Review Article

Anticancer and health protective properties of citrus fruit components

Jansen Silalahi MAppSc, PhD

Department of Pharmacy, University of Sumatra Utara, Medan, Indonesia

Accumulated evidence from experimental and epidemiological studies indicates that there is a low risk of degenerative diseases, cardiovascular disease, hypertension, cataract, stroke and, in particular, cancers in people with a high intake of fruit and vegetables. This protective effect is assumed to be associated mainly with the antioxidant activities of either individual or interacting bioactive components present in the fruits and vegetables, and with other biochemical and physical characteristics of the identified and unknown bioactive components. The implicated bioactive components present in citrus fruits include vitamin C, β-carotene, flavonoids, limonoids, folic acid, and dietary fibre. A high intake of citrus fruits may reduce the risk of degenerative diseases.

Key words: anticancer activity, antioxidant activity, bioactive components, citrus fruit, limonoids.

Introduction

Diet is believed to play an important role in four major diseases of advanced and transitional economies: cardiovascular disease, cancer, hypertension, and obesity. The degree to which diet is important in the prevention of these diseases is not known. However, a commonly accepted estimate among experts is that at least one third of cancer cases can be attributed to diet and perhaps one half of the cases of heart and artery diseases and hypertension are related to diet.[1,2] Foods that contain components active in disease prevention and that aid specific bodily functions, in addition to being nutritious, are defined as functional foods.[3]

There is strong, consistent evidence that a high intake of fruit and vegetables protects against various cancers. This association is most marked for cancers of the respiratory and digestive tracts.[4,5] Increasing fruit and vegetable consumption by one to two servings daily may reduce cardiovascular risk by 30%. These protective effects of high fruit and vegetable consumption are attributed to the active micronutrients (e.g. vitamins and minerals) and non-nutritive components that are known as phytochemicals. Phytochemicals can be defined as substances found in edible fruits and vegetables that may be ingested daily by humans (in gram quantities), and that exhibit a potential for modulating human metabolism in a manner favourable for the prevention of cancer and other degenerative diseases.[3,6,7]

Any reduction in the risk of disease that is associated with a high antioxidant nutrient intake may result from consuming a mix of foods rich in antioxidants rather than consuming antioxidants as single nutrients.[8,9] Unfortunately, trials of antioxidant vitamin supplementation have been largely discouraging. Several β-carotene supplementation trials have shown either no effect or adverse effects on cardiovascular disease, cancer and total mortality, and a trial of vitamin E in the secondary prevention of coronary heart disease, which reduced non-fatal events by 70%, had no, or possibly, an adverse effect on mortality. Nevertheless, high fruit and vegetable intakes have been consistently associated with protective benefits for several conditions. These include macular degeneration, visual loss, cataracts, respiratory disease and cancers such as breast, stomach and colorectal. The discrepancy between foods and isolated supplementation may be that many other nutrients in food, or their interactions, are implicated in the clinical effects.[7,10]

A great number of epidemiological studies have shown that citrus fruit consumption is protective in a variety of human cancers. It is presumed that most, if not all, of this protective effect is due to vitamin C. However, several studies by Miller *et al.*[11] have suggested that the frequency of citrus fruit consumption is more closely related to risk reduction than vitamin C intake. This suggests that citrus fruits contain not one but multiple cancer chemopreventive agents. Citrus fruits, including oranges, lemons, limes and grapefruits, are a principal source of such important nutrients. They contain vitamin C, folate and dietary fibre, and other bioactive components such as carotenoids and flavonoids, which are suggested to be responsible for the prevention of

Correspondence address: Dr Jansen Silalahi, Jl. Helvetia Raya no 222, Perumnas Helvetia, Medan 20124, Indonesia.
Tel: +66 061 8454221 Fax: +66 061 8455661 and +66 061 8211113
Email: jansensilalahi@eudoramail.com
Accepted 16 July 2001

degenerative disease. Citrus fruits are particularly high in a class of phytochemicals known as the limonoids.[3] Therefore, citrus fruits could be categorised as functional foods containing components shown to have health promoting and anticancer activities. These components include the bitter substances limonoids, ascorbic acid (vitamin C), carotenoids (especially β-carotene), folate, flavonoids and dietary fibres, and have been shown to prevent a variety of cancers and cardiovascular diseases. This paper reviews the protective effects of these bioactive components.

