Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 96 (2006) 131–136
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays

Anna Gliszczyńska-Świgło *

Faculty of Commodity Science, The Poznań University of Economics, Al Niepodległości 10, 60-967 Poznań, Poland

Received 6 September 2004; received in revised form 3 February 2005; accepted 3 February 2005

Abstract

Thiamine (vitamin B1), folic acid (vitamin B9), pyridoxine, pyridoxal and pyridoxamine (vitamin B6) were studied for their antioxidant activity using trolox equivalent antioxidant capacity (TEAC) assay with $ABTS^{\cdot+}$ radical cation and ferric reducing antioxidant power (FRAP) assay. All vitamins tested were able to scavenge $ABTS^{\cdot+}$ radical cation although they reacted with it relatively slowly. The reaction could be described by pseudo-first order kinetics. The highest free radical scavenging activity was found for thiamine, followed by folic acid and vitamin B6 forms. In the FRAP assay, only folic acid showed ability to reduce Fe^{3+} although its activity was found to be very low. The study constitutes a starting point for more detailed study of the antioxidant activity of water-soluble vitamins and their analogues, especially in view of the use of vitamins for food fortification and as nutritional supplements.

Keywords: Water soluble vitamins; Antioxidant activity; TEAC assay; FRAP assay

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$) and peroxyl radical ($ROO^{\bullet}$), are constantly generated in vivo both by aerobic metabolism and exogenous sources such as UV radiation, environmental pollution and the diet. The formation of ROS may cause oxidative stress and destruction of unsaturated lipids, DNA, proteins and other essential molecules. This plays an important role in ageing and the pathogenesis of such degenerative or chronic diseases as arteriosclerosis and cancer (Duthie, Duthie, & Kyle, 2000; Middleton & Kandaswami, 1993). Many epidemiological studies suggest that consumption of fruit- and vegetable-rich diet inversely correlates with the risk of cardiovascular diseases and certain forms of cancer. These chemoprotective effects are, at least in part, related to the antioxidant activities of polyphenolic compounds, carotenoids or vitamins E and C (Hollman & Katan, 1999; Prior & Cao, 2000). Food is also a source of other important bioactive compounds, including water-soluble vitamins, such as folates, thiamine or pyridoxine, pyridoxal and pyridoxamine.

The term "folates" refers to the class of compounds having chemical structure and nutritional activity similar to that of folic acid (pteroyl-L-glutamic acid; vitamin B9). Epidemiological studies have shown that folic acid supplementation can significantly reduce the risk of cardiovascular and hematological diseases (Lindenbaum & Nath, 1980; Verhaar, Stroes, & Rabelink, 2002), neurological and neuropsychiatric disorders (Alpert & Fava, 2003; Manzoor & Runcie, 1976), neural tube defects (Daly, Kirke, Molloy, Weir, & Scott, 1995; Olney

* Tel.: +48 61 8569368; fax: +48 61 8543993.
E-mail address: a.gliszczynska-swiglo@ae.poznan.pl.

