Review Article

Phytonutrient deficiency: the place of palm fruit*

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The oil palm (*Elaeis guineensis*) is native to many West African countries, where local populations have used its oil for culinary and other purposes. Large-scale plantations, established principally in tropical regions (Asia, Africa and Latin America), are mostly aimed at the production of oil, which is extracted from the fleshy mesocarp of the palm fruit, and endosperm or kernel oil. Palm oil is different from other plant and animal oils in that it contains 50% saturated fatty acids, 40% unsaturated fatty acids, and 10% polyunsaturated fatty acids. The fruit also contains components that can endow the oil with nutritional and health beneficial properties. These phytonutrients include carotenoids (α-, β- and γ-carotenes), vitamin E (tocopherols and tocotrienols), sterols (sitosterol, stigmasterol and campesterol), phospholipids, glycolipids and squalene. In addition, it is recently reported that certain water-soluble powerful antioxidants, phenolic acids and flavonoids, can be recovered from palm oil mill effluent. Owing to its high content of phytonutrients with antioxidant properties, the possibility exists that palm fruit offers some health advantages by reducing lipid oxidation, oxidative stress and free radical damage. Accordingly, use of palm fruit or its phytonutrient-rich fractions, particularly water-soluble antioxidants, may confer some protection against a number of disorders or diseases including cardiovascular disease, cancers, cataracts and macular degeneration, cognitive impairment and Alzheimer's disease. However, whilst prevention of disease through use of these phytonutrients as in either food ingredients or nutraceuticals may be a worthwhile objective, dose response data are required to evaluate their pharmacologic and toxicologic effects. In addition, one area of concern about use of antioxidant phytonutrients is how much suppression of oxidation may be compatible with good health, as toxic free radicals are required for defence mechanisms. These food-health concepts would probably spur the large-scale oil palm (and monoculture) plantations, which are already seen to be a major cause of deforestation and replacement of diverse ecosystems in many countries. However, the environmental advantages of palm phytonutrients are that they are prepared from the readily available raw material from palm oil milling processes. Palm fruit, one of only a few fatty fruits, is likely to have an increasingly substantiated place in human health, not only through the provision of acceptable dietary fats, but also its characteristic protective phytonutrients.

Key Words: palm fruit, phytonutrient, antioxidant, polyphenol, flavonoid, cardiovascular disease, cancer, diabetes, cataracts, age-related macular degeneration, phytochemicals

Introduction

There has been a growing research interest in palm, especially palm oil, which is one of the major edible plant oils in the tropical countries. The oil palm (*Elaeis guineensis*) is native to many West African countries, where local populations have used its oil for culinary and other purposes. Large-scale plantations, established principally in tropical regions (Asia, Africa and Latin America), are mostly aimed at the production of oil, which is extracted from the fleshy mesocarp of the palm fruit, and endosperm or kernel oil. Palm oil is different from other plant and animal oils in that it contains 50% saturated fatty acids, 40% unsaturated fatty acids, and 10% polyunsaturated fatty acids. The fruit also contains components that can endow the oil with nutritional and health beneficial properties. These phytonutrients include carotenoids (α-, β- and γ-carotenes), vitamin E (tocopherols and tocotrienols), sterols (sitosterol, stigmasterol and campesterol), phospholipids, glycolipids and squalene. In addition, it is recently reported that certain water-soluble powerful antioxidants, phenolic acids and flavonoids, can be recovered from palm oil mill effluent.

Phytonutrients in the lipid fraction of palm fruit

Palm oil is one of the most widely used cooking oils in West and Central Africa. It is obtained from the mesocarp of the fruits, and composed of 50% saturated fatty acids, 40% monounsaturated fatty acids and 10% polyunsaturated fatty acids. The saturated fat components are trace amounts of lauric and myristic acids, which are cholesterol-raising fatty acids, and a large amount of palmitic acid, which has

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a minimal effect on cholesterol elevation. The principal triglyceride species in palm oil have palmitic acid at the alpha-position of the molecule, and this location confers the non-hypercholesterolaemic property to the oil.