Limonoids

Citrus limonoids are responsible for the bitter taste in citrus fruits. The most prevalent limonoids are limonin and nomilin. They are present in the rutaceous plants that include lemon, lime, orange and grapefruits. An important characteristic of this class of compound is a substituted furan moiety. It has been determined by animal studies that citrus limonoids and derivatives have certain biological activities that may be used as chemopreventive agents for cancer.[11,12]

Glutathione S-transferase (GST) is a major detoxifying enzyme system that catalyses the conjugation of glutathione with electrophiles that induce activated carcinogens. The glutathione conjugates are usually less reactive and more water soluble, and hence, facilitate excretion. An increase in GST activity caused by a substance is therefore an elevation in the mechanism that protects against the noxious effects of xenobiotics, including carcinogens. Many chemicals that are GST enhancers have been found to inhibit chemically induced carcinogenesis.[12,13] The structures of the naturally occurring furanoids that have been found to induce GST activity range from the simple 2-alkyl substituted compound 2-n-heptyl furan and the sulphur analogue 2-n-butyl thiophen, formed during the roasting of meat, to more complex molecules such as kahweol and cafestol, isolated from green coffee beans, and salannin, identified in the seed of the mythical neem tree of India. The presence of the furan moiety appears to be essential for enzyme induction. When the furan ring in cafestol is saturated by hydrogenation, its activity as a GST inducer is lost. In the case of limonin and nomilin, the triterpene structure appears to play a part in determining the relative GST-inducing activity of these compounds. The structure–activity relationships of limonin and nomilin have been studied and discussed. The chemical structures of limonin, nomilin and their glucoside derivatives are shown in Fig. 1.[11,12]

Results from some animal studies for forestomach tumours have indicated that the inhibitory effects of limonin and nomilin follow the same trend as their ability to induce GST activity. Nomilin, being a much better inducer of GST, was more active as an inhibitor of carcinogenesis than the less effective limonin. Similar to the inhibition of forestomach tumours, the protection of lung carcinogenesis by limonoids was positively correlated with the induction of GST activity. Reduction of skin tumours by limonoids has been also shown by animal studies.[12] The results of limonoid studies in animals have been encouraging, and limonoids might someday be used as a cancer chemopreventive agent for humans. However, there are several properties associated with this chemical that may jeopardise its use as a cancer chemopreventive agent for humans. The primary problem is that limonin is intensely bitter. A second problem is that this citrus chemical is only soluble in organic solvents. A third problem is that the concentration of limonin and nomilin in citrus juices is fairly low and, therefore, the overall consumption of these two specific limonoids is not high. These three problems would limit the type of products that limonin might be incorporated into when used as a food additive or supplement.[11]

Limonoid glucosides, however, have also been isolated from citrus. In these molecules, glucose is attached at C-17 by a α-glycosidic linkage (Fig. 1). This modification changes the properties of the molecule, making it tasteless and soluble in water.[11,12] It has been discovered that limonoids exist as their glycosidic form at very high concentrations in juices. In addition, the limonoid glucosides are present at higher concentrations in commercial orange,

