the test system and on the substrate to be protected by the antioxidant.

In the present study, caffeic acid was a stronger antioxidant in inhibiting LDL oxidation (85.1% inhibition at 5 μM), than ellagic acid, which had more free hydroxyl groups. The same order of antioxidant reactivity between these two compounds was found by Laranjinha *et al.* (1994) in an AAPH (2,2'-azobis-2-amidinopropane dihydrochloride)-catalysed LDL oxidation model. They suggested that the low antioxidant activity of ellagic acid compared with caffeic acid could be due to internal hydrogen bonding in ellagic acid between a proximate phenolic OH group and the oxygen atom in (one of) the lactonic ring(s). Presumably a chain-breaking, hydrogen atom-donating antioxidant mechanism, such hydrogen bonding would reduce the ability of ellagic acid to donate a phenolic hydrogen atom to a peroxyl radical (Laranjinha *et al.*, 1994).

The differences in antioxidant activities towards LDL oxidation observed here could also be ascribed to other factors, including differences in solubilities and partitioning behaviour between the aqueous and lipid phases in the LDL system. Thus, the physicochemical properties of antioxidants are known to affect their

antioxidant efficacy in complex, multiphase systems (Frankel *et al*., 1994). Furthermore, the copper-mediated oxidation of tryptophan residues in the LDL-apolipoprotein B was shown to play an important role in initiating lipid oxidation in LDL particles (Giessauf *et al*., 1995). Therefore, as we suggested previously (Teissedre *et al*., 1996), structural features conferring differences in protein binding may affect the antioxidant activity of flavonoids and phenolic acids in inhibiting oxidation of LDL.

The wide mixture of phenolic antioxidants found in wine and plant foods may interact to produce synergistic protection against LDL oxidation (Kinsella *et al*., 1993). In this study, it was established that none of the compounds exerted any antioxidant synergism together at the levels tested. However, in both two-compound and three-compound combinations, ellagic acid exerted a significant antagonistic effect when it was combined with catechin. Ellagic acid is a dimeric condensation product derived from hexahydroxydiphenic acid (Shahidi and Naczk, 1995). The hydroxyl groups of ellagic acid appear unavailable for antioxidant reactivity. However, the presence of carbonyl-groups on both lactone rings of ellagic acid may induce hydrogen-bonding or even reactive interactions with other dihydroxy phenols. The antagonistic effect observed with catechin may thus be due to hydrogen-bonding between catechin *o*-dihydroxyls and ellagic acid carbonyls, which block the hydrogen-donating ability of catechin. However, more detailed studies are necessary to substantiate this hypothesis.

REFERENCES

De Whalley, C. V., Rankin, S. M., Hoult, J. R. S., Jessup, W. and Leake, D. S. (1990) Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem Pharmacol* **39**, 1743–1750.

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

Frankel, E. N., German, J. B. and Davis, P. A. (1992) Headspace gas chromatography to determine human low density lipoprotein oxidation. *Lipids* **27**, 1047–1051.

Frankel, E. N., Huang, S.-W., Kanner, J. and German, J. B. (1994) Interfacial phenomena in the evaluation of antioxidants: bulk oils versus emulsions. *Journal Agric Food Chem* **42**, 1054–1059.

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

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

Giessauf, A., Steiner, E. and Esterbauer, H. (1995) Early destruction of tryptophan residues of apolipoprotein B is a vitamin E independent process during copper-mediated oxidation of LDL. *Biochim Biophys Acta* **1256**, 221–232.

Goodwin, T. W. and Mercer, E. I. (1992). Plant phenolics. In *Introduction to Plant Biochemistry*, 2nd edn, Pergamon Press, Oxford, pp. 528–567.

Herrmann, K. (1989) Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Crit Rev Food Sci Nutr* **28**, 315–347.

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B. and Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **342**, 1007–1011.

Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B. S., Toshima, H., Feskens, E. J. M., Hollman, P. C. H. and Katan, M. B. (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* **155**, 381–386.

Ho, C.-T. (1992). Phenolic compounds in food. In *Phenolic Compounds in Food and their Effects on Health I*, ed. C. T. Ho, C. Y. Lee and M-T. Huang. ACS Symposium series 506, Washington, DC, pp. 2–7.

Hudson, B. J. F. and Lewis, J. I. (1983) Polyhydroxy flavonoid antioxidants for edible oils. Structural criteria for activity. *Food Chem* **10**, 47–55.

Kanner, J., Frankel, E., Granit, R., German, B. and Kinsella, J. E. (1994) Natural antioxidants in grapes and wines. *Journal Agric Food Chem* **42**, 64–69.

Kinsella, J. E., Frankel, E., German, B. and Kanner, J. (1993) Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol* **47**, 85–89.

Knekt, P., Järvinen, R., Reunanen, A. and Maatela, J. (1996) Flavonoid intake and coronary mortality in Finland: a cohort study. *Br Med Journal* **312**, 478–481.

Lanningham-Foster, L., Chen, C., Chance, D. S. and Loo, G. (1995) Grape extract inhibits lipid peroxidation of human low density lipoprotein. *Biological & Pharmaceutical Bulletin* **18**, 1347–1351.

Laranjinha, J. A. N., Almeida, L. M. and Madeira, V. M. C. (1994) Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation. *Biochem Pharmacol* **48**, 487–494.

Macheix, J.-J., Fleuriet, A. and Billot, J. (1990) *Fruit Phenolics*. CRC Press, Boca Raton, FL.

Montgomery, D. C. (1991) *Design and Analysis of Experiments*, 3rd edn. John Wiley, New York, pp. 28–30.

Nardini, M., D'Aquino, M., Tomassi, G., Gentili, V., Di Felice, M. and Scaccini, C. (1995) Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radic Biol Med* **19**, 541–552.

Orr, J. R., Adamson, G. L. and Lindgren, F. T. (1991) Preparative ultracentrifugation and analytic ultracentrifugation of plasma lipoproteins. In *Analysis of Fats, Oils and Lipoproteins*, ed. E. G. Perkins. American Oil Chemists'Society, Champaign, IL, pp. 524–554.

Osawa, T., Ide, A., Su, J.-D. and Namiki, M. (1987) Inhibition of lipid peroxidation by ellagic acid. *Journal Agric Food Chem* **35**, 808–812.

Rice-Evans, C., Miller, N. J. and Paganga, G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**, 933–956.

Shahidi, F. and Naczk, M. (1995) *Food Phenolics*. Technomic Publishing, Basel, Switzerland, pp. 94–97.

Steinberg, D (1992) Metabolism of lipoproteins and their role in the pathogenesis of atherosclerosis. *Atherosclerosis Rev* **18**, 1–6.

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

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