Palm oil is used directly in a variety of food processes without undergoing a hydrogenation process, in which some of the *cis*- double bonds are transformed to the *trans*-configuration. Therefore, it is worth noting that palm oil does not contain any *trans*- unsaturated fatty acid isomers. The minor components of fresh palm oil are carotenes, vitamin E, sterols, phospholipids, glycolipids and squalene.

a) Carotenes found in palm oil are α-, β- and γ-carotenes. They are precursors of vitamin A, which prevents night blindness, aids maintenance of tissues and promotes growth.
b) Phytosterols present in palm oil are sitosterol, stigmasterol and campesterol. These lipophilic sterols are easily absorbed in the gastrointestinal tract, and then converted through a series of enzymatic reactions into cholesterol, which is a major precursor of steroid hormones.
c) Squalene, present in palm oil, when in excess amounts has been found to possess a negative feedback inhibition activity on the function of HMG-CoA reductase, an enzyme involved in the production of cholesterol in the liver.
d) Vitamin E found in palm oil is composed mainly of tocopherols and tocotrienols. They act as potent antioxidants that make it relatively stable to oxidation. Both animal and human studies show that tocotrienols could reduce plasma cholesterol, apolipoprotein B, thromboxane B2, and platelet factor IV. They could also inhibit or delay the oxidative deterioration of cellular membranes.

Sundram and colleagues (2003) have described the chemistry of palm oil in more detail in this issue.[1]

Phytonutrients in the water fraction of palm fruit

Unlike the lipid fraction, only limited information is available on the water fraction of palm fruit. The first report was presented by Sundram and colleagues of the Malaysian Palm Oil Board at the PIPOC International Palm Oil Congress in 2001.[2] Large amounts of various phenolic acids and flavonoids are retained in the palm oil mill effluent resulting from the heat inactivation of polyphenol oxidase enzyme during the milling process. Many phenolic compounds are effective antioxidants because of their free radical scavenging properties and because they are chelators of metal ions; thus, they may protect tissues against free oxygen radical and lipid peroxidation.[3] It is believed that these water-soluble polyphenols are produced by plants in order to protect against autoxidation.[4]

Dietary polyphenols

Polyphenols are present in all plants, and, thus, are in the diet. There are more than 8,000 phenolic structures that have been identified that vary structurally from being a simple molecule (e.g phenolic acids with a C6 ring structure) to being highly polymerised compounds (such as tannins). The main classes of polyphenols are phenolic acids, flavonoids and the less common stilbenes and lignans.

Phenolic acids

The major class of phenolic acids is the hydroxy cinnamic acids, which are found in almost every plant. The major representative of hydroxy cinnamic acids is caffeic acid, which occurs in foods mainly as chlorogenic acid, an ester with quinic acid. Coffee is a major source of chlorogenic acid in the human diet. Chlorogenic and caffeic acids are antioxidants in vitro, and they might inhibit the formation of mutagenic and carcinogenic *N*-nitroso compounds[5], and chlorogenic acid can inhibit DNA damage.[6] The inverse association between coffee intake and colon cancer in some epidemiologic studies might be explained in part by the chlorogenic acid present in coffee.[7,8]

Flavonoids

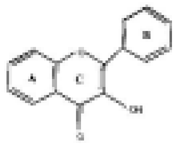
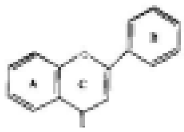
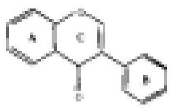
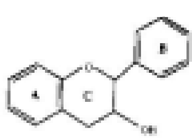
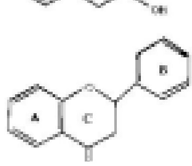
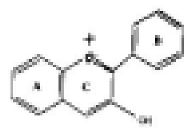
Flavonoids are polyphenolic compounds that occur ubiquitously in plant foods and have structural variations in the one of three rings (C6-C3-C6 backbone structure) that characterises the different types namely, flavonols, flavones, isoflavones, flavanols (catechins), flavanones and anthocyanins (see Table 1).

Polyphenols in palm fruit: their possible role in the prevention of chronic diseases

Sundram and colleagues evaluated the antioxidant activity of the flavonoid-phenolic rich crude extract against Trolox (a water-soluble analogue of vitamin E), and found it to have a substantial antioxidant activity.[2] It was also tested against gallic acid in a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical generating system, and was found to have free radical scavenging activity superior to that of green tea extract on an equivalent weight basis. Furthermore, it was also demonstrated that this palm extract protected against Cu^{2+} induced human LDL oxidation, as effective as α-tocopherol and α-tocotrienol.