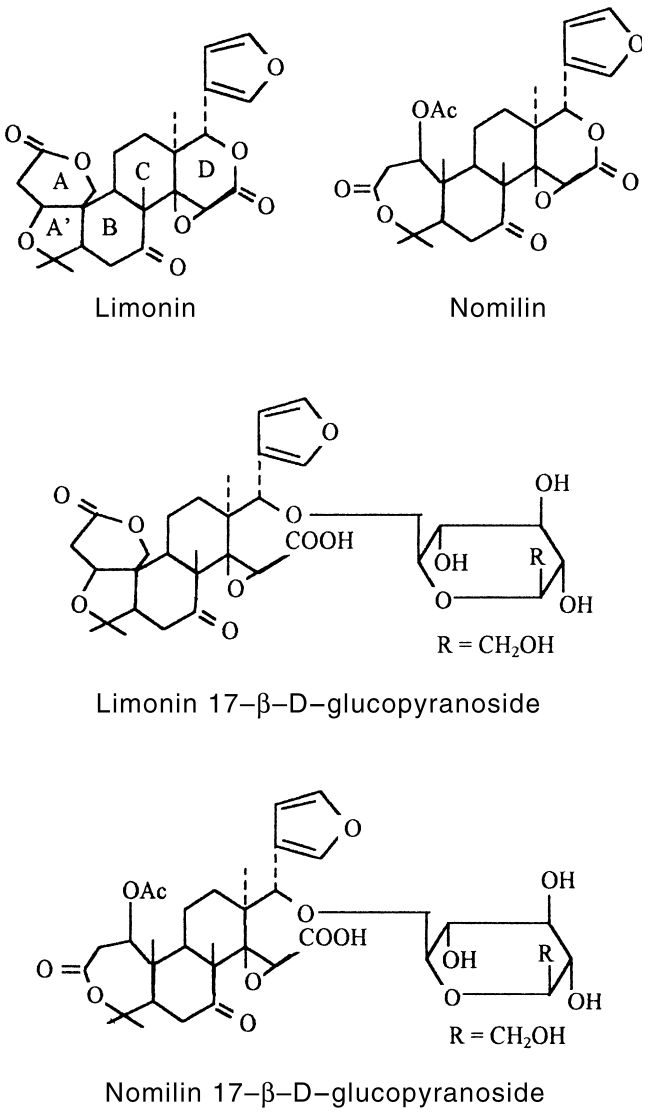

Figure 1. Chemical structures of limonin, nomilin and their glucoside derivatives.

grapefruit and lemon juices, containing 320, 190 and 82 p.p.m., respectively, compared to less than 1–2 p.p.m. of limonin and nomilin in most citrus juices.[11] Animal studies have indicated that limonin and limonoid glucoside limonin 17-α-D-glucopyranoside (LG) are active as cancer chemopreventive agents. Moreover, many other structure-related limonoids such as ichigan, isoobacunoic, obacunone, and others, act as good GST inducers.[11,12]

Flavonoids

Flavonoid structure is based on a 2-phenyl-benzo[a]pyrane or flavane nucleus. This nucleus is defined by having a system of two benzene rings (A and B), which are connected by an oxygen-containing pyrane ring (C). The flavonoids (including compounds such as flavones, isoflavones, flavonols and flavanes) are a group of low molecular weight polyphenolic compounds that are widely distributed in plants.[14–16]

Oxidants may contribute to carcinogenesis both by causing mutations and by stimulating cell division. The oxidative hypothesis of carcinogenesis claims that many carcinogens can generate free radicals that damage cells, setting these cells to malignant changes. DNA contains reactive groups in its bases that are highly susceptible to free radical attack and oxidative DNA damage can lead to deleterious mutations. Most oxidative lesions are efficiently repaired by specific DNA glycosylases, but unrepaired lesions accumulate with age. When the cells divide, the lesions become fixated and mutations and cancer may result. Oxidation is also believed to contribute to cardiovascular disease in two ways: by oxidative changes in blood lipoproteins in the long term development of atherosclerosis and by oxidative processes contributing to immediate tissue damage that occurs during heart attack or stroke.[17–19]

Flavonoids have been shown to be able to act as antioxidants by scavenging free radicals, an activity related to their phenol rings containing hydroxyl groups. Flavonoids have the ability to act as reducing agents, making them capable of donating hydrogens to free radicals and causing their removal. Flavonoids can also act as singlet oxygen quenchers and as chelators of transition metals such as copper and iron, which are known pro-oxidants in foods. The ability of monomeric phenolics to act as antioxidants is dependent on extended conjugation, number and rearrangement of phenolics substituents, and molecular weight. For example, the flavonoids with the most hydroxyl groups are most easily oxidised and for simple flavonoid oligomers, the degree of polymerisation is correlated with the ability to scavenge free radicals. Quercetin is a flavonoid that has a strong hydrogen-donating activity due to its levels of hydroxylation (six OH groups) and the location of the hydroxyl groups in the primary activity sites. It also contains structural requirements allowing it to serve as a metal chelator.[16,20,21]