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.02.018

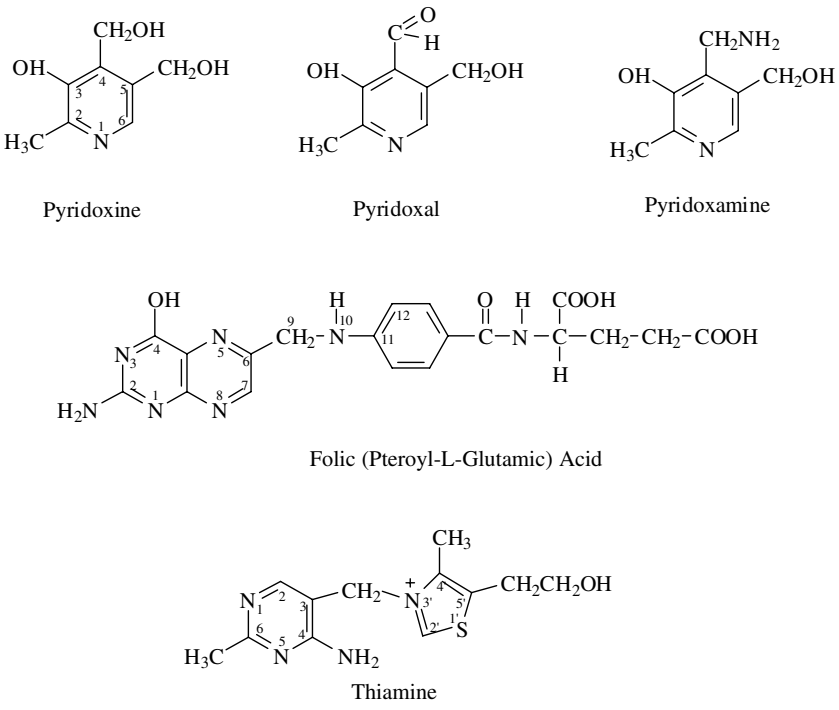

Fig. 1. Structures of vitamins tested.

& Mulinare, 2002) and different forms of cancer (Akoglu, Faust, Milovic, & Stein, 2001; Duthie, Narayanan, Brand, Pirie, & Grant, 2002; Giovannucci, 2002; Zhang et al., 1999). One of the known risk factors for cardiovascular disease is elevated plasma total homocysteine. Folic acid, due to its homocysteine-lowering potential is considered as potentially protective against cardiovascular disease (Boushey, Beresford, Omenn, & Motulsky, 1995; Nygard et al., 1997). It was, however, suggested that folate may have a direct antioxidant role in vivo, which is independent of any indirect effects through lowering of homocysteine levels (Nakano, Higgins, & Powers, 2001). It was also proposed that presumed protective effects of folic acid in the pathogenesis of other degenerative diseases could be associated with its antioxidant activity (Joshi, Adhikari, Patro, Chattopadhyay, & Mukherjee, 2001; Nakano et al., 2001). It was shown that folic acid and its physiological forms can effectively scavenge different free radicals and inhibit lipid peroxidation (Joshi et al., 2001; Rezk, Haenen, van der Vijgh, & Bast, 2003). Activity of both folic acid and pyridoxine, one of the forms of vitamin B6, against the radical-mediated oxidative damage in human whole blood was also reported (Stocker, Lesgards, Vidal, Chalier, & Prost, 2003). No data are available on possible antioxidant activity of other than pyridoxine forms of vitamin B6.

Thiamine (vitamin B1) has been found to protect against lead-induced lipid peroxidation in rat liver and kidney (Senapati, Dey, Dwivedi, Patra, & Swarup, 2000). It may scavenge $O_2^{-\bullet}$ and $^{\bullet}OH$ directly and thus affect the cellular response to oxidative stress (Jung & Kim, 2003). It was also reported that thiamine deficiency results in selective neuronal death in animal models. The neuronal death was associated with increased free radical production, suggesting that oxidative stress processes may play an important early role in the brain damage associated with thiamine deficiency; however, the mechanism of the possible antioxidant activity of thiamine is unknown (Todd & Butterworth, 1999).

Thus, the objective of the present study was to determine the antioxidant activities of folic acid, thiamine, pyridoxine, pyridoxal and pyridoxamine (Fig. 1), using trolox equivalent antioxidant capacity (TEAC) assay with $ABTS^{\bullet+}$ radical cation and ferric reducing antioxidant power (FRAP) assay. This report constitutes a starting point for more detailed study on the antioxidant activity of water-soluble vitamins and their analogues in different in vitro model systems, generating various free radicals or oxidation conditions.

2. Materials and methods

2.1. Materials

Vitamin B1 (thiamine hydrochloride), vitamin B6 (pyridoxine hydrochloride), and ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) diammonium salt were purchased from Fluka (Buchs, Switzerland), pyridoxal hydrochloride was from Serva (Heidelberg, New York); pyridoxamine, folic acid

(pteroyl-L-glutamic acid), 2,4,6-tripyridyl-s-triazine (TPTZ) and microperoxidase-8 (MP8) were from Sigma (St. Louis, MO, USA). Trolox® was from Aldrich (Steinheim, Germany) and ascorbic acid (vitamin C) was from Merck (Darmstadt, Germany).