Owing to its high content of phytonutrients with demon-strable antioxidant properties, the possibility exists that palm fruit offers some health advantages by reducing lipid oxidation, oxidative stress and free radical damage. Mounting evidence indicates protective effects of high consumption of polyphenol-rich foods, such as fruits, vegetables and beverages (tea and red wine) reported to play a role in a number of age-related diseases, such as cardiovascular disease, certain cancers, cataracts and macular degeneration, cognitive impairment and Alzheimer's disease.[9-15] Furthermore, flavonoids may also be operative by mechanisms that apparently are not directly dependent upon their antioxidant properties. A wide range of different biological activities, including anti-bacterial, anti-inflammatory and anti-carcinogenic effects mediated by different mechanisms, are associated with flavonoid compounds.[16] Therefore, it is likely that use of palm fruit or its phytonutrient-rich fractions may confer some protection against these disorders or diseases.

Table 1. The main classes of flavonoids

Class	Basic structure	Compounds	Food sources
Flavonols		Quercetin, myricetin, kaempferol	Apples, onions, tea, berries, olives, broccoli, lettuce, red wine, cocoa, chocolate
Flavones		Apigenin, luteolin	
Isoflavones		Genistein, diadzein	Soy
Flavanols (Catechins)		(−)-epigallo-catechin-3-gallate (EGCG)	Tea, red wine, cocoa/ chocolate
Flavanones		Naringenin, hesperitin	Citrus fruits
Antho-cyanidins		Cyaniding, pelargonidin	Blueberries red cabbage purple sweet potatoes

Cardiovascular disease (CVD)

Several population studies have reported an inverse association between the intake of dietary flavonoids (from a variety of plant foods, such as apples, onions and tea) and risk of coronary heart disease (CHD).[17-21] A protective effect of dietary flavonoid intake on stroke incidence was first demonstrated by the Zutphen Study after 552 men were followed up for 15 years.[20] The consumption of black tea, a good source of catechins, was inversely associated with stroke risk. For those studies that have reported an inverse association, putative mechanism of action include inhibition of LDL oxidation (measured in vitro)[22,23], and reduction of cytotoxicity of modified LDL to the vessel wall.[24,25] LDL is modified by free radicals that oxidise the polyunsaturated fatty acids in the LDL molecule. Modified LDL is easily absorbed by macrophages and is toxic to the vessel endothelium.[26] This may ultimately lead to the formation of athero-sclerotic plaques. There is emerging evidence that phenolic compounds have anti-thrombotic effects that appear to be the result of reduced susceptibility of platelet aggregation, reduced synthesis of prothrombotic and pro-inflammatory mediators, decreased expression of adhe-sion molecules, and tissue factor activity.[27, 28]

Furthermore, antioxidants including polyphenolic compounds may have a role in endothelial-dependent vasodilation by preserving the biological activity of endothelium-derived nitric oxide (NO).[29] NO is an essential molecule in the regulation of vascular tone via the stimulation of vascular smooth muscle cell relaxation and concomitant vasodilation. NO also exerts a number of other anti-atherogenic effects, including inhibition of leukocyte-endothelial interaction, smooth muscle cell proliferation, and platelet aggregation. The increased oxidative stress, particularly increased production of superoxide radicals and elevated levels of oxidised LDL, can attenuate the biological activity of NO. It has been demonstrated that vitamin C, an antioxidant vitamin, can spare intracellular thiols, which in turn can stabilise NO through the formation of biologically active S-nitroso-thiols.[29]

However, while the protective effects of phyto-nutrients against CVD have been proposed to be attri-butable to their antioxidant properties, the results of controlled trials using antioxidant vitamins (vitamin E and β-carotene) are inconsistent. In the Physicians' Health Study in which more than 22,000 apparently healthy US male physicians took either 50mg β-carotene or placebo every second day for 12 years, β-carotene had no effect on the incidence of CHD.[30] Amongst the 1862 Alpha Tocopherol Beta Carotene (ATBC) Study participants with a history of myocardial infarction, both vitamin E and β-carotene and their combination decreased the incidence of nonfatal myocardial infarction compared with the placebo group (14%-38%), but increased the incidence of fatal CHD (33%-75%).[31] Similarly, in the Cambridge Heart Antioxidant Study, 2002 patients with angiographically proven coronary atherosclerosis were given vitamin E supplements 400 or 800 IU/day for an average of 17 months.[32] Vitamin E supplementation reduced the risk of nonfatal myocardial infarction by 77%, but there were 29% more deaths, including more fatal myocardial infarctions, in the group who received vitamin E supplements.

