Biochemical Basis for Functional Ingredient Design from Fruits

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Abstract

Functional food ingredients (nutraceuticals) in fruits range from small molecular components, such as the secondary plant products, to macromolecular entities, e.g., pectin and cellulose, that provide several health benefits. In fruits, the most visible functional ingredients are the color components anthocyanins and carotenoids. In addition, several other secondary plant products, including terpenes, show health beneficial activities. A common feature of several functional ingredients is their antioxidant function. For example, reactive oxygen species (ROS) can be oxidized and stabilized by flavonoid components, and the flavonoid radical can undergo electron rearrangement stabilizing the flavonoid radical. Compounds that possess an orthodihydroxy or quinone structure can interact with cellular proteins in the Keap1/Nrf2/ARE pathway to activate the gene transcription of antioxidant enzymes. Carotenoids and flavonoids can also exert their action by modulating the signal transduction and gene expression within the cell. Recent results suggest that these activities are primarily responsible for the health benefits associated with the consumption of fruits and vegetables.

INTRODUCTION

Fruits are important components of the human diet and are consumed in varying amounts, either fresh or in the processed form. Many vegetables are in the true sense fruits; some are harvested and used at an immature stage when they are horticulturally mature, whereas others are left to ripen until their seeds are mature (e.g., members of Cucurbitaceae, such as squash and cucumbers). Fruits that occur wild may have been consumed by human beings during early stages of evolution, and with the adoption of settled life, selection of useful fruits may have resulted in the array of various genotypes and cultivars of fruits available at present. Irrespective of their organoleptic qualities, fruits provide basic nutrition, including carbohydrates, vitamins, and minerals. Also, several secondary plant products that constitute fruit components show utility in food processing (e.g., pectin, anthocyanins, and soluble and insoluble fiber) and possess health regulatory properties. Fruit ingredients, including polyphenols (e.g., anthocyanins, flavonoids, and phenolic components), carotenoids (e.g., lycopene, carotene, and xanthophylls), and soluble and insoluble fibers, all have been linked to some form of disease preventive action in the human body (Paliyath et al. 2011, 2012). Consequently, these components, collectively termed as functional food ingredients or nutraceuticals, have been the foci of research on their health benefits and their mode of action. Thus, it is important to understand the biochemical basis of their mechanism of action to help design functional food ingredients with enhanced efficacy.

BIOCHEMISTRY OF FRUIT COMPONENTS

In general, fruits contain large amounts of water distributed in various cellular compartments. Familial characteristics provide several variations in the biochemical composition of fruits. Starch is the primary storage ingredient in fruits such as banana, mango, jack fruit, and kiwi, and during fruit ripening, the hydrolysis of starch results in softening and increase in sugar content of the fruits. Activation of metabolic pathways leads to characteristic changes in the biochemical composition of fruits. Fruits such as apple, tomato, and grape contain a high amount of organic acids, and conversion of these organic acids into sugars through gluconeogenesis increases the organoleptic attributes of these fruits. Carbohydrates, e.g., cellulose and pectin, provide firmness and cellular architecture and organization to the fruits. During fruit ripening, degradation of these polymers into smaller water-soluble molecules results in fruit softening. Enzymatic breakdown of pectin (tomato) and cellulose (avocado) leads to the generation of smaller water soluble fibers. These fibers have a tremendous influence on health as modifiers of food viscosity during its transit through the intestine and as prebiotics, promoting the growth of a health beneficial probiotic bacterial population in the large intestine.

Carbohydrates

Carbohydrates are formed by the photosynthetic reduction of carbon dioxide through several reductive pathways, including the Calvin cycle, C_4 pathway, and the Crassulacean acid metabolism. The products of CO_2 fixation and reduction are carbohydrate monomers, such as pentoses (ribose, ribulose) and hexoses (glucose, fructose), that are also channelled into other metabolic pathways. Polymerization of several sugar derivatives leads to various storage components, such as starch, a polymer of glucose, and inulin, a polymer of glucose and fructose, as well as structural components cellulose and pectin, containing a variety of sugar monomers.

Glucose and its isomer fructose, as well as their phosphorylated forms (glucose-6-phosphate, glucose-1,6-diphosphate, fructose-6-phosphate, and fructose-1,6-diphosphate), are the major components in the metabolic pool and are used for the biosynthesis of carbohydrate polymers.

Among storage forms of carbohydrates, starch is the main component in most fruits. Starch occurs in two molecular forms in the starch grain, amylose and amylopectin. There are several enzymes involved in the biosynthesis of starch, including ADP-glucose pyrophosphorylase, starch synthase, and a starch-branching enzyme involved in amylopectin biosynthesis. ADP-glucose pyrophosphorylase catalyzes the reaction between glucose-1-phosphate and ATP-generating ADP-glucose and pyrophosphate. Starch synthase uses ADP-glucose as a substrate to add glucose molecules to the amylose or amylopectin chain, resulting in an increase in their degree of polymerization. The starch branching enzyme introduces glucose molecules through α-1,6 linkages, which further get extended into linear amylose units with α-1,4 glycosidic linkages. Thus, the added glucose branch points (α-1,6 linkages) serve as sites for further elongation by starch synthase, thus resulting in a branched starch molecule, also known as amylopectin.

