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Review

Antioxidants in fruits and vegetables – the millennium's health

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Summary Some of the most exciting research in the last decade has been the discovery of a group of nutrients, which have protective effects against cell oxidation. These naturally occurring compounds impart bright colour to fruits and vegetables and act as antioxidants in the body by scavenging harmful free radicals, which are implicated in most degenerative diseases. Epidemiological studies have established a positive correlation between the intake of fruits and vegetables and prevention of diseases like atherosclerosis, cancer, diabetes, arthritis and also ageing. So pronounced has been their effect on ageing that they have been called 'fountains of youth'. Fruits and vegetables have thus had conferred on them the status of 'functional foods', capable of promoting good health and preventing or alleviating diseases. Phenolic flavonoids, lycopene, carotenoids and glucosinolates are among the most thoroughly studied antioxidants. The present review highlights the potential of fruits and vegetables rich in antioxidants, their health benefits and the effect of processing on the bioavailability of these nutrients. The paper also reviews some of the important methods used to determine the antioxidant activity.

Keywords Antioxidant activity, carotenoids, flavonoids, free radicals, phenols, processing methods, vitamins.

Introduction

In recent years human health has assumed an unprecedentedly important status. Increased interests in nutrition, fitness and beauty have exaggerated concerns over diet and human health. A new diet-health paradigm is evolving which places more emphasis on the positive aspects of diet. Foods have now assumed the status of 'functional' foods, which should be capable of providing additional physiological benefit, such as preventing or delaying onset of chronic diseases, as well as meeting basic nutritional requirements. Nutritional studies are now concentrating on examining foods for their protective and disease preventing potential (Nicoli *et al.*, 1999) instead of negative attributes such as micro-organism count, adulterants, fatty acids and inorganic pollutant concentration. Recently phytochemicals in fruits and vegetables have attracted a great deal of attention mainly concentrated on their role in preventing diseases caused as a result of oxidative stress. Oxidative stress, which releases free oxygen radicals in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and many other auto-immune diseases besides ageing. These phytochemicals act as antioxidants, scavenge free radicals and act as saviours of the cell. Epidemiological studies have consistently shown that there is a clear significant positive associations between intake of fruits and vegetables and reduced rate of heart diseases mortality, common cancers and other degenerative diseases as well as ageing (Steinmetz & Potter, 1996; Garcia-Closas *et al.*, 1999; Joseph *et al.*, 1999; Dillard &

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German, 2000; Prior & Cao, 2000; Wargovich, 2000). The strongest evidence is related to reduced risk of cancers of the mouth and pharynx, oesophagus, lung, stomach and colon. The available data also gives strong support to a protective role for fruits and vegetables in protection against pancreas, bladder and breast cancer (American Institute of Cancer Research., 1997). This is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotic compounds.

Free radicals and antioxidants

Free radicals

It is a paradox that oxygen, which is considered as essential for life, is also reported to be toxic. Its toxicity is because of the process that unleashes the free radicals. The term *free radical* seems to appear a lot lately in everything, from vitamin brochures to cosmetic advertisements. Free radicals are unstable highly reactive and energized molecules having unpaired electrons. Examples of oxygen derived free radicals include super oxide (O_2^-), hydroxyl (OH^-), hydroperoxyl (HOO^-), peroxyl (ROO^-) and alkoxyl (RO^-) radicals. Other common reactive oxygen species (ROS) produced in the body include nitric oxide (NO^-) and the peroxynitrite anion ($ONOO^-$) (Prior & Cao, 2000). Free radicals react quickly with other compounds, trying to capture the electrons needed to gain stability. Generally free radicals attack the nearest stable molecules, 'stealing' its electrons. When the molecule that has been attacked and loses its electron it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, initiating lipid peroxidation which results in destabilization and disintegration of the cell membranes or oxidation of other cellular components like proteins and DNA, finally resulting in the disruption of cells (Halliwell *et al*., 1995). Oxidation caused by free radicals sets reduced capabilities to combat ageing and serious illness, including cancer, kidney damage, atheroscelrosis and heart diseases (Ames, 1983).

Some free radicals arise normally during metabolism. Sometimes the body's immune system's cells purposefully create them to neutralize viruses and bacteria. However environmental factors such as pollution, radiation, cigarette smoke and herbicides can also generate free radicals. Thus free radicals on one hand can produce beneficial effects but can also induce harmful oxidation and cause serious cellular damages, if generated in excess.

Antioxidants

To deal with the free radicals or so called ROS, the body is equipped with an effective defence system which includes various enzymes and high and low molecular weight antioxidants. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-stealing reaction. The antioxidants do not themselves become free radicals by donating electrons because they are stable in either form. These act as scavengers and play the housekeeper's role by mopping up free radicals before they get a chance to create havoc in a body. Thus they may well be defined as the substances that are capable of quenching or stabilizing free radicals.

Antioxidants have also been suggested to have a well defined role as *preservatives*. These have been defined by the US Food and Drug Administration (FDA) as substances used to preserve food by retarding deterioration, rancidity or discoloration caused by oxidation (Dziezak, 1986). Lipid peroxidation is an important deteriorative reaction of foods during processing and storage. Toxic substances formed by lipid peroxidation may lead to adverse effects such as carcinogenesis, cell DNA mutagenesis and ageing. Antioxidants therefore, according to their mode of action, have also been classified as the compounds that terminate the free radical chain in lipid oxidation by donating electrons or hydrogen to fat containing a free radical and to the formation of a complex between the fat chain and a free radical. Antioxidants stop the free radical chain of oxidative reactions by contributing hydrogen from the phenolic hydroxyl groups, themselves forming stable free radicals that do not initiate or propagate further oxidation of lipids (*Free Radical Terminators*). Some of the important synthetic antioxidants of this class are butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), *tert*- butylhydroquinone (TBHQ), propyl gallate (PG) and tocopherols.

Not all antioxidant activity is conferred by free-radical interceptors. Reducing agents that function by transferring hydrogen atoms have also been categorized as oxygen scavengers. Some of these are ascorbyl palmitate, sulphites, ascorbic acid, glucose oxidase and erythorbic acid. To be effective in foods these must be added during manufacturing or to the finished products. No single antioxidant offers a panacea to oxidative deterioration for all food products. The selection of an appropriate antioxidant appears to be determined by compatibility between the effect and the food-related properties of the product. Thus, in general synthetic antioxidants are compounds with phenolic structures and various degrees of alkyl substitutions. Tocopherol and ascorbic acid are both extensively used as natural antioxidants but their activity is much lower than synthetic antioxidants (Nishina *et al*., 1991).

Antioxidants are also referred to as *chelators* which bind metal ions such as copper and iron that catalyse lipid oxidation; *oxygen scavengers* or those compounds that react with oxygen in closed systems and *secondary antioxidants* which function by breaking down the hydroperoxides (Shahidi & Wanasundam, 1992).

In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (Frankel *et al*., 1995; Gazzani *et al*., 1998; Yen *et al*., 1998). This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties (Duh *et al*., 1992; Yen *et al*., 1996; Miyake & Shibamoto, 1997). A number of plant constituents have been recognized to have positive effects when tested against the oxygen reactive compounds in biological systems. Many plant by-products such as peanut hulls, carob seeds, citrus peels and seeds and malt root extracts have also been exploited for their antioxidative potentials (Bocco *et al*., 1998; Bonnely *et al*., 2000). Thus the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects (Steinberg, 1991; Ames *et al*., 1993). Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases (Renaud & de Lorgeril, 1992; Fuhrman *et al*., 1995), cancers (Dragsted *et al*., 1993; Wargovich, 2000), neurodegenerative diseases, the most well known of which are Parkinson's and Ahlzeimer's diseases (Okuda *et al*., 1992; Clarke, 1999; Joseph *et al*., 1999), as well as inflammation (Lietty *et al*., 1976; Joseph *et al*., 1999) and problems caused by cell and cutaneous ageing (Ames *et al*., 1993; Gaulejac *et al*., 1999a; Prior & Cao, 2000).