Cancer

Epidemiologic studies consistently have demonstrated an inverse relation between flavonoid consumption and risks of certain types of cancer.[33-37] Several in vitro cell culture and in vivo experiments have shown that flavonoids may interrupt various stages of the cancer process.[37] However, in most studies, supplements of vitamins C and E, and β-carotene do not decrease DNA damage.[38,39]

It appears that these phytonutrients may act in a variety of ways beyond their antioxidant properties to interfere with carcinogenesis, such as protecting DNA from oxidative damage, deactivating carcinogens, and inhibiting the expression of mutated genes and the activity of enzymes that promote carcinogenesis, as well as promoting detoxification of xenobiotics.[40-42] Catechins in green tea, particularly the major (−)-epigallocatechin-3-gallate (EGCG), possess significant chemopreventive activity.[43] It is evident that EGCG protects normal cells from genotoxic or carcinogenic assault and is capable of eliminating tumour cells, by eliciting a variety of cellular and molecular responses which include antimutagenic activity[44], suppression of oxidative DNA damage[45], and induction of apoptosis in tumour cells.[46,47]

Diabetes

Non-insulin dependent diabetes mellitus (NIDDM) or type 2 diabetes is increasingly common throughout the world. The World Health Organization (WHO) has predicted that between 1997 and 2025, the number of diabetics will double from 143 million to about 300 million. The leading cause of mortality and morbidity in people with NIDDM is CVD caused by macro- and microvascular degeneration. A number of studies have suggested that enhanced oxidation is the underlying abnormally responsible for some of the complications of diabetes. Studies in human and laboratory animals with NIDDM indicate that vitamin E and lipoic acid supplements lessen the impact of oxidative damage caused by dysregulation of glucose metabolism.[48-50]

Diseases of the eye

Cataracts, age-related macular degeneration and other eye diseases, such as diabetic retinopathy, are probably linked to the effects of oxygen radicals derived from light or metabolic reactions. Cataracts develop as the protein making up the lens of the eye is damaged by free radicals, oxidants and UV-light.[51] High consumption of fruit and vegetables is associated with delayed development of various forms of cataract. The same beneficial relationship to vision pertains to increased intakes of antioxidants, such as vitamins C, E and β-carotene, and to plasma antioxidant status.[52-54] Thus, it appears that assuring optimal antioxidant intake can extend lens function. In addition to achieving beneficial effects from long-known antioxidant vitamins such as vitamins E and C and folic acid, some protection may be conferred by other antioxidant phytonutrients, such as flavonoids, especially catechin, and other polyphenols.[55]

Similar to cataracts, epidemiological and intervention studies suggest that low antioxidant intake may be associated with the occurrence of neovascular age-related macular degeneration (AMD), the leading cause of irreversible vision loss in the developed world.[56-58] That is because the retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light. The Age-Related Eye Disease Study (AREDS) Research Group recently reported significant reduction in the progression of certain categories of age-related macular degeneration (AMD) with the use of high-dose antioxidant and zinc supplementation.[59-61]

Alzheimer's disease

The aetiology of Alzheimer's disease (AD) is not well understood; therefore neither prevention strategies nor long-term effective treatments are available for this disease. Based on laboratory and clinical studies, it appears that reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are generated extracellularly and intracellularly by various mechanisms are among the major intermediary risk factors that initiate and promote neurodegeneration in AD. Therefore, it is proposed that antioxidant supplements could be useful in the prevention of AD, and as an adjunct to standard therapy in the treatment of AD. However, results from prospective studies are inconsistent. One study showed that, after 5,395 dementia-free elderly were followed for a mean of 6 years, high dietary intake of vitamin C and vitamin E decreased the risk of AD.[62] In contrast, no relationships were observed between the AD risk and the intake of vitamins C and E and carotenes in supplemental or dietary (non-supplemental) form or in both forms in a study where 980 participants were followed for an average of 4 years.[63] The value of antioxidant phytonutrients present in palm fruit for AD prevention is ambiguous, and will remain so until properly designed human trials have been performed.