A characteristic difference between starch and cellulose is that the starch molecule contains glucose linked by α-1,4-glycosidic linkages, whereas cellulose is made up of glucose units linked by β-1,4-glycosidic linkages, which drastically alters the digestibility and ability to crystallize. A consequence of this is that in mammals starch is digestible by enzymes in the gastrointestinal tract, whereas cellulose can only be digested by bacteria that occur in the ruminant animals.

The cell wall is a complex structure composed of cellulose and pectin, and contains hexoses (glucose, galactose, rhamnose, and mannose) and pentoses (xylose and arabinose). A characteristic feature of pectin that cements the cellulose molecules together to form the cell wall is that it contains acidic derivatives of sugars, the glucuronic and galacturonic acids (Negi & Handa 2008). The acidic moieties in pectin enable the chelation of calcium ions forming bridges between pectin strands. This helps in rigidifying cell wall structure. A macromolecular model proposed by Keegstra et al. (1973) describes the cell wall as a polymeric structure constituted of cellulose microfibrils and hemicellulose embedded in the apoplastic matrix in association with pectic components and proteins. Cellulose is biosynthesized by β-1,4-glucan synthase enzyme complexes localized on the plasma membrane. The enzyme uses uridine diphosphate glucose (UDPG) as a substrate, and by adding UDPG units to small cellulose units, it increases the polymerization of the cellulose chain. Other polymeric structures in the cell wall include hemicelluloses, and based on their composition, they are categorized as xyloglucans, glucomannans, and galactoglucomannans. The cellulose chains assemble into microfibrils through hydrogen bonds to form crystalline structures. Pectin is biosynthesized from UDP-galacturonic acid and includes several molecular forms, such as galacturonans and rhamnogalacturonans, that form the acidic fraction of pectin. The neutral fraction of the pectin comprises polymers, including arabinans (polymers of arabinose), galactans (polymers of galactose), and arabinogalactans (containing both arabinose and galactose). All these polymeric components form a complex three-dimensional network stabilized by hydrogen bonds, ionic interactions involving calcium, phenolic components (e.g., diferulic acid), and hydroxyproline-rich glycoproteins (Negi & Handa 2008). The cell wall and pectic components contribute to the fiber fraction of the diet and are important as prebiotics for the growth of probiotic bacteria that are beneficial for colon health.

Proteins

In general, fruits are not major sources of dietary proteins. The chloroplasts and mitochondria are the major organelles that contain both structural proteins and enzymes. The structural proteins include the light-harvesting complexes in chloroplasts and the respiratory enzyme/protein complexes in mitochondria. Ribulose-bis-phosphate carboxylase/oxygenase (Rubisco) is the most abundant enzyme in photosynthetic tissues. In general, fruits do not store proteins as an energy source. Bell pepper and tomato have a higher level of chloroplast proteins.

Among dry fruits, seeds are a major source of protein. Members of the family Leguminosae (Fabaceae) produce seeds that are rich in various types and proportions of proteins. Soybeans are highly utilized for processing into a variety of functional food products. Soybeans contain proteins in the range of 40% or higher. The unique physicochemical properties of soy proteins, such as gel formation, emulsification, foaming, and hydrophilic nature, are the basis for the production of processed foods, e.g., tofu and koori-tofu (Maruyama et al. 2006).

Soy seed proteins primarily are salt-soluble globulins. On the basis of their sedimentation coefficient, soy proteins are grouped into 7S (β-conglycinin) and 11S globulins (glycinin), constituting nearly 80% of the seed proteins. The nutritional quality and processing characteristics of soy proteins are influenced by the ratio of 11S globulin to 7S globulin (Derbyshire et al. 1976, Wright 1987). Other proteins in soybean include γ-conglycinin and basic 7S globulin.

The 7S globulin or β-conglycinin is a trimeric glycoprotein with a relative molecular mass ranging from 150 kDa to 200 kDa. The three subunits designated α, α′, and β possess a core region showing high homology, whereas the α and α′ subunits possess extended regions from the core (Maruyama et al. 1998). These subunits are also glycosylated to various extents.

Glycinin or the 11S globulin comprises six subunits with a relative molecular mass ranging from 300 kDa to 400 kDa. Each subunit is composed of an acidic and a basic polypeptide, the acidic moiety having a molecular mass ranging from 30 kDa to 40 kDa, and the basic polypeptide having a molecular mass of 18 kDa to 22 kDa. The acidic and basic chains are linked by a disulfide bond. Glycinins may have great variation, as they are often constituted by several subunits in various proportions, giving rise to the diversity (Utsumi et al. 1981). The functional diversity of soybean proteins may also arise from such diversity.