Certain flavonoids possess anti-inflammatory, antiallergenic, anticarcinogenic and antiproliferative activities, as well as antiviral and cell differentiating properties. These compounds may affect several enzyme systems critically involved in cell activation phenomena. Depending on structure, certain flavonoids also possess antioxidant, vitamin C-sparing and radical-scavenging activities.[22] It has been established that cell types that are involved in immunity and inflammation (e.g. lymphocytes, macrophages, mast cells, basophils, neutrophils and platelets) can be affected by particular flavonoids.[22,23]

There is also solid evidence to indicate that certain flavonoids have anticarcinogenic activity due to their ability to induce the hepatic enzymes affecting the metabolism of carcinogens (e.g. benzo[a]pyrene), and their effect on the metabolic activation of benzo[a]pyrene. Interestingly, the metabolites that are produced following enzyme induction tend to metabolise benzo[a]pyrene towards the formation of less carcinogenic metabolites.[22] Citrus flavonoids include nobiletin and tangeretin, and certain other polyphenols possess anticancer activity both *in vivo* and *in vitro*, acting through a variety of mechanisms.[22,24]

β-Carotene

β-Carotene is one of the better-known carotenes because of its high vitamin A activity and its wide distribution in nature. Theoretically, one β-carotene molecule can be cleaved symmetrically by an enzyme in the intestinal mucosa to produce two molecules of retinaldehyde. In reality, the actual conversion is much less efficient because of other competing reactions that cleave β-carotene non-symmetrically.[10,25] More than 40 other carotenoids (such as α-carotene, 5,6- and 5,8-epoxides of β-carotene, cryptoxanthin and β-apocarotenals) also exhibit some vitamin A activity because at least a portion of each molecule can be cleaved into retinaldehyde and converted into retinol. Sixteen of these carotenoids with pro-vitamin A activity have been found in citrus. The pro-vitamin A content of most citrus cultivars is derived primarily from β-cryptoxanthin, β-carotene and α-carotene, and each has a different pro-vitamin A activity. One very interesting feature of β-carotene is the feedback mechanism for vitamin A levels in the body. During the absorption process, vitamin A levels are adequate. No additional β-carotene is converted into vitamin A and this unchanged β-carotene is absorbed into the body. Thus deleterious accumulation to unhealthy, excessive levels of vitamin A from β-carotene is inhibited.[19,25]

The protective effects of β-carotene, at least from food, although not necessarily as an isolated molecule, are thought to occur through one or more of several modes. These modes include singlet oxygen quenching (photo protection), antioxidant protection and enhancement of the immune response. Singlet oxygen is a highly reactive form of oxygen that participates in reactions that can alter or destroy important cellular components such as membranes, enzymes and nucleic acids (e.g. DNA). β-Carotene may function as a redox reagent, an immunological regulator or by increasing cell-to-cell communications.[10] There is substantial evidence that β-carotene is capable of influencing all of these mechanisms, at least *in vitro*. β-Carotene is an uncommon type of biological redox reagent in that it best reduces

oxidation products at low partial pressure of oxygen. This low pressure is relevant to many physiological tissues. β-Carotene is a physiological modulator with the capacity to act as a singlet oxygen quencher. It can also function as an antioxidant or a reductant, depending on reaction conditions. *In vivo*, it appears to function most often as an antioxidant and singlet oxygen quencher. As such, it decreases lipoprotein and DNA oxidation, which are detrimental to human health. Lipoprotein oxidation is thought to initiate atherogenesis. DNA and lipoprotein oxidation may be related to cancer and other degenerative diseases.[10,25] β-Carotene has been shown to be effective in protecting lipid membranes from free radical damage, particularly under low oxygen partial pressure. This finding is of particular significance as enzyme or chain-breaking antioxidants are most effective at high oxygen pressure, and lower oxygen concentrations are typically found in capillary beds of tissues far removed from direct exposure to oxygen. Thus β-carotene might complement other protective antioxidants such as a vitamin C and vitamin E, which are most effective at normal oxygen pressures.[19,25]

Carotenoids have been shown to enhance both specific and non-specific immune functions in addition to enhancing tumour immunity. It has been postulated that carotenoids may enhance activity by (i) quenching excessive reactive species formed by various immunoactive cells, (ii) quenching immunosuppressive peroxides and maintaining membrane fluidity, (iii) helping to maintain membrane receptors essential for immune functions, and (iv) acting in the release of immunomodulatory lipid molecules such as prostaglandins and leukotrienes.[10,25] Considerable caution is required in extrapolating from these phenomena of isolated carotenoids, and other anti-oxidants, to reach outcomes. Indeed, for β-carotene alone there is now considerable concern about its pro-tumorigenic properties.