The chemical characteristics of antioxidants, including their solubility, regenerative ability, structure/activity relationships and bioavailability are important factors when considering their role in human health. What dietary constituents are responsible for this activity are not known with certainty, but well characterized antioxidants including vitamin C and E or β-carotene, are often assumed to contribute to the observed protection against diseases (Gey *et al*., 1991; Stahelin *et al*., 1991; Steinberg, 1991; Ames *et al*., 1993; Willet, 1994; Burning & Hennekens, 1997; Velioglu *et al*., 1998). However, results from intervention trials, where diets have been supplemented with antioxidants, have not conclusively demonstrated protection (Van Poppel *et al*., 1995; Hennekens *et al*., 1996; Omenn *et al*., 1996; Prieme *et al*., 1997). Recent epidemiological evidence indicates that the putative beneficial effects of a high intake of fruits and vegetables against the diseases of cancer and ageing may not be exclusively because of these antioxidants (Hertog *et al*., 1992; Knekt *et al*., 1997), but other phytochemicals contained in fruits and vegetables may be equally important (Bidlack, 1998). An adequate intake of these free radical scavengers is necessary for efficient prevention of these degenerative diseases (Block *et al*., 1992; Kanner *et al*., 1994). The other natural antioxidants can be phenolic compounds, such as flavonoids, and phenolic acids or nitrogen compounds, such as alkaloids, chlorophyll derivatives, amino acids and amines. These may act as singlet oxygen quenchers or chain breaking antioxidants. Certain phytochemicals seem to halt cancer at its inception by blocking enzymes that potentiate cancer or by preventing the various carcinogens that initiate cancer. Other phytochemicals stop carcinogens from damaging cells, tissues and organelles by helping the body to produce enzymes that destroy carcinogens. Still others suppress the spread of cancer by interfering with the reproduction of cells that already have been exposed to carcinogens. Strong evidence exists that one class in particular, the plant phenolics, can prevent DNA adduction, presumably by presenting alternative targets for attack by carcinogens (Newmark *et al*., 1984; Teel & Castonguay, 1992). Recently, Wargovich (2000) has made an attempt to provide a comprehensive view of the evidence for and mechanism of action of fruits and vegetables in reducing the risk for cancer.

Scientists have also shown that antioxidants like vitamin E and β-carotene may be beneficial in helping to delay initial episodes of general immune disorders in some patients by extending the period between infection and appearance of clinical symptoms. An example is HIV infection and appearance of AIDS, where the antioxidants can help in limiting drug toxicity and decreasing production of drug-resistant HIV strains (Baranowitz *et al*., 1996). The preponderance of evidence in human nutrition and medical research substantiates a role for dietary antioxidants in health maintenance and disease prevention and is an impetus for researchers to consider monitoring antioxidant compounds in fruits and vegetables (Bidlack, 1998).

Fruits and vegetables as a source of natural antioxidants

Fruits and vegetables contain significant levels of biologically active components that impart health benefits beyond basic nutrition (Oomah & Mazza, 2000). They are a major source of dietary antioxidants that increase the plasma antioxidant capacity resulting in inhibition of atheroscelerosis related diseases in humans (Cao *et al*., 1998). Consumption of fruits and vegetables has thus been associated with lower incidence and lower mortality rates caused by cancer in several human cohort and case–control studies for all common cancer types (Doll, 1990; Ames *et al*., 1993; Dragsted *et al*., 1993; Willet, 1994). The antitumorigenic effects of vegetables were also found in experiments using cells (Maeda *et al*., 1992) and animals (Bresnick *et al*., 1990; Wattenberg & Coccin, 1991). There has been highly significant negative correlation between total intake of fruits and vegetables, cardio- and cerebrovascular diseases and mortality (Acheson & Williams, 1983; Verlangieri *et al*., 1985). Vegetarians and nonvegetarians with a high intake of fruits and vegetables have been reported to have reduced blood pressure (Ascherio *et al*., 1992).

The most thoroughly investigated dietary components in fruits and vegetables acting as antioxidants are fibre, polyphenols, flavonoids, conjugated isomers of linoleic acid, D-limolene, epigallocatechin, gallate, soya protein, isoflavanones, vitamins A, B, C, E, tocopherols, calcium, selenium, chlorophyllin, alipharin, sulphides, catechin, tetrahydrocurecumin, seasaminol, glutathione, uric acid, indoles, thiocyanates and protease inhibitors (Karakaya & Kavas, 1999). These compounds may act independently or in combination as anticancer or cardio-protective agents by a variety of mechanisms.

Discussed below are some of the most important natural antioxidants present in fruits and vegetables that have been shown to play a crucial role in prevention of various diseases.

Polyphenols

Polyphenols are secondary plant metabolites and confer on fruits and vegetables both desirable and undesirable food qualities. Historically these were considered as antinutrients because some (tannins) were shown to have adverse effects in human metabolism, but recently the recognition of antioxidative properties of these phenolics has evoked a rethinking towards the health benefits of these secondary metabolites (Bravo, 1998). Polyphenols account for the majority of antioxidant activity when compared with ascorbic acid in fruits (Wang *et al*., 1996; Deighton *et al*., 2000).

The antioxidant properties of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans *et al*., 1997). A polyphenol substance can be defined as an antioxidant only if it fulfills two conditions, firstly, when present in low concentration relative to the substrate to be oxidized it can delay, retard or prevent the oxidation or free radical mediated oxidation of a substrate and secondly that the resulting radicals formed after scavenging must be stable. Polyphenols can be stabilized through intramolecular hydrogen bonding or by further oxidation.

Polyphenols, depending on their precise structure, act as hydrogen donating antioxidants and also act as chelators of metal ions, preventing metal catalysed formation of initiating radical species. (Salah *et al*., 1995). It is known that the degree of glycosylation significantly affects the antioxidant properties of the compound, for example, aglycones of quercetin and myricetin were more active than their glycosides (Hopia & Heinonen, 1999). The molecular scavenging mechanism of these molecules is closely related to their stereo chemical structure (Gaulejac *et al*., 1999a). Catechins and their epimers serve as powerful antioxidants for directly eliminating super oxide anion radicals. These are basically flavonoids and some related compounds (Williamson *et al*., 1998). The flavonoids are a large class of compounds ubiquitous in plants usually occurring as glycosides. Cereals as well as vegetables and fruits have been identified as a possible major dietary source of variety of flavonoids (Meltzer & Malterud, 1997; Andlauer & Furst, 1998; Prior & Cao, 2000). Of the flavonoids, high molecular weight plant polyphenols (tannins) show the most benefits to human health (Hagerman *et al*., 1998). Anthocyanins, flavanols and isoflavones, have been associated with anticarcinogenic activity in animal and cell systems (Huang & Ferraro, 1991; Papas, 1999). Hagerman *et al*. (1998) have proposed that tannins could have unique roles as both antioxidants and protectors of other nutrients from oxidative damages. Evidence is accumulating demonstrating the absorption of dietary flavonoids in humans and also their significant contributions to the antioxidant capacity of fruits and vegetables. These compounds could, therefore, be responsible for the health benefits observed with increased consumption of fruits and vegetables (Prior & Cao, 2000).