Lipids

Other than avocado and olive, fresh edible fruits are not rich sources of storage lipids. These two fruits are rich in triacylglycerols, and several health benefits are related to the consumption of avocado and olive oil. Phospholipids, sterols, and waxes that provide an external barrier to the fruits are the major forms of lipids in fruits. The major phospholipids that constitute the biomembranes include phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol. As the fruit ripens, the phospholipids are degraded releasing the fatty acids (Paliyath et al. 2008b). In fruits rich in chloroplasts, chloroplast degradation leads to the accumulation of plastoglobuli that are rich in lipids. Monogalactosyl diacylglycerols and digalactosyl diacylglycerols in the thylakoid membrane are broken down to release fatty acids. The major fatty acids in fruits include palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and lower levels of linolenic (18:3) acids. Oleic, linoleic, and linolenic acids show increasing degrees of unsaturation.

The oil content of avocado varies among various cultivars. This ranges from 2.5% to 8% in West Indian cultivars, 10% to 13% in Guatemalan cultivars, and 15% to 25% or higher in Mexican cultivars by fresh weight (Knight 2002). Avocado fruits of varieties grown in California, such as Fuerte and Hass, have yielded oil at 15% to 25% or higher. By contrast, olives used for oil extraction can yield 18% to 25% oil. In general, avocado oil and olive oil are rich in monounsaturated fatty acids, such as oleic acid, with lower levels of palmitic acid, linoleic acid, and palmitoleic acid. These four fatty acids constitute 95% of the lipid fraction in avocado, with oleic acid (C18:1) ranging from 40% to 80%, palmitic acid (C16:0) ranging from 7.2% to 25%, linoleic acid (C18:2) ranging from 6% to 18%, and palmitoleic acid (C16:1) ranging from 0% to 8%. By comparison, olive oil contained primarily oleic acid (83%), linoleic acid (7%), palmitic acid (6%), and stearic acid (C18:0; 4%). Several cardiovascular health benefits of avocado and olive oil have been attributed to the high proportion of oleic acid.

Secondary Plant Products

Secondary plant products constitute a diverse array of chemical compounds synthesized by the plants for various physiological purposes, including attraction through color and smell, and protection from pathogens and environmental conditions. As hunter-gatherers humans could have adapted to the consumption of plants and their products with aesthetic appeals, which may have provided them nutrition as well as protection from diseases. Horticultural selection of appealing plants during the transition to agrarian habits may have helped the adoption of several cultivars of fruits and vegetables used today. Anthocyanins are major color components in several fruits, including berries, e.g., blueberry, elderberry, grape; pome fruits, e.g., apple; and tender fruits, e.g., cherry and plum. Vegetables that are purple in color (such as red cabbage, purple carrots, and purple tomatoes) also contain high levels of anthocyanins. In yellow tomato and carrot, carotenoid derivatives, e.g., β-carotene and xanthophylls, are the major component that provide the yellow-red coloration. Ripe tomato fruits and the reddish interior pulp of ripe watermelon are rich in lycopene. The anthocyanins and carotenoids provide a range of health benefits. By virtue of their structure, they are strong antioxidants. β-carotene is catabolized into vitamin A derivatives (retinol, retinal) in the human body. These components can modulate the biochemical processes in the cell as well as influence the activity of signal transduction systems and gene expression, resulting in positive health benefits. Anthocyanins and carotenoids are only a few of the several classes of secondary plant products. Others are nonprotein amino acids, alkaloids, isoprenoid components (terpenes), ester volatiles, and several additional organic compounds with diverse structures. Information on the health benefits of several of these components has recently been published (Paliyath et al. 2011).

Flavonoids and anthocyanins. Flavonoids are one of the most ubiquitous groups of compounds among secondary plant products. Greater than 4,000 structural variations of flavonoids have been identified, most of which possess multiple physiological functions in plants and health regulatory functions in humans. The flavonoid moiety contains three ring structures designated A, B, and C (**Figure 1**). The A and C rings form the benzopyran nucleus of the molecule, with the 3, 5, and 7 positions being hydroxylated and further conjugated to sugars. The B ring is linked to the C ring at the 2 position. Flavonoids are grouped into flavonols, flavones, flavanols, flavonones, anthocyanidins, and isoflavonoids. Flavonoids have been reported to show several health regulatory functions, including antibacterial, antiviral (Ratty & Das 1988), anti-inflammatory, antiallergic (Hanasaki et al. 1994, Hope et al. 1983, Middleton & Kandaswamy 1993), and vasodilatory actions (Duarte et al. 1993). They also inhibit lipid peroxidation (Ratty & Das 1988, Salvayre et al. 1988), platelet aggregation (Beretz et al. 1986, Beretz & Cazenave 1988, Bourdillat et al. 1987, Gryglewski et al. 1987, Tzeng et al. 1991), capillary permeability and fragility (Torel et al. 1986), and the activity of enzymes, such as cyclooxygenase and lipooxygenase (Hodnick et al. 1988, Hope et al. 1983), involved in inflammation. Multiple roles of flavonoids have been related to their ability to act as antioxidants (Cavallini et al. 1978, Fraga et al. 1987, Hanasaki et al. 1994, Robak & Gryglewski 1988), metal ion chelators (Afanas'ev et al. 1989), and modulators of cell biochemistry and gene expression (Andersen & Jordheim 2006).