2.2. Trolox equivalent antioxidant capacity assay

The antioxidant activities of vitamins were measured by the TEAC (trolox equivalent antioxidant capacity) assay, performed essentially as described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993) and Rice-Evans and Miller (1994) with some modifications introduced by Tyrakowska et al. (1999). The TEAC value is based on the ability of the antioxidant to scavenge the blue-green coloured $ABTS^{\cdot+}$ radical cation relative to the $ABTS^{\cdot+}$ radical cation scavenging ability of the water-soluble vitamin E analogue, Trolox® (Miller et al., 1993; Rice-Evans & Miller, 1994). In the present study, microperoxidase-8 (MP8), instead of metmyoglobin, was used to generate the $ABTS^{\cdot+}$ radical cation in PBS (potassium phosphate-buffered saline), pH 7.4. MP8 (final concentration 0.2 μM) and ABTS (final concentration 3.0 mM) in PBS were mixed and the reaction was initiated by the addition of hydrogen peroxide (final concentration 0.1 mM). The mixture was incubated at 30 °C for 1 h. The $ABTS^{\cdot+}$ radical cation solution thus obtained was diluted with PBS (v/v) to give an absorbance of about 0.8 at 734 nm. Vitamins and Trolox® were added as 1% (v/v) solutions of 100 times concentrated stock solutions in DMSO (folic acid), PBS (other vitamins) or methanol (Trolox®) to give the final concentration required (Tyrakowska et al., 1999). For each experiment, a solvent blank was run. The decrease in absorbance caused by vitamins, measured at 6 min, reflected the $ABTS^{\cdot+}$ radical cation scavenging capacity and was plotted against the concentration of the antioxidant. The TEAC value represents the ratio between the slope of this linear plot for scavenging of $ABTS^{\cdot+}$ radical cation by the vitamin compared to the slope of this plot for $ABTS^{\cdot+}$ radical cation scavenging by Trolox® (expressed in ΔA per mM of vitamin or Trolox®), used as an antioxidant standard. Moreover, for 6- and 30-min-measurements, the radical-scavenging activity was expressed in terms of IC_{50} value (the concentration of the antioxidant required to scavenge 50% of $ABTS^{\cdot+}$ radical cation) calculated by a linear regression analysis.

2.3. Determination of rate constants of the reaction of vitamins with $ABTS^{\cdot+}$ radical cation

A solution of $ABTS^{\cdot+}$ radical cation was prepared as described in Section 2.2. Vitamins were added to the solution of $ABTS^{\cdot+}$ radical cation as 1% (v/v) solutions of 100 times concentrated stock solutions in DMSO (folic acid) or PBS (other vitamins) to give the final concentrations required. For each experiment, a solvent blank was run. Glass cuvettes, sealed with a teflon cap, were used for all measurements, which were performed at 25 ± 0.5 °C. The decrease in absorbance caused by tested compounds, measured every 2 min for 30 min, reflected the $ABTS^{\cdot+}$ radical-scavenging capacity. The pseudo first-order rate constants (k) were calculated as a slope of the linear plot: $[\ln(A/A_o)]$ versus time in seconds. The second-order rate constants (K) of the reaction of $ABTS^{\cdot+}$ radical cation with vitamins were derived from the linear dependence between the pseudo-first order rate constants (k) and the concentrations of the vitamins (Atkins, 2001).

2.4. The ferric reducing antioxidant power assay

FRAP assay was carried out by the method of Benzie and Strain (1996) with minor modification. The method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}-TPTZ). Vitamins were added as 2% (v/v) solutions of 50 times concentrated stock solutions to 10 mM ferric-TPTZ reagent and the increase in absorbance at 593 nm was measured at 8 min. $FeSO_4 \cdot 7H_2O$ was used as a standard. The concentrations of vitamins and $FeSO_4 \cdot 7H_2O$ were chosen to give an absorbance value not higher than 1. The FRAP value represents the ratio between the slope of the linear plot for reducing Fe^{3+}-TPTZ reagent by the vitamin compared to the slope of this plot for $FeSO_4$ (expressed in ΔA per mM).

2.5. Statistical analysis of data

Data were presented as means ± SD of at least triplicate experiments. Analysis of variance was performed on the data obtained. Significance of differences between means was determined by least significant differences (LSD) at $P \leqslant 0.05$.