Fruits and vegetables rich in polyphenols

Fruits

Most of the work on antioxidant potential of fruits has been limited to grapes and berry fruits and juices. Flavonoid and other phenolics present in grapes and grape products have been shown to possess anticarcinogenic, antiinflammatory, antihepatotoxic, antibacterial, antiviral, antiallergic, antithrombic and antioxidative effects (Meyer *et al*., 1998a; Gaulejac *et al*., 1999b). Commercial grape juice has been shown to give the health benefits of grape phenolics in inhibiting low density lipoprotein (LDL) oxidation (Day, 1998).

Grapes and wine contain large amounts of polyphenols the major being, caftaric acid, tartaric acid ester of caffeic acid, flavan-3-ol catechin and the blue-red pigment malvidin-3-glucoside, the major anthocyanin (Macheix *et al*., 1990). Unlike other classes of flavonoids, monomeric flavan-3-ols are generally found in free rather than glycosylated or esterified form in fruit. Among commercial juices, grape has been shown to have the highest antioxidant activity followed by grapefruit juice, tomato, orange and apple (Wang *et al*., 1996).

Berries are not only delicious, low energy food, but also a rich source of antioxidant vitamins, fibre and various phenolic compounds. The majority of berries studied have a similar or higher levels of flavonoids and phenolic acids than the commonly consumed fruits (Torronen, 2000). Cranberry juice has been recognized for a long time as efficacious in the treatment of urinary tract infections and oxidative effects (Schmidt & Sobota, 1998). Proanthocyanidins or condensed tannins present in blue berries have been identified as compounds responsible for preventing urinary tract infections caused by *E. coli*. Black berries extracts were more active than red raspberries, sweet cherry or blue berries in LDL oxidation whereas sweet cherries were more active than others in liposome oxidation (Heinonen *et al*., 1998a). Main flavonoid subgroups in berries are anthocyanins, proanthocyanins, flavonols and catechins (Wang *et al*., 1997). Phenolic acids present in berries and fruits are hydroxylated derivatives of benzoic acid and cinnamic acid (Macheix *et al*., 1990). The 3-glucosides and 3-galactoside of delphindin, petunidin, cyanidin and peonidin are the primary anthocyanins that have been identified. A strong correlation has been observed between antioxidant activity and total phenolics (Cao *et al*., 1996; Wang *et al*., 1996; Prior *et al*., 1998). Berries with strong purple colour such as crowberry, aronia, bilberry and whortle berry have higher phenolic concentrations than yellowish rowanberries and cloudberries but no significant difference between their antioxidant activities has been observed. Blue berries are one of richest source of antioxidants studied so far with antioxidant capacity as high as 45.9 μmol Trolox equivalent g^{-1}. Difference in antioxidant activity when tested under conditions may be because of differences in activities of phenolic compounds and their antagonistic and synergistic reactions with other antioxidants. Strawberry is reported to have fifteen times higher total antioxidant capacity than Trolox in an artificial peroxyl radical model system (Wang *et al*., 1996). Extracts of blackberries, black and red currants, blueberries, and black and red raspberries possessed a remarkable high scavenging activity towards chemically generated super oxide radicals (Constantino *et al*., 1992). Hydroxycinnamic acids typically present in fruits have been shown to inhibit LDL oxidation *in vitro* (Meyer *et al*., 1998b).

Prunes and prune juices are another excellent source of dietary antioxidants (Donovan *et al*., 1998). Hydroxycinnamates, neochlorogenic and chlorogenic acid are predominant antioxidants exhibiting LDL oxidation.

In recent years, pomegranate juice, yet another polyphenol rich juice has become more and more popular because of the attribution of important biological, antioxidative and antimuoral actions (Lansky *et al*., 1998; Schubert *et al*., 1999; Gil *et al*., 2000).

The genus *Citrus* is another group characterized by a substantial accumulation of flavonone glycosides, which are not found in many other fruits. The limonoids, a group of chemically related triterpene derivatives present in large citrus fruits and vegetables has been demonstrated as the preventative agent against a variety of human cancers and atherosclerosis (Crowell, 1997; Gould, 1997; Vinson *et al*., 1998; Uedo *et al*., 1999). Citrus seeds and peels have also been shown to possess high antioxidant activity (Bocco *et al*., 1998). Terpenes react with free radicals by partioning themselves into fatty membranes by virtue of their long carbon side chains. Lemon peel contains two flavonone glycosides, hesperidin and eriocitrin. In grape fruit, narinigin is predominant and is accompanied by narirutin. Naringin is also predominant in the juice of sour orange, along with other neohesperidosides. Only 7-β-rutinosides are present in sweet orange, hesperidine being the dominant flavanone glycoside. Citrus fruits also contain several flavones, such as nobiletin and sinensetin in orange peel. Another family of phenolics found in citrus are the phenylpropanoids, in particular hydroxy-cinnamates. The most widely distributed phenolic components in plant tissues are hydroxycinnamic acids, such as p-coumaric acid, caffeic acid and ferulic acid (Rice-Evans *et al*., 1997). Phytosterols are another important terpene subclass and two sterol molecules that are synthesized by plants are β-sitosterol and its glycoside. In animals these two molecules exhibit antiinflammatory, antineoplastic, antipyretic and immune modulating activity (Hertog *et al*., 1993; Mimaki *et al*., 1998; Bouic & Lamprecht, 1999). In the body, phytosterol can compete with cholestrol in the intestine for uptake and aid in the elimination of cholestrol from the body, thus reducing serum or plasma total cholestrol and LDL cholestrol (Jones *et al*., 1999).

In a recent finding Liu (2000) has shown that phytochemicals, like phenolic acids and flavonoids from apples have very strong antioxidant activity in colon and liver cancer cells. Anticancerous effects was significantly greater when the skin of the apple was included in the test samples. Rechner (2000) has also reported on the antioxidant capacity of apples.

Rubus species with antioxidant capacity ranging from 0 to 25.3 μmol Trolox eq g^{-1} [Trolox equivalent antioxidant capacity (TEAC)] and high phenol concentration, up to 4.5 g kg^{-1}, has been recommended for improvement of nutritional value through germplasm enhancement programmes (Deighton *et al*., 2000).

Wine

Wine assumes an important role in the diet partly because of phenomenon called the ’French paradox’. The French paradox refers to the epidemiological finding that in certain parts of France coronary heart disease mortality is low despite a high intake of saturated fats and relatively high plasma cholesterol levels in the population (Renaud & de Lorgeril, 1992). Red wine has been shown to inhibit *in vitro* oxidation of human LDL (Frankel *et al*., 1993, 1995; Teissedre *et al*., 1996). The ability of wine phenolics to inhibit LDL oxidation have been suggested as a possible mechanism explaining the ‘French paradox’ (Kinsella *et al*., 1993).

The phenolics substances in wine mainly originate from grapes and include nonflavonoids such as hydroxycinnamates, hydroxybenzoates and stilbene in addition to flavonoids such as flavan-3-ol (catechin), anthocyanin, flavonols and polyphenol tannins. However the phenolic profile of wine is not the same as that of fresh grapes because significant changes take place during processing for wine making (Singleton, 1982). Meyer *et al.* (1997) showed that phenolic compounds extracted from fresh grapes showed high antioxidant activity, in fact it was of same order as that of wine.