Chalcones and dihydrochalcones have open ring structures. Their occurrence in the free form is generally rare (Tomás-Barberán & Clifford 2000). Chalcones may undergo prenylation as observed in xanthohumol, which is a component found in hops and beers (Zhao et al. 2005). Apigenin and luteolin are the most common flavones (Valant-Vetschera & Wallenweber 2006) and are found in vegetables, including celery and parsley. Flavones possess a double bond between positions 2 and 3, and a keto group at position 4 of the C ring. Hydroxyl groups are also observed at positions

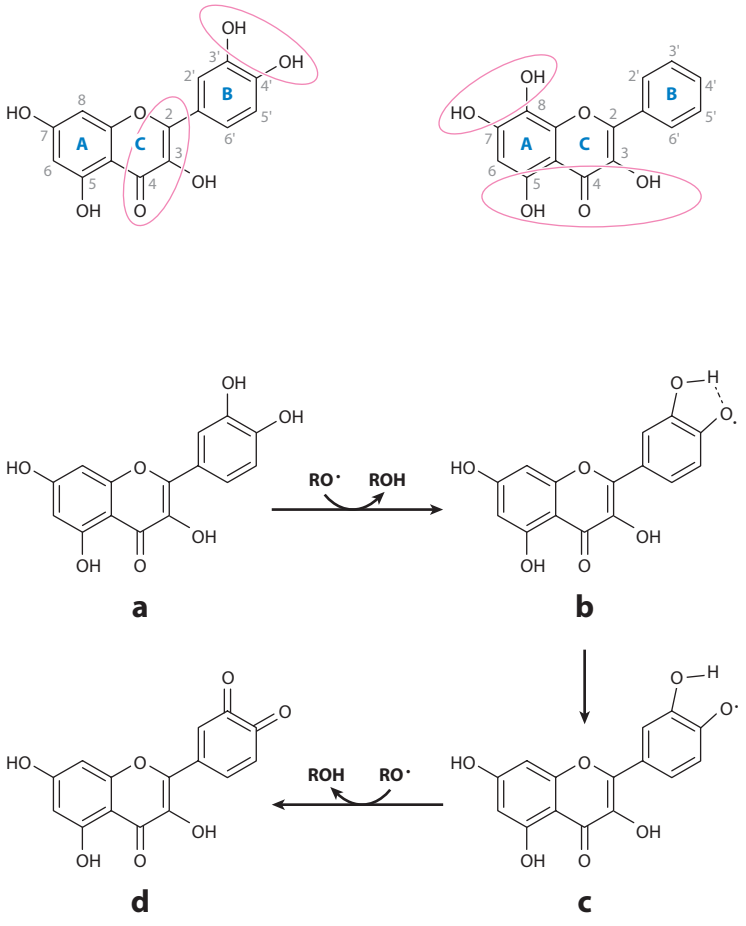

Figure 1

Basic structural features that characterize antioxidant functionality in polyphenols. (*top*) Areas circled in pink show features that are critical for efficient antioxidant function. Letters A, B, and C denote the ring structures of the flavonoid moiety (A and C for the benzopyran part of the molecule, and B for the phenolic ring attached to position 2 of the benzopyran ring). Structures (*a–d*) depict oxidation-dependent changes in the structure of flavonoids. (*b*) Structure depicted shows a flavonoid radical stabilized by a hydrogen bond. (*d*) Further oxidation of the flavonoid radical leads to the formation of an orthodiquinone.

5 and 7 of ring A, and 3′ and 4′ positions of ring B. Tangeretin and nobiletin, found in the peels of citrus fruits, are polymethoxylated. Flavonols, quercetin and kaempferol, are nearly ubiquitous in fruits, vegetables, and grains (Valant-Vetschera & Wallenweber 2006). Structurally, flavonols possess a hydroxyl group on position 3 of ring C as compared with the flavones. This is a common site for glycosylation among flavonoids.

Flavanones and flavanonols are structurally similar to flavones except that the bond between positions 2 and 3 in ring C is saturated. Flavanones that are hydroxylated at position 3 in the C ring are termed as flavanonols, e.g., Taxifolin in citrus fruits (Grayer & Veitch 2006, Kawaii et al. 1999).

Among flavonoids, catechins, or flavan-3-ols, form a highly diverse group with complex structures and health benefits, and include compounds such as tea polyphenols and cocoa.

Characteristically, catechins possess a hydroxyl group attached at position 3 of the C ring. Catechins exist as epimers because the hydroxyl bond exists either above or below the plane of the ring, giving rise to two epimeric forms, (−)-epicatechin and (+)-catechin. Tea polyphenols, gallocatechin, and epigallocatechin contain an additional hydroxyl group on ring B (Harnly et al. 2006). Green tea is enriched in gallic acid esters of the catechins, whereas black tea contains theaflavin and its derivatives, which also exist as polymers. Flavanols can exist as polymeric forms, generally referred to as procyanidins, proanthocyanidins, or condensed tannins. Procyanidins may be 2 to 60 units long linked by a carbon-carbon bond between the position 8 carbon (C8) of the terminal unit and C4 of the neighbor, in a sequential fashion. Isoflavones form a unique subgroup of flavonoids with their B ring characteristically linked to position 3 of the C ring. Soy or soy products are highly enriched in isoflavones (daidzein, genistein) (Lee et al. 2007).