3. Results and discussion

Several assays have been introduced for the measurement of the antioxidant activity of single compound and/or complex mixtures (Benzie & Strain, 1996; Cao, Vedron, Wu, Wang, & Prior, 1995; Fogliano, Verde, Randazzo, & Ritieni, 1999; Miller et al., 1993; Rice-Evans & Miller, 1994). Miller et al. (1993) and Rice-Evans and Miller (1994) have developed the so-called TEAC (trolox equivalent antioxidant capacity) assay, which has attracted much interest because it enables high-throughput screening of potential antioxidant activity of single compounds and biological matrices, such as plasma, as well as food components, food extracts or beverages (Gliszczyńska-Świgło & Tyrakowska, 2003; Miller, Diplock, & Rice-Evans, 1995;

Miller & Rice-Evans, 1997; Proteggente et al., 2002; Rice-Evans, Miller, & Paganga, 1996; Tyrakowska et al., 1999). This assay is based on the antioxidant's ability to react with $ABTS^{\bullet+}$ radical cation generated in the assay system. In contrast, the ferric reducing antioxidant power (FRAP) assay measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. This assay is also commonly used for the routine analysis of single antioxidants and total antioxidant activity of plant extracts (Gao, Björk, Trajkovski, & Uggla, 2000; Halvorsen et al., 2002; Schlesier, Harwat, Böhm, & Bitsch, 2002).

A few literature data suggest that some water soluble vitamins may act as antioxidants (Joshi et al., 2001; Jung & Kim, 2003; Nakano et al., 2001; Rezk et al., 2003; Senapati et al., 2000; Stocker et al., 2003). In the present study, thiamine, folic acid, pyridoxine, pyridoxal, and pyridoxamine were chosen as model compounds to study the antioxidant capacities of water-soluble vitamins using TEAC and FRAP assays.

Table 1 presents the results of the antioxidant activity of folic acid, thiamine, pyridoxine, pyridoxal, pyridoxamine and ascorbic acid as a reference vitamin. It was found that all vitamins tested were able to scavenge $ABTS^{\bullet+}$ radical cation although their antioxidant activities measured at 6 min and expressed as the TEAC value or IC_{50} were relatively low as compared to vitamin C. The exception could be thiamine. Its radical-scavenging activity, expressed as the TEAC value or IC_{50}, is only about 3–4-fold lower than that of vitamin C. The TEAC values of thiamine and folic acid are comparable to such natural antioxidants as some carotenoids (Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996) and polyphenols, e.g., naringin and monosubstituted benzoic acid derivatives (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995; Rice-Evans et al., 1996). The TEAC values of vitamin B6 forms, measured at 6-min of incubation with $ABTS^{\bullet+}$ radical cation, was found to be similar. However, it was observed that all vitamins tested act as free radical-scavengers relatively slowly and their reactivity could be described by pseudo-first order kinetics. Thus, the pseudo first-order rate constant (k) was obtained by following the decrease in absorbance of the $ABTS^{\bullet+}$ radical cation within 30 min at 734 nm. Fig. 2 shows the straight lines of dependence between the pseudo first-order rate constants (k) and the concentrations of vitamins. From the slope of lines, the second-order rate constants (K) of the reactions of $ABTS^{\bullet+}$ radical cation with appropriate vitamins were estimated to be 19.4 M^{-1} s^{-1} for thiamine, 4.1 M^{-1} s^{-1} for folic acid, 2.5 M^{-1} s^{-1} for pyridoxine, 2.8 M^{-1} s^{-1} for pyridoxal and 2.0 M^{-1} s^{-1} for pyridoxamine. The reaction of thiamine with $ABTS^{\bullet+}$ radical cation was found to be 4.7- ~10-fold faster than those of folic acid and vitamin B6 forms. The second-order rate constants for vitamin B6 forms indicate that pyridoxamine is the least active form.

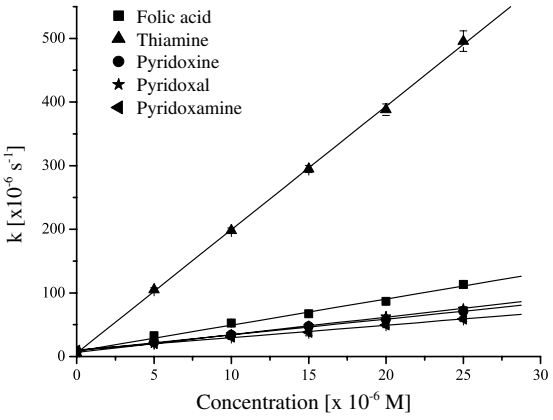

Fig. 2. Dependence between the pseudo-first order rate constants (k) and concentrations of vitamins under study.