Antioxidant activity of wines made from mixtures of black currants and crowberries were superior to other berry and fruit wines as well as to red wine and equally as active as tocopherol. Red wine has great antioxidant potential, because of phenolic compounds (tannins and anthocyanins) which are present in sufficient quantities to ensure optimum free radical scavenging activity of the compounds and even combined action between them leading to synergistic effect of these polyphenols (Gaulejac *et al.*, 1999a). The protective effect of red wines on the hepatic cells in the livers of rats which were bombarded with free radicals have also been shown during immunochemical tests (Gaulejac *et al.*, 1999b).

Vegetables

There have been only a few reports on the antioxidant activity of vegetables (Prior & Cao, 2000). Garlic, broccoli (Al-Saikhon *et al.*, 1995; Cao *et al.*, 1996), mushroom, white cabbage and cauliflower (Gazzani *et al.*, 1998), kidney and pinto beans (Vinson *et al.*, 1998), beans, beet and corn (Kahkonen *et al.*, 1999) have been reported to have high antioxidant activity. Besides this other vegetables such as kale, spinach, brussel sprouts, alfalfa sprouts, broccoli, beets, red bell-pepper, onion, corn, eggplant, cauliflower and cucumber are also rich source of antioxidants (Prior & Cao, 2000). High levels of quercetin have been found in onion, kale, tomato and certain varieties of lettuce (Hertog *et al.*, 1992; Crozier *et al.*, 1997; Justesen *et al.*, 1998). The level of kempferol is high in kale, broccoli and endive (Justesen *et al.*, 1998; Price *et al.*, 1998a).

Garlic extracts are also used as potential cardiovascular and anticancer agents. Allicin, produced when garlic cloves are crushed, spontaneously decomposes to form sulphur containing compounds with chemo-preventive activity. Mushrooms, white cabbage, cauliflower and garlic had been shown to have strong protective activity against a number of diseases (Gazzani *et al.*, 1998). *Allium* vegetables are reported to contain high levels of flavonoids. Flavanols which scavenge free radicals have also been shown to be present in onion skin (Suh *et al.*, 1999).

The brain may be particularly vulnerable to the damaging effects of free radicals because it is relatively deficient in antioxidants to begin with. A diet rich in fruit and vegetables may help age related neurogenerative diseases, the most well known of which are Parkinson's and Ahlzeimer's diseases (Okuda *et al.*, 1992). According to a recent US study, animals fed with blueberry, strawberry, or spinach extracts, showed fewer age-related motor changes and outperformed their study counterparts on memory tests. In addition the groups receiving these supplements showed signs of the presence of vitamin E in their brains. Whether the results found in this study will also prove true for humans remains to be studied. Spinach is regarded as the 'brain food' needed to avoid memory loss and Ahlzeimer's disease. It is believed that the phytochemicals present in these extracts may have properties that increase cell membrane fluidity, allowing important nutrients and chemical signals to pass in and out of the cell, thereby reducing inflammatory processes in the tissues. (Joseph *et al.*, 1999; Clarke, 1999).

Recently, special attention has also been accorded to edible vegetables that are rich in plant secondary metabolites responsible for induction of detoxifying enzymes (e.g. glutathione-S-transferase, quinone reductase, and epoxide hydrolase), which inactivate reactive carcinogens by destroying their reactive centres or by conjugating them with endogenous ligands, thereby triggering their elimination from the body (Talalay, 1992). Among crucifers (e.g. broccoli, cabbage, cauliflower, etc.) this inducer activity is principally because of highly reactive *thiocyanates*. The anticarcinogenic activity of isothiocyanate, a sulphoraphane present in broccoli, was demonstrated in a rat mammary tumour model (Zhang *et al.*, 1994). Glucosinolates, which are thought to be anticarcinogenic, are very stable precursors of isothiocyanates, typically present in crucifers at very high levels and their hydrolysis by myrosinase is a prerequisite for observed biological activity (Williamson *et al.*, 1998; Fahey & Stephenson, 1999).

Spices and herbs

Besides vegetables, work on spices and herbs also suggests the presence of phenolic antioxidative and antimicrobial constituents. Several studies confirmed that many leafy spices, especially those belonging to the Labiatae family such as sage, rosemary, oregano and thyme show strong antioxidant activity (Nakatani, 1997; Hirasa & Takemasa, 1998).

Other phytochemicals present in fruits and vegetables implicated as antioxidants

These substances include fat soluble antioxidants, such as vitamin E, coenzyme Q10, lycopene, β-carotene, α- carotene and water soluble antioxidants including vitamin C.

Carotenoids, vitamin E and vitamin C

Several carotenoids such as β-carotene, lycopene, lutein and zeaxanthine are known to exhibit antioxidant activity, but β-carotene has been the most thoroughly studied. Collectively vitamin C, vitamin E and β-carotene are referred to as the antioxidant vitamins. All work both singly and synergistically to prevent or delay oxidative reactions that lead to degenerative diseases, including cancer, cardiovascular diseases, cataracts and other diseases (Elliot, 1999). Studies have indicated that significant numbers of the world population are not consuming the levels of antioxidant vitamins needed to prevent oxidative damage because they do not eat enough fruits and vegetables. Some studies have shown that smokers with diets high in carotenoids have a lower rate of lung cancer development than their counterparts whose carotenoid intake is relatively low. Other research efforts have suggested that diets high in carotenoids may also be associated with a decreased risk of breast cancer. Also, vitamin C has been found to prevent the formation of N-nitroso compounds, the cancer causing substances from nitrates and nitrites found in preserved meats and some drinking water.

The relevance of carotenoids to human nutrition and health has historically been confined to those possessing pro-vitamin A activity such as α-carotene and β-carotene. A positive correlation between consumption of food rich in carotenoids and a lowered risk of developing certain types of cancer (Block *et al.*, 1992; Van Poppel, 1993; Ramon *et al.*, 1993; Potischman *et al.*, 1994; Giovanucci *et al.*, 1995; Appel & Woutersen, 1996; Narisawa *et al.*, 1996; Steinmetz & Potter, 1996; Pool-Zobel *et al.*, 1997), oxidative stress (Kohlmeier *et al.*, 1997) or chronic disease and health benefits (Kohlmeier & Hastings, 1995; Baranowitz *et al.*, 1996; Moriguchi *et al.*, 1996) have been reported. The rather long lifetime of the carotenoid derived free radicals indicates that compounds are relatively stable and the parent carotenoids may hence act as chain-breaking antioxidants. β-carotene has been shown to protect lipids from free radical auto-oxidation. β-carotene is also an effective quencher of singlet oxygen during inhibition of photoxidation by reacting with peroxyl radicals, thereby inhibiting propagation and promoting termination of oxidation termination chain reaction (Britton, 1995). More recently a core human study in European Union has demonstrated that increased consumption of carotene rich fruits and vegetables increased LDL oxidation resistance and higher plasma concentration of total carotene was associated with lower DNA damage and higher repair activity (Southon, 2000).