Apart from the typical flavonoids, there are several other polyphenolic components that provide antioxidant and health regulatory functions. Pterostilbenes are an important group of polyphenols. Red grape and wine contain resveratrol, a stilbene that is a strong antioxidant and shows the ability to alter gene expression and delay aging (Calabrese et al. 2008). Ellagitannins are highly active antioxidant components present in pomegranate, strawberry, and several other berry fruits.

Red wine has traditionally been used as a source of flavonoids, especially among the Mediterranean population. "French Paradox" is a term coined for the low incidences of cardiovascular mortality observed among the Mediterranean population associated with the dietary habit of red wine consumption. Red wine polyphenols inhibit low density lipoprotein (LDL) oxidation in vitro (Kondo et al. 1994) and increase antioxidant capacity in humans (Whitehead et al. 1995). Red wine polyphenols also decrease breast cancer cell proliferation in vitro (Damianaki et al. 2000) and delay the onset of mammary tumors in transgenic mice (Clifford et al. 1996). Selective cytotoxicity of red wine polyphenols to MCF-7 human breast cancer cell lines, with marginally cytostatic response toward normal cells has been reported (Hakimuddin et al. 2004, 2006, 2008). These studies show that consumption of foods enriched with red wine polyphenols may provide increased health benefits. As well, the selective cytotoxicity of polyphenolic compounds is detrimental to breast cancer cells, and hence, this specificity could be useful in adjuvant cancer therapeutics.

Among the fruits and vegetables commonly consumed, grapes and their products, including wine, juice, and raisins, may be the most important source of dietary anthocyanins (Wang et al. 1997). Grapes, wines, and grape byproducts contain large amounts of phenolic compounds, mostly flavonoids at high concentrations ranging from 1,000 to 1,800 mg kg^{-1} (Macheix et al. 1990, Singleton 1982). Large amounts of anthocyanins derived from red grapes contribute to the anthocyanin content of red wine, and these have been shown to contribute to a strong protection against plasma LDL oxidation (Frankel et al. 1995). Studies evaluating the antioxidant effects of specific phenolics toward LDL oxidation support these observations (Teissedre et al. 1996, Tera et al. 1994).

Grape flavonoids have been ingested from the fruits and various grape products for centuries and are considered relatively nontoxic (Waterhouse & Walzem 2005). Grapes are rich sources of phenolic compounds, both flavonoids and nonflavonoids. The most abundant classes of grape flavonoids include flavan-3-ols (catechins), anthocyanins (in red grapes), and flavonols, whereas the most abundant class of nonflavonoids is the hydroxycinnamates (Waterhouse & Walzem 2005). Grapes also contain small amounts of gallocatechins. However, in most cases the largest proportion of flavan-3-ols is found in the oligomeric (procyanidins) and polymeric forms (condensed tannins). Flavan-3-ols are not found in grapes as glycosides.

Anthocyanin pigments cause the diverse coloration of grape cultivars, resulting in skin colors varying from translucent to red and black (Nunez et al. 2004). All the forms of anthocyanins, along with those with modifications of the hydroxyl groups, are routinely present in the red and dark

varieties of grapes. Anthocyanins predominantly exist in grapes as glucosides formed through the conjugation of the flavonoid component, an anthocyanidin, with glucose. The sugar component increases the chemical stability and water solubility of the anthocyanidin. Each anthocyanin may be further complexed with acetic acid, coumaric acid, or caffeic acid, which are bonded to the sugar component (Nunez et al. 2004). Differences between individual anthocyanins include the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, the nature and number of sugars attached to the molecule and the nature and number of aliphatic or aromatic acids attached to the sugars in the molecule. Hydroxylation and methylation pattern of the B ring give the characteristic differences in properties between various anthocyanins. The structures of anthocyanin aglycones are consistent for all grapes and primarily include malvidin and varying amounts of delphinidin, cyanidin, peonidin, and petunidin. In the red wine varieties, e.g., merlot, pinot noir, and cabernet sauvignon, anthocyanin content may vary between 1,500 and 3,000 mg kg^{-1} fresh weight. In some high anthocyanin–containing varieties, such as Vincent, Lomanto, and Colobel and their hybrids, the anthocyanin levels can exceed 9,000 mg kg^{-1} fresh weight (Jacob et al. 2008).

Many fruits have a tart taste during the early stage of development, which is termed astringency and is characteristic of fruits such as banana, kiwi, and grape. The astringency is due to the presence of tannins and several other phenolic components in fruits. Tannins are polymers of flavan-3-ols (catechin and epicatechin) and phenolic acids (such as caffeoyl tartaric acid and coumaroyl tartaric acid). Tannin content decreases during ripening, making the fruit palatable.