Ascorbic acid

Vitamin C (ascorbic acid), which must be obtained from the diet, is an essential micronutrient required for normal metabolic functioning of the body. Therefore, a deficiency of this vitamin results in the symptoms of scurvy, and death. This potentially fatal disease can be prevented with as little as 10 mg of vitamin C per day, an amount easily obtained through consumption of fresh fruits and vegetables. However, the current recommended dietary allowance (RDA) for vitamin C is set at 60 mg per day to provide an adequate margin of safety, as 60 mg/day would prevent the development of scurvy for about one month in a diet lacking vitamin C.[13,18] Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine and neurotransmitters. A deficiency in vitamin C results in a weakening of collagenous structures, causing tooth loss, joint pains, bone and connective tissue disorder and poor wound healing, all of which are characteristic of scurvy.

Vitamin C is an important water-soluble antioxidant in biological fluids. It readily scavenges reactive oxygen and nitrogen species such as superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitroxide radicals and hydrochlorous acid, thereby effectively protecting other biomolecules from oxidative damage. Vitamin C can also act as a coantioxidant by regenerating α-tocopherol (vitamin E) from the α-tocopheroxyl radical produced via scavenging of lipid-soluble radicals. This is a potentially important function because *in vitro* experiments have shown that α-tocopherol can act as a pro-oxidant in the absence of coantioxidants such as vitamin C.[8,13] Vitamin C may protect against cancer through several mechanisms, in addition to inhibiting DNA oxidation. One potential mechanism is chemoprotection against mutagenic compounds such as nitrosamines, which can be formed by reaction of nitrite or nitrate (common in food and cigarette smoke) with amines and amides.[26,27] Vitamin C prevents the reaction of nitrites with amines and amides that form potent carcinogenic nitrosamines within the digestive tract, and prevents oxidation of specific chemicals to their active carcinogenic forms.[18,19,25] Vitamin C reduces the *in vivo* nitrosation by scavenging nitrite and hence, preventing its reaction with amines to form nitrosamines. Concentrations of faecapentaenes, faecal mutagens (toxic metabolites formed by colonic fermentation) that have been implicated in colon cancer, are also reduced by vitamin C.[18,28,29] It has been shown that vitamin C protects against *in vivo* oxidation of lipids and DNA in humans, particularly in persons exposed to enhanced oxidative stress, such as smokers. It therefore reduces the incidence of and mortality from two of the most prevalent human diseases: cardiovascular disease and cancer. This role of vitamin C in lowering disease incidence is most likely derived from its antioxidant activity, although other mechanisms may also contribute.[18,19] Therefore, Carr and Frei[18] have suggested that the RDA for vitamin C should be doubled from 60 to 120 mg/day.

Folic Acid

Folic acid compounds and their derivatives are water-soluble vitamins that are collectively referred to as folate or folacin. Folate is a generic term referring to a family of related compounds. All of these compounds represent modifications of the simplest form of the vitamin, folic acid (pteroylglutamic acid, PteGlu). The name 'folic acid' is derived from the Latin word for 'green leaves', *folium*. Folates play an important metabolic role as coenzymes in amino acid metabolism and nucleic acid synthesis. Tetrahydrofolate is an active form of this vitamin.[13,30] Folate is critically important for growth, and for this reason it is required in increased amounts during pregnancy and during lactation to transfer the mother's vitamins to the milk. Citrus fruit and their juices and green vegetables such as broccoli, spinach and bell peppers are good sources of folate.[13,30] The principal forms of folate in citrus are the reduced 5-methyl tetrahydrofolate (monoglutamate) form and polyglutamate derivatives. There is strong scientific evidence supporting a link between folic acid intake and the prevention of neural tube defects in infants. Elevated

blood homocysteine levels are found in patients with coronary heart disease, cerebral vascular diseases, peripheral vascular disease and venous thromboembolism. In individuals with elevated homocysteine levels, an inverse relationship has been observed between homocysteine and folate levels in serum.[25]