The IC_{50} values of vitamins tested, determined at 30 min of incubation of vitamins with $ABTS^{\bullet+}$ radical cation, were from 3- to 5-fold lower than the appropriate IC_{50} values obtained at 6 min. The radical-scavenging activities of folic acid and vitamin B6 forms were only about 5-fold and 5–11-fold lower than that of vitamin C, respectively. The IC_{50} value found for thiamine was very similar to the IC_{50} value of vitamin C (Table 1).

In the FRAP assay, only folic acid revealed the ability to reduce Fe^{3+} to Fe^{2+}, however its activity was about 25-fold lower than that of vitamin C.

Altogether, the results obtained indicate that some water-soluble vitamins, especially thiamine and folic acid, may act as potent antioxidants although they scavenge free radicals such as $ABTS^{\bullet+}$ radical cation relatively slowly. However, antioxidant activities of

Table 1
Antioxidant activities of thiamine, folic acid, pyridoxine, pyridoxal and pyridoxamine as compared to ascorbic acid

Vitamin	TEAC value	$ABTS^{\bullet+}$ IC_{50} (μM) at 6 min	$ABTS^{\bullet+}$ IC_{50} (μM) at 30 min	FRAP value
Thiamine	0.32 ± 0.01	57.4 ± 3.0	19.9 ± 1.6	–
Folic acid	0.06 ± 0.01	291 ± 3.5	73.0 ± 1.9	0.04 ± 0.01
Pyridoxine	0.03 ± 0.00[a]	623 ± 25.1[a]	120 ± 5.3[a]	–
Pyridoxal	0.03 ± 0.00[a]	601 ± 13.6[a]	112 ± 6.5[a]	–
Pyridoxamine	0.03 ± 0.00[a]	694 ± 8.6	156 ± 6.7	–
Ascorbic acid	0.99 ± 0.05	14.6 ± 0.7	14.5 ± 0.7	0.98 ± 0.05

[a] Not significantly different within the column ($P \leqslant 0.05$).

water-soluble vitamins could become important in view of nutritional supplementation and fortification of food especially with thiamine and folic acid.

References

Akoglu, B., Faust, D., Milovic, V., & Stein, J. (2001). Folate and chemoprevention of colorectal cancer: is 5-methyl-tetrahydrofolate an active antiproliferative agent in folate-treated colon-cancer cells?. *Nutrition, 17*, 652–653.

Alpert, J. E., & Fava, M. (2003). Nutrition and depression: the role of folate. *Nutrition Review, 55*, 145–149.

Atkins, P. W. (2001). *Chemia fizyczna*. Warszawa: PWN.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Boushey, C. J., Beresford, S. A., Omenn, G. S., & Motulsky, A. G. (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *The Journal of the American Medical Association, 274*, 1049–1057.

Cao, G., Vedron, C. P., Wu, A. H. B., Wang, H., & Prior, R. L. (1995). Automated-assay of oxygen radical absorbance capacity with the COBAS FARA-II. *Clinical Chemistry, 41*, 1738–1744.

Daly, L. E., Kirke, P. N., Molloy, A., Weir, D. G., & Scott, J. M. (1995). Folate levels and neural tube defects. Implications for prevention. *Journal of the American Medical Association, 274*, 1698–1702.

Duthie, G. G., Duthie, S. J., & Kyle, J. A. M. (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition Research Reviews, 13*, 79–106.

Duthie, S. J., Narayanan, S., Brand, G. M., Pirie, L., & Grant, G. (2002). Impact of folate deficiency on DNA stability. *Journal of Nutrition, 132*, 2444S–2449S.

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry, 47*, 1035–1040.

Gao, X., Björk, L., Trajkovski, V., & Uggla, M. (2000). Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *Journal of the Science of Food and Agriculture, 80*, 2021–2027.

Giovannucci, E. (2002). Epidemiologic studies of folate and colorectal neoplasia: a review. *Journal of Nutrition, 132*, 2350S.

Gliszczyńska-Świgło, A., & Tyrakowska, B. (2003). Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. *Journal of Food Science, 68*, 1844–1849.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition, 132*, 461–471.

Hollman, P. C. H., & Katan, M. B. (1999). Dietary flavonoids: intake, health effects and bioavailability. *Food and Chemical Toxicology, 37*, 937–942.