Increased interest in tomato products has been created by the fact that their consumption has been correlated with a reduced risk of some type of cancer and ischaemic heart disease (Parfitt *et al.*, 1994; Khachik *et al.*, 1995; Gerster, 1997; Bramley, 2000; Lavelli *et al.*, 2000). Therefore other carotenoids, such as lycopene have recently emerged as efficient singlet oxygen quencher (DiMasico *et al.*, 1989; Franceschi *et al.*, 1994; Levy *et al.*, 1995). Lycopene has been shown in *in vivo* research to inhibit carcinogenesis in specific animal model systems (Nagasawa *et al.*, 1995; Narisawa *et al.*, 1996) as well as in human cell cultures (Matasushima-Nishikawa *et al.*, 1995). The inhibitory role of lycopene in mammary tumorigenesis of mice (Mitimura *et al.*, 1996) and colon carcinogenesis in rats (Narisawa *et al.*, 1996) are well documented. Lycopene's ability to act as an antioxidant and as a scavenger of free radicals that are often associated with carcinogenesis is potentially a key to its beneficial effects on human health (Khachik *et al*., 1995). It may prevent carcinogenesis and atherogenesis by interfering positively with oxidative damage to DNA and lipoproteins (Gerster, 1997; Clinton, 1998). It may also inhibit the formation of LDL cholestrol's oxidised products, which in turn have been suggested to participate in the early stages of coronary heart diseases (Ojima *et al*., 1993; Diaz *et al*., 1997; Weisburger, 1998). Besides lycopene, nonesterified forms of xanthophylls and capsanthin have been also found to be good antioxidants (Miki, 1991; Hirayama *et al*., 1994; Matasufuji *et al*., 1998).

Vitamin E is the most abundant lipid soluble antioxidant and protects the lipid portions of the cell, especially cellular membranes. The naturally occurring tocopherols (α, β, γ, δ) and tocotrienols are synthesized by plants (Papas, 1999). All chlorophyll-a containing tissues contain tocopherols, primarily in the chloroplasts. Tocotrienols have been identified in a number of plant tissues, ranging from kale and broccoli to cereal grains and nuts (Piironen *et al*., 1986). Tocopherols scavenge free radicals by reacting with lipid peroxyl radicals to produce a tocopheroxyl radical. Evidence exists that these can prevent atherosclerosis by interfering with the oxidation of LDL, a factor associated with increased risk of heart diseases (Stampfer *et al*., 1993). It provides vital antioxidant protection for cell membranes, where it works together with both vitamin C and coenzyme Q10. Although vitamin E does not show anticancerous activity in animals, a recent clinical chemoprevention study suggests that supplemental vitamin E might decrease risk of prostate cancer, and epidemiological studies support a protective role against colon cancer. γ-Tocopherol may be more effective in quenching dangerous radicals derived from peroxynitrite, a product of inflammation.

Coenzyme Q10- ubiquinol is a very easily oxidized lipid soluble molecule that is made by the body and also derived from food. It is found mainly in the inner membrane of plant mitochondria. It helps to maintain vitamin E in its active form. It has been found to be more active than vitamin E in protecting LDL from oxidation *in vitro*.

Vitamin C is a water soluble antioxidant. It is easily oxidized to form a free radical, semidehydroascorbic acid, that is relatively stable. Further oxidation generates diketogluconic acid which has no biological function. The antioxidant activity of ascorbic acid is caused by the ease of its loss of electrons, making it very effective in biological systems. Because it is an electron donor it serves as a reducing agent for many reactive oxidant species. It protects compounds in the water soluble portions of cells and tissues, and reduces tocopherol radicals back to their active form at the cellular membranes. However in the presence of free iron ions, it can generate the dangerous ferrous ions, a crucial catalyst of oxidative damage. Fortunately, supplementation studies demonstrate that, at least under ordinary circumstances in which iron is properly sequestered in storage and transport proteins, vitamin C exerts a net antioxidative effect. Vitamin C deficiency exacerbates atherogenesis in animal models. Risk of oesophageal, pancreatic and lung cancer also appear to be lower in those people with ample intake of vitamin C or fruits and vegetables (Nishina *et al*., 1991; Wargovich, 2000).

However some conflicting reports on the health benefits of antioxidant vitamins also exist. It is difficult to know what to believe and whose advice to follow. Many clinical studies performed have however, shown no direct beneficial effects of these vitamins on various forms of lung cancers and cardiovascular diseases. Vitamin E can also have an anticoagulant effect that can promote excessive bleeding. No benefit from supplementation with vitamin E has been reported in clinical trials conducted by α-tocopherol, β-carotene Cancer Prevention Study Group (ATBC) (1994) on lung cancer among heavy smokers. In addition, the overall death rate of recipients of β-carotene was 8% higher and those who took vitamin E had a higher frequency of haemorrhagic stroke. A significant incidence of more deaths from coronary heart disease among those who took β-carotene and vitamin A have also been reported (Rapola *et al*., 1997). Another study found no evidence that supplementing with vitamin C, vitamin E, or β-carotene prevented various forms of cancer and cardiovascular diseases (Greenberg *et al*., 1994; Hennekens *et al*., 1996; Omenn *et al*., 1996). Similar studies performed by different group of researchers have again shown that treatments with vitamin E, β-carotene or a placebo had no apparent beneficial effect on cardiovascular outcomes (Hennekens *et al.*, 1994; Lee *et al.*, 1999). Other published studies on age related macular degeneration (AMD) have had conflicting results with some finding correlations and others finding none between the incidence of disease and consumption of foods rich in antioxidants (Smith *et al.*, 1999). Although adverse effects on lung cancer in heavy smokers were noted for the β-carotene treated groups in α-tocopherol, β-carotene Cancer Prevention Study Group (ATBC) (1994) and CARET (Omenn *et al.*, 1996) studies, the levels given were 20 and 30 mg, respectively, or four to five times the proposed fortification level. Elliot (1999) has discussed the applications of antioxidant vitamins in a comprehensive manner and has suggested the recommended permissible doses of various vitamin antioxidants and the methods of their delivery.

The various health benefits based on combined evidence from animal, epidemiological and clinical studies have been elucidated in the case of cardiovascular, cancer, cataracts, immune functions, arthritis and Alzheimer's disease. Thus, smoking cessation and other lifestyle factors would have a far greater effect on the efficiency of these vitamin antioxidants in controlling the incidence of lung cancer and coronary heart disease. In view of these findings, the most prudent and scientifically supportable recommendation for the general population is to consume a balanced diet with emphasis on antioxidant-rich fruits and vegetables and whole grains (Tribble, 1999). It is best to remember that vitamin and mineral supplements should never be used as substitutes for a healthy, well balanced diet. If we over supplement our body by taking much higher doses of these vitamins, adverse effects may creep in. Vitamin A and E are fat soluble, meaning the excess amounts are stored in the liver and fatty tissues, instead of being quickly excreted, creating a risk of toxicity.

Dietary fibres

Dietary fibres from fruits and vegetables have been associated with alterations of the colonic environments that protect against colorectal diseases and have been shown to have antioxidative properties (Calixeto, 1998). A review by Smith *et al.* (1998) points out that different dietary fibres have markedly different cancer protective effects, and that the differences may be related to the differential bacterial fermentation of fibre in the colon to short-chain fatty acids, especially butyric acid. Dietary sugarbeet fibre also suppresses cholestrol synthesis in a rat liver and intestine model (Hara *et al.*, 1999).