Isoprenoids and carotenoids Isoprenoids possess a basic five carbon skeleton in the form of 2-methyl-1,3-butadiene (isoprene), and larger molecules contain multiple numbers of this basic unit. There are two separate pathways through which isoprenoids are biosynthesized, namely the acetate/mevalonate pathway (Bach et al. 1999) localized in the cytosol and the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway, localized in the chloroplast (Rohmer pathway) (Rohmer et al. 1993). Isoprenoids derived through the acetate/mevalonate pathway are biosynthesized by the condensation of three acetyl CoA molecules to form HMG-CoA, (3-hydroxy-3-methyl-glutaryl CoA). HMG-CoA undergoes further reduction by the key regulatory enzyme of the pathway HMG CoA reductase, forming mevalonate. A two-step phosphorylation of mevalonate by kinases generates isopentenyl pyrophosphate (IPP), which serves as the basic five carbon condensational unit of terpenes. Further, isomerization of IPP to dimethylallylpyrophosphate (DMAPP) is mediated by the enzyme IPP isomerase. Condensation of IPP to DMAPP results in the synthesis of C_{10} (geranyl) pyrophosphate, and sequential addition of further IPP molecules results in the formation of C_{15} (farnesyl) and C_{20} (geranylgeranyl) pyrophosphates. Monoterpenes are formed from the C_{10} pyrophosphates, sesquiterpenes from C_{15} pyrophosphates, and diterpenes from C_{20} pyrophosphates. Monoterpenes are major volatile components of several fruits, especially citrus fruits. In citrus fruits, these components include limonene, myrcene, and pinene, among others. Monoterpenes can further be metabolically converted into derivatives, such as geranial, neral (aldehydes), geraniol, nerol, linalool, terpineol (alcohols), and esters that include geranyl acetate and neryl acetate. Monoterpenes, e.g., limonene, and their derivatives show strong anticancer properties (Crowell et al. 1994, Crowell & Gould 1994, Elegbede et al. 1984, Gould 1997, Stark et al. 1995).

Carotenoids, which are major isoprenoid components in the human diet, are biosynthesized through the Rohmer pathway from the precursors pyruvate and glyceraldehyde-3-phosphate. DOXP is a key metabolite formed by the condensation of these two molecules. Enzymatic reduction of DOXP ultimately leads to the formation of IPP. Just as in the acetate-mevalonate pathway, subsequent condensation of IPP and DMAPP results in the formation of several types

of isoprenoids. The color changes in fruits during ripening result from the changes in the relative composition of carotenoids, unmasking the brightly colored xanthophyll pigments. Lycopene is the major carotenoid pigment that accumulates during ripening of tomato. Lycopene is formed by the condensation of two geranylgeranyl pyrophosphate (C_{20}) moieties catalyzed by the enzyme phytoene synthase. In young fruits, lycopene is converted to β-carotene by the action of the enzyme sesquiterpene cyclase. However, during ripening, the levels and activity of sesquiterpene cyclase are decreased, which results in the accumulation of lycopene in the chromoplast providing the red color. Carotene biosynthesis is not inhibited in yellow varieties of tomatoes, and during fruit ripening, chlorophyll pigments are degraded, exposing the yellow carotenoids. β-carotene is a major pigment in melons with an orange flesh. The melons may also contain other carotenoids, such as α-carotene, δ-carotene, phytofluene, phytoene, lutein, and violaxanthin. Lycopene is the predominant component in red-fleshed melons, whereas in yellow-fleshed melons, xanthophylls and β-carotene predominate. In general, carotenoids are strong antioxidants. Lycopene has been suggested to provide protection from cardiovascular diseases and cancer (Giovannucci 1999). Lutein, a xanthophyll, has been proposed to play a protective role in the retina, maintaining vision and preventing age-related macular degeneration.

FUNCTIONAL PROPERTIES OF INGREDIENTS

From the breadth of action of the functional ingredients in fruit, it is conceivable that the structural diversity of various components inherently results in their functional diversity. From the voluminous literature available at present, we are able to understand the unique aspects as well as the similarity in the mechanisms of action of various components. Many studies have been conducted under in vitro conditions, and the conclusions drawn from such studies may not be fully extendable into in vivo conditions. For example, several functional ingredients have been proposed to be strong antioxidants by their ability to scavenge ROS. However, absorption of these components may be extremely low as observed in the case of polyphenols. So, the question arises as to whether these components may act as antioxidants in vivo. It has become increasingly clear that functional ingredients may act by a variety of mechanisms within the body: as antioxidants, as modulators of biochemical reactions and signal transduction systems, and as regulators of gene expression (Paliyath et al. 2011).