Joshi, R., Adhikari, S., Patro, B. S., Chattopadhyay, S., & Mukherjee, T. (2001). Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radical Biology and Medicine, 30*, 1390–1399.

Jung, I. L., & Kim, J. G. (2003). Thiamine protects against paraquat-induced damage: scavenging activity of reactive oxygen species. *Environmental Toxicology and Pharmacology, 15*, 19–26.

Lindenbaum, J., & Nath, B. J. (1980). Megaloblastic anaemia and neutrophil hypersegmentation. *British Journal of Haematology, 44*, 511–513.

Manzoor, M., & Runcie, J. (1976). Folate-responsive neuropathy: report of 10 cases. *British Medical Journal, 1*, 1176–1178.

Middleton, E., Jr., & Kandaswami, Ch. (1993). The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In J. B. Harborne (Ed.), *The flavonoids: Advances in research since 1986* (pp. 619–652). London: Chapman and Hall.

Miller, N. J., Rice-Evans, C. A., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science, 84*, 407–412.

Miller, N. J., Diplock, A. T., & Rice-Evans, C. A. (1995). Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *Journal of Agricultural and Food Chemistry, 43*, 1794–1801.

Miller, N. J., Sampson, J., Candeias, L. P., Bramley, P. M., & Rice-Evans, C. A. (1996). Antioxidant activities of carotenes and xanthophylls. *FEBS Letters, 384*, 240–242.

Miller, N. J., & Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chemistry, 60*, 331–337.

Nakano, E., Higgins, J. A., & Powers, H. J. (2001). Folate protects against oxidative modification of human LDL. *British Journal of Nutrition, 86*, 637–639.

Nygard, O., Nordrehaug, J. E., Refsum, H., Ueland, P. M., Farstad, M., & Vollset, S. E. (1997). Plasma homocysteine levels and mortality in patients with coronary artery disease. *New England Journal of Medicine, 337*, 230–236.

Olney, R. S., & Mulinare, J. (2002). Trends in neural tube defect prevalence, folic acid fortification, and vitamin supplement use. *Seminars in Perinatology, 26*, 277–285.

Prior, R. L., & Cao, G. (2000). Flavonoids: diet and health relationships. *Nutrition in Clinical Care, 3*, 279–288.

Proteggente, A. R., Pannala, A. S., Paganga, G., van Buren, L., Wagner, E., Wiseman, S., et al. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research, 36*, 217–233.

Rezk, B. M., Haenen, G. R. M. M., van der Vijgh, W. J. F., & Bast, A. (2003). Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. *FEBS Letters, 555*, 601–605.

Rice-Evans, C. A., & Miller, N. J. (1994). Total antioxidant status in plasma and body fluids. *Methods in Enzymology, 234*, 279–293.

Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*, 375–383.

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

Senapati, S. K., Dey, S., Dwivedi, S. K., Patra, R. C., & Swarup, D. (2000). Effect of thiamine hydrochloride on lead induced lipid peroxidation in rat liver and kidney. *Veterinary and Human Toxicology, 42*, 236–237.

Schlesier, K., Harwat, M., Böhm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research, 36*, 177–178.

Stocker, P., Lesgards, J.-F., Vidal, N., Chalier, F., & Prost, M. (2003). ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochimica et Biophysica Acta, 1621*, 1–8.

Todd, K., & Butterworth, R. F. (1999). Mechanisms of selective neuronal cell death due to thiamine deficiency. *Annals of the New York Academy of Sciences, 893*, 404–411.

Tyrakowska, B., Soffers, A. E. M. F., Szymusiak, H., Boeren, S., Boersma, M. G., Lemańska, K., et al. (1999). TEAC antioxidant activity of 4-hydroxybenzoates. *Free Radical Biology and Medicine, 27*, 1427–1436.

Verhaar, M. C., Stroes, E., & Rabelink, T. J. (2002). Folates and cardiovascular disease. *Arteriosclerosis Thrombosis and Vascular Biology, 22*, 6–13.

Zhang, S., Hunter, D. J., Hankinson, S. E., Giovannucci, E. L., Rosner, B. A., Colditz, G. A., et al. (1999). A prospective study of folate intake and the risk of breast cancer. *Journal of the American Medical Association, 281*, 1632–1637.