Pro-oxidant activities

It is generally accepted that certain components and some plant materials have beneficial antioxidant effects; however, this is often complicated by the realisation that these compounds can have adverse effects. A majority of antioxidants present in plant foods or food additives are capable of stimulating free-radical damage to non-lipid components, carbohydrates and DNA in vitro, and may therefore exert pro-oxidant actions in biological systems.[64] One area of concern about use of antioxidant phytonutrients is how much suppression of oxidation may be compatible with good health, as toxic free radicals are required for defence mechanisms. Like other antioxidant phyto-nutrients, under certain conditions flavonoids may also behave as pro-oxidants.[65] However, if the extent of pro-oxidant activity of these compounds is limited, this could suggest that the effects are unlikely to present a problem in biological systems.

Dietary antioxidants can exert a number of effects in vivo, such as promoting the synthesis of endogenous antioxidant defences by up-regulation of their biosynthesis and/or increased gene expression.[66] For example, the presence of vitamin E in the dietary supplement may preserve the pool of endogenous glutathione, and therefore the glutathione levels were elevated in antioxidant-fed mice. The vitamin E supplements can indeed modify gene expression induced by heat shock in vivo as well as protect animal tissues against oxidative stress by enhancing the level of endogenous antioxidants and inducing heat shock protein-70 gene expression.

Because there are many biological activities attributed to phytonutrients present in palm fruit, especially polyphenols, some of which could be beneficial or detrimental depending on specific circumstances, further studies in both the laboratory and with populations are warranted.

Conclusions

The cultural link between the medicinal values of a plant and its value as food has repercussions on a whole set of studies dealing with the nutritional values of certain edible plants. Knowing the chemical composition is an indispensable precondition for their promotion. The question might be asked as to whether enough consideration has been given to food component contributions to bodily functions and to health protection. These food-health concepts would probably spur the large-scale oil palm (and monoculture) plantations, which are already seen to be a major cause of deforestation and replacement of diverse ecosystems in many countries. However, the environmental advantages of palm phytonutrients are that they are prepared from the readily available raw material from palm oil milling processes.

In conclusion, palm fruit is likely to have an increasingly substantiated place in human health, not only through the provision of acceptable dietary fats, but also its characteristic protective compounds.