Antioxidant Function

Oxidative stress is imposed on the body's cells when the formation of reactive oxygen species (ROS) overwhelms the bodily defense mechanisms mediated through antioxidants and antioxidant enzyme systems. Oxidative stress from reactive oxygen species generated through various mechanisms is always present in organisms that are dependent on oxygen for their life processes. Continued existence of stress and ROS can result in damage to cell membranes, proteins, and DNA. Increased production of ROS has been linked to the development of several chronic degenerative diseases. Cardiovascular diseases, including ischemia-reperfusion injury, cancer, diabetes, neurodegenerative diseases, arthritis, and inflammatory diseases, (e.g., inflammatory bowel disease), have all been linked to oxidative stress (Ames et al. 1993, Aw 1999, Rezaie et al. 2007, Roessner et al. 2008, Siegers et al. 1984).

Utilization of oxygen for life processes invariably results in the generation of ROS during biochemical reactions. ROS and the byproducts generated from such processes can negatively affect the cellular homeostasis through disruption of structure/function of constituent molecules (Harris 1992). In eukaryotes, superoxide anion ($O_2^{\cdot -}$) is primarily produced when electrons leaking

from the mitochondrial electron transport chain reduce oxygen during tissue injury by xanthine oxidase via auto-oxidation reactions in the presence of transition metal ions, during cytochrome P450 cycling, and at inflammatory sites by activated neutrophils and phagocytes via reduced nicotinamide adenine dinucleotide phosphate oxidase (Halliwell & Gutteridge 2007a). Under physiological conditions, it is estimated that of the 90% of inhaled oxygen consumed by the mitochondria, approximately 1% to 3% of the oxygen molecules are converted into superoxide (Brookes et al. 2002), with H_2O_2 formed almost immediately following $O_2^{\cdot -}$ production.

Hydrogen peroxide can react with Fe^{2+} or Cu^{+} via the Fenton reaction to form the extremely damaging hydroxyl radical ($^{\cdot}OH$) (Imlay et al. 1988). During inflammation, the enzyme myeloperoxidase in phagocytes catalyzes the formation of HOCl from H_2O_2 or $^{\cdot}OH$ (Proctor 1996). Activated phagocytes are also induced to produce high sustained levels of nitric oxide radical ($NO^{\cdot}$) (Carr et al. 2000, Mayer & Hemmens 1997), which react with $O_2^{\cdot -}$ to produce the powerful reactive nitrogen species, peroxynitrite ($OONO^-$). $OONO^-$ can directly oxidize lipoproteins (Heinecke 1998) or give rise to other species that promote oxidation (Eiserich et al. 1998, Radi et al. 2001).

The inability of the body's immune system to control and downregulate ROS and inflammation is a common feature of chronic inflammatory conditions. The ROS can react with the unsaturated bonds in membrane lipids, causing lipid peroxidation and affecting membrane fluidity and function. Another consequence of membrane fluidity changes is the loss of receptor and transport protein function and membrane leakage of ions ultimately leading to cell lysis (Paliyath & Thompson 1988). Sulfur-containing amino acids in enzymes and proteins are prime targets of ROS, resulting in cross-linking, inactivation, and denaturation of the target molecules. DNA is highly susceptible to ROS attack, which can cause mutations that may lead to the development of cancer (Grisham & McCord 1986, Halliwell & Gutteridge 1985, Sies & Cadenas 1985, Slater 1972).

ROS include molecules with differing degrees of reactivity. These can be in the form of free radicals or nonradicals; however, they all contain oxygen in an activated form. Common ROS observed in biological systems include $O_2^{\cdot -}$, hydrogen peroxide (H_2O_2), $^{\cdot}OH$, and hypochlorous acid (HOCl). The dismutation of the ROS depends on the pH of the biological environment, the availability of chemical antioxidants, and the levels and activities of endogenous antioxidant enzymes and their cofactors, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase, and glutathione peroxidase (GPX). Hydrogen peroxide, formed from superoxide by SOD action, is membrane permeable through aquaporin channels (Bienert et al. 2007). Thus, H_2O_2 can alter the function of redox-sensitive signaling molecules, such as kinases, and transcription factors, including nuclear factor kappa-B (NFκB) and activator protein-1 (AP-1), affecting cell signaling and gene expression (Halliwell & Gutteridge 2007b). In Caco-2 human intestinal adenocarcinoma cells, an increased synthesis of interleukin-8, a proinflammatory cytokine under the transcriptional control of NFκB, has been observed in response to H_2O_2 (Yamamoto et al. 2003).

Antioxidant function of biological molecules can delay or prevent the oxidation of substrates, including DNA, proteins, and lipids, even when they usually occur at lower concentrations than oxidizable substrates (Halliwell & Gutteridge 2007a). In biological systems, there are a variety of components that protect the system from ROS. These reactions may be independent or through coordinated action of several antioxidant enzymes. SOD converts the superoxide radical to H_2O_2 and O_2. CAT converts H_2O_2 to O_2 and H_2O. SOD occurs as several isozymes in the chloroplast, mitochondria, and cytosol, whereas CATs are present in mitochondria and peroxisomes. The antioxidant enzymes SOD, GPX, and CAT work in tandem to remove most superoxides and peroxides before they react with metal ions to form more reactive free radicals, e.g., $^{\cdot}OH$. GPX is the major enzyme that removes the superoxide generated by SOD in the mitochondria and

cytosol by oxidizing glutathione (GSH) into its oxidized form (glutathione disulfide). Peroxidative chain reactions initiated by free radicals that escape the antioxidant defenses are terminated by chain-breaking water- or lipid-soluble antioxidants (vitamins C and E) (Mates et al. 1999).