Influence of processing on the dietary antioxidants from fruits and vegetables

The health promoting capacity of fruits and vegetables depends strictly on processing history of the food. It is well known that naturally occurring antioxidants could be significantly lost as a consequence of processing and storage. Operations such as peeling, cutting and slicing have been shown to induce rapid enzymatic depletion of several naturally occurring antioxidants (McCarthy & Mattehus, 1994). In general thermal treatments are believed to be the main cause of the depletion in natural antioxidants in food (Jonsson, 1991). A blanching time of 1 min has been recommended for green leaves of sweet potato to retain high antioxidant activity (Chu *et al.*, 2000). The concentration of nutrients and their biological activity may be changed by their environmental variables as well as by processing methods (Greefield & Southgate, 1992; Salvini *et al.*, 1998). By and large processing is believed to be responsible for losses in natural antioxidant activity and the impact of processing has been evaluated by measuring the residual activity of some enzymes commonly taken as indicators of processing damage (Lund, 1979; Miller *et al.*, 1995). But recent research has now established that food processing has also some positive roles which improves the quality and health properties of the processed food. This is mainly attributed to the increased bioavailability of some antioxidants and some products known to have antioxidative capacity. The impact of processing on the antioxidant activity of fruits and vegetables is a neglected area and scarce information is available. The consequence of food processing and preservation procedure on the overall antioxidant activity of the food are generally the result of different events.

Hence the evaluation of processing factors influencing the antioxidant activity is imperative in optimizing the technological conditions to increase or preserve their activity and bioavailability. Recent reviews by Nicoli *et al.* (1999) and Klein & Kurilich (2000) give an excellent picture of the antioxidant activity as influenced by processing.

Carotenoids

Antioxidant status of processed products is a direct function of the varietal types. Appreciable differences in antioxidant status have been observed among varieties of vaccinum, rubus, apple, tomatoes and raspberry species (Prior *et al.*, 1998; Ancos *et al.*, 2000b; Deighton *et al.*, 2000; Scalfi *et al.*, 2000). The most notable positive effect of processing on the overall quality or health capacity of food is the increased bioavailability of β- carotene resulting in an increased antioxidant status. The antioxidant levels of processed and pureed carrots were higher than their fresh counterparts. The antioxidant levels increased immediately after heat processing by 34.3% declined during storage but never returned to the original levels found in canned carrots. Furthermore incorporation of periderm tissue in carrot puree resulted in not only an increase in the antioxidant activity but also increased processing efficiency and yields (Talcott *et al.*, 2000). The bioavailability of carotenoids increased fivefold in the processed products as a consequence of moderate heating or enzymatic disruption of vegetable cell structure (Southon, 2000). In general the antioxidant activity of the juices from different vegetables increased appreciably after heat treatment (Gazzani *et al.*, 1998; Southon, 1998; West & Castenmiller, 1998). The effect of various methods of cooking on the levels of carotenoids has been extensively studied. It has been conclusively shown that while epoxy carotenoids were somewhat sensitive to heat treatment, lutein and hydrocarbons such as neurosporene, α- and β-carotene, lycopene, phytofluene and phytoene survive heat treatment (Miki & Akatsu, 1971; Khachik *et al.*, 1992; Boileau *et al.*, 1999). Thermal processing has been shown to convert all *trans*- form of β-carotene to *cis* forms (Chandler & Schwartz, 1987). Such isomerization induced by exposure to light and processing treatments results in loss of carotenoid activity. A unique characteristic of carotenes is their association with the plant matrix or with carotenoproteins (Boileau *et al.*, 1999). Following heating the carotenes are released for example release of lycopene in tomatoes is needed for their effective utilization (Giovannucci, 1999).

Ascorbic acid

Loss of ascorbic acid as a result of blanching, cooking, pasteurization, sterilization, dehydration and freezing has been reported in a number of cases (Lathrop & Leung, 1980; Van den Broeck *et al.*, 1998). In a recent study in fresh and processed vegetables storage at 4 °C resulted in a slight decline in ascorbic acid in both carrots and broccoli but substantial losses were apparent in green beans. In contrast microwave cooking had no significant effect on the loss of vitamin C (Howard *et al.*, 1999). Few comparisons of vitamin E stability in raw and processed plant foods appear in the literature where conflicting reports have appeared regarding the losses during processing (Klein & Kurilich, 2000).

Flavonoids and phenolic acids

Influence of processing (boiling, frying, canning, juicing) and storage on the quality and quantity of flavonol glycosides have been reported in onion (Price *et al.*, 1997; Hirota *et al.*, 1998), broccoli (Price *et al.*, 1998a), green beans (Price *et al.*, 1998b), apples (Price *et al.*, 1999) and berries (Hakkinen *et al.*, 2000). Effects of domestic processing and storage on the flavonoids revealed that myrecietin and kaempferol were more susceptible to losses during storage (Hakkinen *et al.*, 2000). Processing berries into jam did not lead to any appreciable change in their flavonoids and phenolic acids (Amakura *et al.*, 2000).

Ancos *et al.* (2000a, 2000b) reported slight decreases in the anthocyanin content, ellagic acid, vitamin C and total phenolics during freezing of berry fruits. Freezing also produced a slight decrease in antioxidant activity but during frozen storage no changes in antioxidant activity were observed. Drying temperatures of 100 and 140 °C resulted in significant reduction in both total extractable and condensed tannins, resulting in a decrease of 28–50% in antioxidant activity in red grape pomace peels (Larrauri *et al.*, 1997). The total flavonoids content remained constant during storage in both air dried and fresh spinach stored in modified atmosphere (Gil *et al*., 1999; Gil & Tomas-Barberan, 1999). Industrially processed pomegranate juice had higher antioxidant activity than pomegranates processed in the laboratory and obtained only from the arils. This was mainly attributed to the presence of punicalagin, a tannin which gets incorporated into the juice from the fruit rind during industrial processing (Gil *et al*., 2000).

The antioxidant properties of polyphenols may change as a consequence of their oxidation state. Polyphenols with an intermediate oxidation state can exhibit higher radical scavenging activity than nonoxidized polyphenols. The concentration and oxidation of the phenolic acids, accentuated by thermal processing and storage conditions at elevated temperatures significantly impacted the antioxidant activity of carrot puree (Talcott *et al*., 2000). The higher antioxidant activity of the partially oxidized polyphenols could be attributed to their increased ability to donate a hydrogen atom from the aromatic hydroxyl group to a free radical and/or to the capacity of their aromatic structure to support unpaired electrons through delocalization around the π-electron system. Processing or prolonged storage can thus promote or enhance the progressive enzymatic or chemical oxidation of phenolic compounds. The increased antioxidant properties of certain oxidized polyphenols have been observed by Kikugava *et al*. (1990). Recently it was reported that antioxidant properties of red wine increased or decreased depending on the storage conditions (Tubaro & Ursini, 1996).

Redox reactions such as those occurring between different natural antioxidants and lipid oxidation products (Namiki, 1990; Halliwell *et al*., 1995; Mortensen & Skibsted, 1997; Wijewickreme & Kitts, 1998) have also been found to affect the antioxidant properties of different compounds. These events, which mainly take place when different food matrices are mixed together (e.g. aqueous phase and lipid phase), have almost unpredictable consequences on the overall antioxidant properties and food stability. Processing can promote or enhance these reactions. It has been recently shown that when a small amount of olive oil was mixed with tomato puree, the ascorbic acid content decreased after a few hours of storage. This was because of the ability of these compounds to reduce the radical forms of α-tocopherol contained in the lipid matrix (Nicoli *et al*., 1999). This hypothesis is confirmed by the lower standard redox potential value of the ascorbyl radical forms of α-tocopherol radical. In addition when studying the antioxidant properties of complex foods, it must be taken into account that water-soluble antioxidants could protect lipids soluble antioxidants, because of the so-called polar paradox (Porter *et al*., 1977). The interaction between the vegetable matrix and the lipid fraction becomes more evident when heated.