Dietary Fibre

Dietary fibre is commonly defined as 'plant polysaccharides and lignin, which are resistant to hydrolysis by the digestive enzymes of man'. They are generally classified into two groups: soluble and insoluble dietary fibres. Soluble fibres are highly fermentable and are associated with carbohydrate and lipid metabolism, while insoluble fibres contribute to faecal bulk and reduce transit time. Pectin, cellulose and hemicellulose, with only trace amounts of lignin, are the predominant components of dietary fibre from most fruits. Citrus fruits are a particularly rich source of the soluble dietary fibre pectin, which occurs both in edible portions of fruit and the inedible residues such as peel, rag and core. Consumption of citrus fruit can contribute significant quantities of pectin in a diet. Dietary incorporation of pectin appears to affect several metabolic and digestive processes; those of principal interest are the effects on glucose absorption and cholesterol levels.[3,21]

The mechanisms by which fibre can influence colon cancer include physical dilution of colon content, absorption of bile acids and carcinogens, decreased transit time, altered bile acid metabolism and the effects of fermentation, namely, the production of short-chain fatty acids, lowering of pH and stimulation of bacterial growth. Carbohydrate that escapes digestion is a potential substrate for colonic fermentation. The products of fermentation include gases (CO_2, CH_4, H_2), short-chain fatty acids (SCFA; acetate, propionate and butyrate), lactic acid and branched-chain fatty acids (isobutyric, isovaleric).[29,31] Tomomatsu[28] and Bird[32] have reported that two types of active bacteria in the colon include indigenous beneficial bifidobacteria and detrimental or putrefactive microflora. As with undigested carbohydrate, protein that reaches the colon undigested is metabolised by the colonic microflora to end products that include isoderivatives of SCFA (isobutyric and isovaleric), phenol, cresol, indoles, amines, ammonia and phenylated SCFA, many of which have adverse effects. Ammonia may promote tumorigenesis by stimulating cell proliferation and favouring growth of malignant cells in preference to normal cells. Volatile phenols (p-cresol and phenol), produced from bacterial metabolism of the aromatic amino acids phenylalanine and tyrosine, are promoters of skin cancers and have been implicated in development of both bladder and bowel cancers.[29] Bacteria known to participate in the formation of these metabolites are *Escherichia coli*, *E. clostridia*, *Streptococcus faecalis*, and others.[28] Conversely, fermentable carbohydrates may protect against the build up of undesirable by-products of undigested protein metabolism. A number of studies have shown that fermentable non-starch polysaccharides (NSP) can lower levels of faecal ammonia. A high fibre diet may also lower urinary phenol, cresol and N-nitroso compounds. The mechanism for this action may be through the preferred use of carbohydrates as an energy substrate by rapidly replicating colonic bacteria. Proliferating bacteria use undigested protein for their nitrogen requirements and thus acts as 'nitrogen sinks'.[29] Dietary fibre may increase faecal bulk and reduce transit time. The increase in faecal mass is due to an increase in both bacteria and water content. An increase in stool mass is important in relief of constipation and in the prevention of diverticulosis and anorectal disorders such as haemorrhoids. Bulkier stool may also enable a dilution of potential toxic compounds, with important implications for reducing the risk of bowel cancer. Insoluble dietary fibres are thought to exert their protective effect against colon cancer by absorbing carcinogens in the gastrointestinal tract. These absorbed carcinogenic agents can then be carried out of the body, minimising their potential for carcinogenesis.[33,34]