References

1. Sundram K, Sambanthamurthi R, Tan YA. Palm fruit chemistry and nutrition. Asia Pac J Clin Nutr 2003; 12 (3): 355-362.
2. Sundram K, Sambanthamurthi R, Tan YA. Composition and nutritional characteristics of a water soluble antioxidant rich extract from oil palm processing. 2001 PIPOC International Palm Oil Congress. Food Technology & Nutrition Conference, 20-22 August 2001: 250-3.
3. Shahidi F, Naczk M. Food Phenolics: Sources, Chemistry, Effects, Application. Lancaster: Technomic Publishing, Pennsylvania, 1995.
4. Reische DW, Lillard DA, Eitanmiller RR. Antioxidants. In: Akoh CC, Min Db, eds. Food Lipids – Chemistry, Nutrition and Biotechnology. New York: Marcel Dekker, 1998; 423-48.
5. Kono Y, Shibata H, Kodama Y, Sawa Y. The suppression of the *N*-nitrosating reaction by chlorogenic acid. Biochem J 1995; 312: 947-53.
6. Kasai H, Fukada S, Yamaizumi Z, Sugie S, Mori H. Action of chlorogenic acid in vegetables and fruits as a inhibitor of 8-hydroxydeoxyguanosine formation in vitro and in a rat carcinogenesis model. Food Chem Toxicol 2000; 38: 467-71.
7. La Vecchia C, Ferraroni M, Negri E, D’Avanzo B, Decarli A, Levi F, Franceschi S. Coffee consumption and digestive tract cancer. Cancer Res 1989; 49: 1049-51.
8. Tavani A, Pregnolato A, La Vecchia C, Negri E, Talamini R, Franceschi S. Coffee and tea intake and risk of cancers of the colon and rectum: a study of 3530 cases and 7057 controls. Int J Cancer 1997; 73: 193-7.
9. Criqui MH, Ringel BL. Does diet or alcohol explain the French paradox? Lancet 1994; 344: 1719-23.
10. Tijburg LBM, Mattern T, Folts JD, Weigeber UM, Katan MB. Tea flavonoids and cardiovascular disease: a review. Crit Rev Food Sci Nutr 1997; 37: 771-85.
11. Ness AR, Powles JW. Fruit and vegetables and cardio-vascular disease: a review. Int J Epidemiol 1997; 6: 1-13.
12. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc 1996; 96: 1027-39.
13. Yang CS, Wang ZY. Tea and cancer. J Natl Cancer Inst 1993; 85: 1038-49.
14. Christen WG, Glynn RJ, Hennekens CH. Antioxidants and age-related eye disease. Current and future perspectives. Annals Epidemiol 1996; 6: 60-6.
15. Ortega RM, Requejo AM, Andres P, Lopez-Sobaler AM, Quintas ME, Redondo MR, Navia B, Rivas T. Dietary intake and cognitive function in a group of elderly people. Am J Clin Nutr 1997; 66: 803-9.
16. Middleton E Jr, Kandaswami C, Theoharides TC. The effect of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000; 52: 673-751.
17. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993; 342: 1007-11.
18. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic B, Toshima H, Feskens EJM, Hollman PCH, Katan MB. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155: 381-6.
19. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in post-menopausal women. Am J Epidemiol 1999; 149: 943-9.
20. Keli SO, Hertog MGL, Feskens EJM, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch Intern Med 1996; 156: 637-42.
21. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, Hakulinen T, Aromaa A. Flavonoid intake and risk of chronic disease. Am J Clin Nutr 2002; 76: 560-8.
22. Frankel EN, Kanner J, German JB, Parks E, Kinsela JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993; 341: 454-7.
23. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994; 344: 793-5.
24. Landolfi R, Mower RL, Steiner M. Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Biochem Pharmacol 1984; 33: 1525-30.
25. Negre-Salvayre A, Salvayre R. Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. Free Radical Biol Med 1992; 12: 101-6.
26. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witzum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915-24.
27. Rotondo S, de Gaetano G. Protection from cardiovascular disease by wine and its derived products. Epidemiological evidence and biological mechanisms. World Rev Nutr Diet 2000; 87: 90-113.
28. Wollin SD, Jones PJH. Alcohol, red wine and cardiovascular disease. J Nutr 2001; 131: 1401-4.
29. Carr A, Frei B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. Free Radical Biol Med 2000; 28: 1806-14.
30. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996; 334: 1145-9.
31. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Randomised trial of α-tocopherol and β-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet 1997; 349: 1715-20.
32. Stephens NG, Parsons A, Schofield PM, et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 1996; 347: 781-6.
33. Gandini S, Merzenich H, Robertson C, Boyle P. Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. Eur J Cancer 2000; 36: 636-46.
34. Feskanich D, Ziegler RG, Michaud DS, Giovannucci EL, Speizer FE, Willett WC, Colditz GA. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 2000; 92: 1812-3.