Maillard reaction products

Thermal treatments, although generally believed to be the main cause of depletion of natural antioxidants, can also induce the formation of further compounds with antioxidant properties as occurs during the development of Maillard reaction products (MRPs). Antioxidant depletion in thermally treated fruits and vegetables may also be attributed to consumption of ascorbic acid and polyphenols as reactants in the Maillard's reaction (Kaanane *et al*., 1988; Djilas & Milic, 1994). Recent research strongly suggests that MRPs, formed as a consequence of intense heat treatment or prolonged storage, generally exhibit strong antioxidant properties, generally chain breaking and oxygen scavenging activities (Eichner, 1981; Anese *et al*., 1993; Nicoli *et al*., 1997; Wijewickreme *et al*., 1999). Lipid oxidation rates were significantly slowed down when MRPs were added or formed during heating (Severini *et al*., 1994; Bressa *et al*., 1996). According to extensive literature, the antioxidant activity of MRPs can be mainly attributed to the high molecular weight brown compounds, which are formed in the advanced stages of reaction. The changes in the overall antioxidant properties of tomato juice samples and model solutions as a consequence of heat treatments were studied by Anese *et al*. (1999). While a decrease in the antioxidant potential was found for short heat treatments, a recovery of these properties was found during prolonged heat treatment. The initial reduction in the antioxidative activity can be attributed not only to thermal degradation of naturally occurring antioxidants but also to formation of early MRPs with pro-oxidant properties. The gain in antioxidant activity coincided with the formation of brown MRPs. The antioxidative capacity of aged citrus juice was stronger than that of BHA and increased as the orange juice became more and more discolored. (Lee, 1992). Nicoli *et al.* (1997) suggested that the loss of antioxidant vitamins in food (tomato puree) during processing could be compensated for by the appearance of other antioxidants. Specifically they suggest that MRPs might have both antioxidant and antimutagenic characteristics (Namiki, 1990; Yen & Tsai, 1993; Usman & Hosono, 1997).

Thus during processing the antioxidant compounds may undergo physical and chemical changes that alter their potential benefits. Generally thermal processing results in a decrease in ascorbate and tocopherols and an increase in carotenes. As other micronutrient phytochemicals may act in concert with vitamins, future studies should be considered to investigate the effects of processing on other naturally occurring antioxidants.

Antioxidant evaluation methods

A wide range of methods are currently used to assess antioxidant capacity of fruits and vegetables (Halliwell *et al.*, 1995). The most popular being the ORAC (oxygen radical absorbance capacity) method of Cao *et al.* (1995) and Wang *et al.* (1996) which measures total antioxidant capacity in biological systems. This sensitive assay measures the protection provided by antioxidants against either hydroxyl or peroxyl radicals. Different free radicals generators or oxidants can be used in the ORAC assay. The peroxyl radical is a common free radical found in the body and used in the antioxidant assays. This is slightly less reactive than OH and thus possesses an extended half-life of seconds instead of nanoseconds. The assay is unique in that it measures both inhibition time and inhibition degree of free radical action by antioxidants using an area under curve technique for quantification. Other similar methods (Wayner *et al.*, 1987; Glazer, 1990; Miller *et al.*, 1993) use either the inhibition time at fixed inhibition degree or the inhibition degree at the fixed time as the basis for quantitating the results.

The automated ORAC assay is done by using the COBAS FARA II centrifugal analyser with a fluorescent attachment. In this assay, R- or β – phycorerythrin is used as a target of free radical attack, with 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) and Cu^{2+} -H_2O_2 used as generators of the peroxyl and hydroxyl radicals, respectively. Trolox (6-Hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid), a water soluble vitamin E analogue is used as the antioxidant standard and ORAC is expressed in units of Trolox equivalents.

The antioxidant activity of some common fruits and vegetables has thus been determined by using the $ORAC_{ROO}$* which measures all traditional antioxidants including ascorbic acid, α-tocopherols, β-carotene, glutathione, bilirubin, uric acid, melatonin and flavonoids.

Assays have also been developed for flavonoid compounds found in biological systems. As many flavonoids are antioxidants and therefore electroactive, multielectrode electrochemical detection coupled with high performance liquid chromatography (HPLC) methods are being used to separate, identify and quantify these compounds. It has been demonstrated that individual fruits and vegetables each have their own HPLC-electrochemical fingerprint, which may be used to provide an evidence of their absorption in to animal tissues after dietary supplementation (Guo *et al.*, 1997).

Sample scavenging assays, such as the TRAP (total reactive antioxidant potential or total radical-trapping antioxidant parameter) (Wayner *et al.*, 1987) and the TEAC assay, have gained popularity because they enable high throughput screening of potential antioxidant capacity. The TEAC assay, originally described by Miller *et al.* (1993) is based on scavenging of long lived radical anions 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical anions ($ABTS^{-}$). In this assay radicals are generated through the peroxidase activity of metamyoglobin in the presence of hydrogen peroxide and can easily be detected spectrophotometrically at 734 nm. The antioxidants are added before the $ABTS^{-}$ formation is initiated by hydrogen peroxide, resulting in a delay in radical formation (lag time) which is measured. A TEAC value can be assigned to all compounds able to scavenge the $ABTS^{\cdot-}$ by comparing their scavenging activity with that of Trolox, a water soluble vitamin E analogue. A modification of this assay, made by introducing horse radish peroxidase for generation of $ABTS^{\cdot -}$, was proposed by Arnao *et al.* (1996). However Strube *et al.* (1997) showed that these pre-additions of antioxidants before radical generation might result in overestimation of antioxidant capacity because of compounds interfering with the formation of $ABTS^{\cdot -}$ and proposed a post-addition protocol in which compounds were added after radical formation. A further improvement in the assay was proposed where there is regeneration of $ABTS^{\cdot -}$ in the presence of a thermolabile azo compound, 2,2′-azobis-(2-amidinopropane) (ABAP) (Berg *et al.*, 1999). Generation of radicals before the addition of antioxidants prevents interference of compounds which affect radical formation. The original TEAC assay could measure only water soluble antioxidants. As foods generally contain both water-soluble and lipid soluble compounds, an effort was also made to evaluate lipid soluble components by solubilizing lipid soluble components in the aqueous TEAC assay medium using suitable solvents. For application of this assay to foods containing both water- and lipid soluble antioxidants choice of proper solvents for the extraction and solubilization of these compounds in aqueous system has to be achieved. Although this assay can be useful in ranking the different antioxidants, it may not give quantitative evaluation of antioxidants.

Gaulejac *et al.* (1999a) measured antioxidant activity in terms of inhibition of generation of superoxide anions (O_2^-) by hypoxanthine-xanthine oxidase system. At pH 7.4, (O_2^-) reduces the tetrazolium blue into formasan blue, but after addition of some radical scavengers the formation of formasan blue is restricted and absorption at 560 nm is decreased. The free radical scavenging activity may be defined by the quantity of antioxidant necessary to inhibit 50% of the O_2^- radical generated by XOD-HPX system. The major limitation of this assay is that a fresh xanthine oxidase solution has to be made after every 30 min because the enzyme loses its activity. Hence this assay is not practical and time consuming if a large number of samples are to be evaluated at a time.

The β-carotene bleaching method (coupled oxidation of β-carotene and linoleic acid) developed by Taga *et al.* (1984) with modifications (Hammerschmidt & Pratt, 1978; Wanasundara *et al.*, 1994) estimates the relative ability of antioxidant compounds in the plant extracts to scavenge the radical of linoleic acid peroxide (LOO*) that oxidizes β-carotene in the emulsion phase. This method has been widely used but with different extraction mediums (Al-Saikhon *et al.*, 1995; Nicoli *et al.*, 1997; Gazzani *et al.*, 1998). In a recent study performed in our laboratory (unpublished), using this method for the measurement of antioxidant activity of different vegetables it has been shown that alcoholic extracts of vegetables gave better antioxidant values as compared with aqueous extracts.