Soluble dietary fibre such as pectin is completely fermented by resident bacterial flora of the lower bowel to produce short-chain fatty acids, primary acetate, propionate and butyrate, which can be utilised by bacteria or absorbed by the intestinal mucose. Short-chain fatty acids exert a number of effects on large bowel metabolism, which seem to be critical to the maintenance of normal colonic function. One effect is a lowering of the luminal pH. This is regarded to be of benefit since a more acidic colonic milieu leads to inhibition of both the growth of exogenous pathogenic organisms and the excessive growth of indigenous detrimental microflora. In addition, toxic basic compounds become ionised under low pH conditions, and so, are not absorbed, whereas secondary bile acids or free fatty acids may become un-ionised, insoluble and less absorbed organic acids.[28,35] Moderate pectin fortification usually results in little faecal bulking. Although pectin supplementation in excess of 30% per day increased stool bulk 33%, this effect was ascribed to an increase in bacterial mass rather than an increase in fibres. In the absence of significant effects on intestinal transit time or stool volume, it is apparent that pectin does not influence fat or cholesterol absorption via dilution or reduced absorption time.[3,29] Yet several studies have shown that dietary supplementation with pectin increases excretion of faecal fat, sterol and bile acids. Increased excretion of bile acids interrupts the enterohepatic circulation, causing an increase in liver functions leading to bile acid synthesis from cholesterol. In this manner, loss of the bile acids serves to reduce the pool of circulating cholesterol.[3,35]

Citrus fruits constitute one of the richest sources of high quality pectin. Citrus fruit peel, particularly the albedo portion, is an especially rich source and serves as a major raw material for pectin manufacture. Consumption of oranges as peeled fruit greatly increases pectin intake, since juice sac walls and capillary membranes are included. By far the most effective sources of citrus pectin and dietary fibre are the peel (albedo), membranes and juice sacs. Of these fractions, peel and membranes are unsuited for consumption due to excessive levels of levels of bitter and astringent compounds such as naringin and limonin.[3]

Conclusions

Bioactive components present in citrus fruits that are implicated in degenerative disease prevention include vitamin C, β-carotene, flavonoids, limonoids, folic acid and dietary fibres. This protective effect results from several biochemical properties of either individual or a combination of bioactive components including antioxidant activity, induction of glutathione-S-transferase, amino acid metabolism, colonic fermentation and facilitating the excretion of toxic compounds. Vitamin C, flavonoids and β-carotene are potential antioxidants protecting against oxidation of biomolecules such as DNA, protein and lipid membranes, thereby reducing the risk of cancers, cataract and cardiovascular diseases. Limonoids may protect against a variety of cancers by inducing GST activity to neutralise carcinogenic free radicals. Folic acid plays an important role in amino acid metabolism and hence, it is a critical factor for growth. Dietary fibre can influence colon cancer by physical dilution of colon content, absorption of bile acids and carcinogens, decreased transit time, altered bile acid metabolism, and the effects of fermentation, namely, the production of SCFA, lowering of pH and stimulation of beneficial bacterial growth and inhibition of detrimental microflora. Dietary supplementation of these bioactive components, however, is not as effective as when they are consumed in the citrus fruits. This suggests that the substantial protective effect might also be the result of an interaction between the bioactive compounds or the other unknown components that still remain for further study.