35. Smith-Warner SA, Spiegelman D, Yaun SS, Adami HO, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE. Intake of fruits and vegetables and risk of breast cancer: a pooled analysis of cohort studies. JAMA 2001; 14: 769-76.
36. Wu K, Willett WC, Chan JM, Fuchs CS, Colditz GA, Rimm EB, Giovannucci EL. A prospective study on supplemental vitamin E intake and risk of colon cancer in women and men. Cancer Epidemiol Biomarkers Prevention 2002; 11: 1298-304.
37. Hollman PCH, Hertog MGL, Katan MB. Role of dietary flavonoids in protection against cancer and coronary heart disease. Biochem Soc Trans 1996; 24: 785-9.
38. Prieme H, Loft S, Nyyssonen K, Salonen JT, Poulsen HE. No effect of supplementation with vitamin E, ascorbic acid or coenzyme Q on oxidative DNA damage estimated 8-oxo-7,8-dihydro-2’-deoxyguanosine excretion in smokers. Am J Clin Nutr 1997; 65: 503-7.
39. Rehman A, Collis CS, Yang M, Kelly M, Diplock AT, Halliwell B, Rice-Evans C. The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. Biochem Biophys Res Commun 1998; 246: 293-8.
40. Rice-Evans CA, Miller NJ. Antioxidant activities of flavonoids as bioactive components of food. Biochem Soc Trans 1996; 24: 790-5.
41. Yang CS, Landau JM, Huang M-T, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr 2001; 83: 2223-35.
42. Hasler CM. Functional foods: their role in disease prevention and health promotion. Food Tech 1998; 52: 63-70.
43. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies. Int J Oncol 1996; 8: 221-38.
44. Roy M, Siddiqi M, Bhattacharya RK. Cancer chemoprevention: tea polyphenol induced cellular and molecular responses. Asia Pac J Cancer Prev 2001;2: 109-16.
45. Weitberg AB, Corvese D. The effect of epigallocatechin gallate and sarcophytol A on DNA strand breakage induced by tobacco-specific nitrosamines and stimulated human phogocytes. J Exp Clin Cancer Res 1999; 18: 433-7.
46. Paschka AG, Butler R, Young CY. Induction of apoptosis in prostate cancer cell lines by the green tea component, (−)-epigallocatechin-3-gallate. Cancer Lett 1998; 130: 1-7.
47. Otsuka T, Ogo T, Asano Y, Suganuma M, Niho Y. Growth inhibition of leukemic cells by (−)-gallocatechin gallate, the main constituent of green tea. Life Sci 1998; 63: 1397-403.
48. Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F. Pharmacologic doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. Am J Clin Nutr 1993; 57: 650-6.
49. Tajiri Y, Grill VE. Interactions between vitamin E and glucose on B-cell functions in the rat: an in vivo and in vitro study. Pancreas 1999; 18: 274-81.
50. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition 2001; 17: 888-95.
51. Bhuyan KC, Bhuyan DK. Molecular mechanism of cataractogenesis. III. Toxic metabolites of oxygen as initiators of lipid peroxidation and cataract. Curr Eye Res 1984; 3: 67-81.
52. Jacques PF, Chylack LT Jr. Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am J Clin Nutr 1991; 53: 352S-5S.
53. Leske MC, Chylack LT, Wu S-Y. The lens opacities case-control study risk factors for cataract. Arch Ophthalmol 1991; 109: 244-51.
54. Jacques PF, Chylack LT Jr, Hankinson SE, Khu PM, Rogers G, Friend J, Tung W, Wolfe JK, Padhye N, Willett WC, Taylor A. Long-term nutrient intake and early age-related nuclear lens opacities. Arch Ophthalmol 2001; 119:1009-19.
55. Thiagarajan G, Chandani S, Sundari CS, Rao SH, Kulkarni AV, Balasubramanian D. Antioxidant properties of green and black tea, and their potential ability to retard the progression of eye lens cataract. Exp Eye Res 2001; 73: 393-401.
56. Goldberg J, Flowerdew G, Smith E, Brody JA, Tso MOM. Factors associated with age-related macular degeneration: an analysis of data from the First National Health and Nutrition Examination Survey. Am J Epidemiol 1988; 128: 700-10.
57. Eye Disease Case-Control Study Group. Antioxidant status and neovascular age-related macular degeneration. Arch Ophthalmol 1993; 111: 104-9.
58. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 2001; 119: 1417-36.
59. Snellen EL, Verbeek AL, Van Den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. Acta Ophthalmol Scand 2002; 80: 368-71.
60. Sackett CS, Schenning S. The age-related eye disease study: the results of the clinical trial. Insight 2002; 27: 5-7.
61. Hyman L, Neborsky R. Risk factors for age-related macular degeneration:an update. Curr Opin Ophthalmol 2002; 13: 171-5.
62. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC. Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA. 2002; 287:3223-9.
63. Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol 2003; 60: 203-8.
64. Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, Lunec J. Vitamin C exhibits pro-oxidant properties [letter] Nature 1998; 392-559.
65. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20: 933-56.
66. Ushakova T, Melkonyan H, Nikonova L, Mudrik N, Grogvadze V, Zhukova A, Gaziev AI, Bradbury R. The effect of dietary supplements on gene expression in mice tissues. Free Radical Biol Med 1996; 20: 279-84.