The thiocyanate method of Mitsuda *et al.* (1966) involves measurement of the peroxide value using linoleic acid as substrate and has also been widely used to measure the antioxidative activity (Kikuzaki & Nakatani, 1993; Larrauri *et al.*, 1997). Two copper catalysed *in vitro* oxidation assays using human LDL and lecithin liposomes. (Heinonen *et al.*, 1998b) have been also employed. These models have been chosen as LDL oxidation is an early event in coronary diseases and liposome oxidation is relevant to oxidation in food systems. The antioxidant activity of grape extracts shown by inhibition of copper catalysed oxidation of human LDL, was assayed by monitoring production of hexanal by static headspace gas chromatography (Meyer *et al.*, 1997).

Antioxidant activity was also measured by oxidizing methyl linoleate in the presence of antioxidants (Hopia *et al.*, 1996; Kahkonen *et al.*, 1999). The formation of conjugated diene between the product and 2,2,4-trimethyl pentane was measured at 234 nm. The antioxidant activity was expressed as percentage inhibition of formation of conjugated diene hydroperoxide.

A deoxyribose assay (Halliwell *et al.*, 1987) has also been used to measure the scavenging activity of the hydroxyl radicals. In this assay a Fenton reaction based model containing Fe^{3+} or Cu^{2+} as catalytic metal were used.

A DNA nick test (Wijewickreme *et al.*, 1999), where the OH* generated by Fenton reactants attacks DNA guanosine residues, results in strand breakage and transformation from native supercoiled to relaxed nicked circular or linear forms. Unno *et al.* (2000), using electron spin resonance spectroscopy along with spin trapping agent, evaluated the scavenging effect of tea catechins and their corresponding epimers against superoxide anion radicals generated by a hypoxanthine and xanthine oxidase reaction system. The hydroxyl radical and the superoxide anion generated in the reaction mixture were trapped with 5,5-dimethylpyrrolidine N-oxide (DPMO), and the resultant DPMO-OH and DPMO-O_2^- were detected by ESR spectrometery.

Recently, Iwai *et al.* (2000) have developed the XYZ- dish method to evaluate antioxidant activity assay for various foods. It is based on emission of ultra weak chemiluminescence (photon) in the presence of an active oxygen species (X), active oxygen scavenging substances (Y) and receptors (Z). The results showed that this method is useful for various foods.

Both *in vivo* and *in vitro* methods which have been used have their own advantages and limitations. Some methods can be used with water soluble extracts while others are useful for water-insoluble fractions. Lavelli *et al.* (2000) measured the antioxidant activity in tomato products by following three model systems: (a) the xanthine oxidase/xanthine system which generates a superoxide radical and hydrogen peroxide (b) the myeloperoxidase/NaCl/H_2O_2 system, which produces hypochloric acid and (c) the linoleic acid/$CuSO_4$ system which promotes lipid peroxidation. The results showed that both hydrophilic and lipophillic fractions responded differently when different methods were used.

Four methods were used to test the antioxidant activity of pomegranate juice including three based on the evaluation of the free-radical scavenging capacity of the juices and one based on measuring their iron-reducing capacity (Gil *et al.*, 2000). The first method generated the $ABTS^+$ by addition of H_2O_2 and horseradish peroxidase (Cano *et al.*, 1998), which is a coloured free radical, whose neutralization was easily followed by reading the decrease in absorbance at 414 nm after the addition of antioxidant. This assay is similar to that described by Rice-Evans & Miller (1994). The second method used a commercially available free radical ($DPPH^+$, 2,2′ diphenyl-1-picrylhydrazyl) which is soluble in methanol (Brand-Williams *et al.*, 1995), and antioxidant activity was measured by decrease in absorbance at 515 nm. The third radical scavenging method generates a coloured free radical ($DMPD^+$) by addition of Fe^{3+} to p-phenylenediamine (Fogliano *et al.*, 1999) and the absorbance at 593 nm was recorded. The fourth Ferric-Reducing Antioxidant Power (FRAP) method was developed to measure the ferric reducing ability of plasma at low pH (Benzie & Strain, 1996). An intense blue colour is formed when the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous form and the absorption at 593 nm was recorded. When measuring the antioxidant activity with the DMPD method an extraordinary high activity was observed compared with other free radical scavenging activity methods. The results clearly show that the presence of some constituents in the extracts neutralizing DMPD free radicals may add to the observed values. The presence of organic acids especially citric acid in some extracts may interfere in the DMPD assay and so this assay should be used with caution with such extracts. These authors recommend the use of DPPH and FRAP assays as easy and accurate methods for measuring the antioxidant activity of fruit and vegetable juices or extracts. The DPPH method is less sensitive than the other methods for hydrophilic antioxidants while FRAP is a simple test with a wide dilution range. Results using the DPPH method further show that the interaction of a potential antioxidant with DPPH· depends on its structural conformation. Therefore this assay system may not give the true picture of total antioxidant capacity of a system.

The measurement of antioxidant activity of biological samples thus largely depends upon the free radical or the oxidants used in the assays and degree and type of substitutions (e.g. glycosylation) of different natural antioxidants. Hydrophilic and lipophilic fraction of tomato extracts behaved differently towards XOD/Xanthine and copper catalysed lipid peroxidation systems used (Lavelli *et al.*, 2000). Hence it is pertinent to use different chemical and biochemical model assays in an efficient extraction medium, instead of relying on a single assay system to assess and compare the antioxidant activity in fruits and vegetable extracts. It is noteworthy to mention that syngeristic effects and concentration may also bring effects that are not observed when individual constituents are tested.

Prospects and scope

The potential health benefits of the phytochemicals and their ability to be incorporated into foods or food supplements as nutraceuticals has impacted the food industry with a big bang (Dillard & German, 2000). The concept of functional food now poses a big challenge for the food and health industry. The food industry can play a significant role in improving nutritional density by providing designer foods that not only provide traditional nutrients (proteins, fats and carbohydrates) but also phytonutrients. The concept of antioxidant status of processed foods is gaining momentum and emerging as an important parameter to assess the quality of the product. With the expansion of the global market and fierce competition amongst multinational companies, the parameter of antioxidant activity will soon secure its place in nutritional labelling. In this context development of a practical method of determining the antioxidant activity for industrial use will become imperative. This will give a further boost to the exploitation of fruits and vegetables and development of nutraceuticals and beverages.

Most of the data now available generally relates solely to the measurement of the natural antioxidant content or to evaluation of its ability to slow down lipid oxidation in accelerated tests. They do not provide comprehensive information on overall antioxidant potential of foods arising from action of compounds via various mechanisms such as chain breaking, oxygen scavenging and metal chelation. The evaluation of total antioxidant properties of foods should therefore, take into account the measurement of these properties and their relative importance in food matrix. This will certainly help scientists, food manufacturers and consumers, as the trend of the future is to move towards functional foods with specific health benefits.

On the research front better understanding of the role and the fate of natural and process-induced antioxidants, as effected by processing methods on both, the food stability and human health is required. Furthermore research evaluating the beneficial role of phytochemicals against degenerative diseases has to be extended to *in vivo* studies besides the *in vitro* studies to draw logical conclusions.

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