References

1. Goldberg I. Introduction. In: Goldberg I, ed. Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. New York: Chapman and Hall, 1994; 1–16.
2. Milner JA. Reducing the Risk of Cancer. In: Goldberg I, ed. Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. New York: Chapman and Hall, 1994; 39–62.
3. Baker RA. Potential Dietary Benefits of Citrus Pectin and Fibre. Food Technol 1994; 11: 133–139.
4. Negri E, Vecchia L, Franceschi S, D'Avanzo B, Parazzini F. Vegetable and fruit consumption and cancer risk. Int J Cancer 1991; 48: 350–354.
5. Austoker J. Cancer Prevention in Primary Care: Diet and Cancer. BMJ 1994; 308: 1610–1614.
6. Watzl B. Health promoting effects of phytochemicals. Proceedings of IUFoST′96 Regional Symposium on Non-Nutritive Health Factors for Future Foods, October 10–11, 1996. Seoul: Korean Society of Food Science and Technology, 1996; 203–222.
7. Khaw KT. Healthy aging. BMJ 1997; 315: 1090–1096.
8. Gillman MW. Enjoy your fruits and vegetables [editorial]. BMJ 1996; 313: 765–766.
9. Zino S, Skeaff M, Williams S, Mann J. Randomized controlled trial of effect of fruit and vegetable consumption on plasma concentration of lipids and antioxidants. BMJ 1997; 314: 1787–1796.
10. Burri BJ. Beta-carotene and Human Health: a Review of current research. Nutr Res 1997; 17: 547–580.
11. Miller EG, Sanders APG, Couvillon AM, Binnie WH, Hasegawa S, Lam LKT. Citrus Limonoids as Inhibitors of Oral Carcinogenesis. Food Technol 1994; 110–114.
12. Lam LKT, Zang J, Hasegawa S. Citrus Limonoid Reduction of Chemically Induced Tumorigenesis. Food Technol 1994; 104–108.
13. Brody T. Nutritional Biochemistry. Sydney: Academic Press, 1994.
14. Chen ZU, Zhu QY, Wong YF, Zhang Z, Chung HY. Stabilizing effect of Ascorbic Acid on Green Tea Cathechins. J Agric Food Chem 1998; 46: 2513–2516.
15. Prior RL, Cao G, Martin A, Sofic E, McEwen J, Obrien C, Lichener N, Ehlenfelt M, Kalt W, Krewer G, Mainland CM. Antioxidant Capacity as Influenced by Total Phenolic and Anthocyanin Content, Maturity and Variety of Vaccinium Species. J Agric Food Chem 1998; 46: 2687–2693.
16. Cuppett S. Plant production of biochemical compounds. INFORM 1998; 9: 588–595.
17. Aruoma OI. Free radicals, Oxidative stress, and Antioxidants in Human Health and Disease. J Am Oil Chem Soc 1998; 75: 199–212.
18. Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999; 69: 1086–1107.
19. Silalahi J. Free Radicals and Antioxidant Vitamins in Degenerative Diseases. J Indon Med Assoc 2001; 51: 16–21.
20. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. J Agric Food Chem 1998; 46: 1887–1892.
21. Silalahi J. Hypocholesterolemic Factors in Foods. A Review. Indon Food Nutr Prog 2000; 7: 26–35.
22. Middleton E, Kandaswami C. Potential Health-Promoting Properties of Citrus Flavonoids. Food Technol 1994; 11: 115–119.
23. Bocco A, Cuvelier M, Richard H, Berset C. Antioxidant Activity and Phenolic Composition of Citrus Peel and Seed Extracts. J Agric Food Chem 1998; 46: 2113–2129.
24. Cheng SJ. Study on Antimutagenicity and Anticarcinogenicity of Green Tea Epicatechins – A Natural Free Radical Scavenger. In: Parker L, Traber MG, Xin W, eds. Proceedings of the International Symposium on Natural Antioxidants: Molecular Mechanisms and Health Effects. Champaign: AOCS Press, 1996; 392–396.
25. Rouseff RL, Nagy S. Health and Nutrition Benefits of Citrus Fruit Components. Food Technol 1994; 11: 125–132.
26. Scanlan RA. Formation and Occurrence of Nitrosamines in Foods. Cancer Res 1983; 43: 2435S–2440S.
27. Fiddler W, Pensabene JW, Gates RA, Adam R. Nitrosamine Formation in Processed Hams as Related to Reformulated Elastic Rubber Netting. J Food Sci 1998; 63: 276–278.
28. Tomomatsu H. Health Effects of Oligosaccharides. Food Technol 1994; 10: 61–64.
29. Muir JG. Location of colonic fermentation events: Importance of combining resistant starch with dietary fibre. Asia Pacific J Clin Nutr 1999; 8: S14–S21.
30. Padh H. Vitamins for Optimal Health. In: Goldberg I, ed. Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. New York: Chapman and Hall, 1994; 279–280.
31. Kritchevsky D. Dietary fibre in health and disease: An overview. Asia Pacific J Clin Nutr 1999; 8: S1–S2.
32. Bird AR. Prebiotics: a role for dietary fibre and resistant starch. Asia Pacific J Clin Nutr 1999; 8: S32–S36.
33. Marshall JR, Martinez ME, Albert DS. Wheat bran as a means of cancer chemoprevention. Asia Pacific J Clin Nutr 1999; 8: S47–S53.
34. Ferguson LR, Harris PJ. Wheat bran and cancer: The role of dietary fibre. Asia Pacific J Clin Nutr 1999; 8: S41–S46.
35. Topping DL. Physiological effects of dietary carbohydrates in the large bowel: Is there a need to recognize dietary fibre equivalents? Asia Pacific J Clin Nutr 1999; 8: S22–S26.