Diet-derived antioxidants and health benefits. Diet is a major source for several essential molecules that act in conjunction with antioxidant enzymes providing protection from the ROS. These include vitamins C and E; carotenoids, such as β-carotene, which is also a precursor for vitamin A; other carotenoids, including lycopene and xanthophylls; flavonoids; and essential minerals (selenium and zinc) that act as cofactors for enzymes. For example, zinc is a cofactor of SOD and selenium is a cofactor of GPX. Polyphenols, especially flavonoids and derivatives, have gained increasing interest because of their numerous biological effects, such as free-radical scavenging, modulation of enzyme activities, and inhibition of cell proliferation, as well as their potential utility as antibiotic, antiallergic, and anti-inflammatory agents (Bravo 1998, Clifford & Brown 2006). They may be involved in the prevention of cardiovascular diseases, cancers, and other degenerative diseases (Scalbert et al. 2005). Because of their wide distribution in foods and beverages of plant origin, polyphenols can be considered as common micronutrients in human diet. Total polyphenol intake may vary depending on food habits and has been estimated to be anywhere between less than a gram to more than 5 g (Clifford & Brown 2006, Saura-Calixto et al. 2007, Saura-Calixto & Diz Rubio 2007). This intake is mainly constituted by hydroxycinnamates and flavonoids, which account for approximately 1/3 and 2/3 of the total intake, respectively (Manach et al. 2004). It has been reported that the level of intake of flavonoids from diet is considerably high as compared with those of vitamin C (70 mg day^{-1}), vitamin E (7–10 mg day^{-1}), and carotenoids (β-carotene: 2–3 mg day^{-1}) (Yamasaki et al. 1997). The variations in consumption of foods and beverages containing flavonoids are mainly responsible for such differences (Clifford & Brown 2006).

Antioxidant properties of flavonoids. Flavonoids have been reported to be efficient scavengers of free radicals, such as $^{\cdot}OH$, $O_2^{\cdot -}$, and lipid peroxide radicals ($LOO^{\cdot}$) (Bors et al. 1990, Cotelle et al. 1996, Hanasaki et al. 1994, Young et al. 2011). Consumption of polyphenol-rich foods has been promoted as a means to prevent the development of chronic diseases as well as reduce the mortality rates caused by chronic diseases. Biochemical actions of flavonoids include the catalytic inhibition of enzymes, including xanthine oxidase (Hanasaki et al. 1994), protein kinases (Ursini et al. 1994), enzymes activated by calmodulin (Pinhero & Paliyath 2001, Paliyath & Poovaiah 1985), cyclooxygenase, lipoxygenase, and NADPH oxidase, all involved in the generation of ROS and the promotion of inflammation. (Brown et al. 1998, Dangles & Dufour 2006, Kandaswami & Middleton 1994, Korkina & Afanas'ev 1997). Flavonoids are efficient chelators of trace metal ions, iron and copper, which enhance the formation of ROS. For example, the Fenton reaction involves the reduction of hydrogen peroxide to highly aggressive $^{\cdot}OH$ in the presence of iron:

$$H_2O_2 + Fe^{2+}(Cu^{+}) \rightarrow {}^{\cdot}OH + OH^{-} + Fe^{3+}(Cu^{2+}).$$

Copper can enhance the action of iron. During ROS scavenging, α-tocopherol can be converted into the α-tocopheroxy radical. Flavonoids may participate in the regeneration of the tocopheroxy radical (Rice-Evans 1995). During the radical scavenging reaction, the flavonoid molecule serves as a donor of a hydrogen atom to the reacting radical as shown in **Figure 1**. The ROS, such as $O_2^{\cdot -}$, $^{\cdot}OH$, peroxyl, or alkoxyl radical, are indicated as $R^{\cdot}$. The flavonoid free radical formed during the reaction possesses more stability than the ROS that donates the electron because of the delocalization of electrons in the benzene ring (**Figure 1**).

As a rule of thumb, the higher the degree of hydroxyl groups present in the flavonoid ring, the stronger will be the free radical scavenging efficiency of a flavonoid. Methylation of the free

hydroxyl groups may result in the reduction of antioxidant activity. Additional structural features that can enhance the free radical scavenging activity include a catechol or pyrogallol group in ring B as observed in anthocyanins, a combination of a double bond at C_2-C_3 and an OH group at C_3, and an oxonium ion (O^+) on ring C (Bors et al. 1990, Cao et al. 1997, Cotelle et al. 1996, Sichel et al. 1991, van Acker et al. 1996).

Antioxidant activity of anthocyanins. Anthocyanins show strong antioxidant activity against free radicals generated during in vitro assays. Because of the hydrophobic nature of the ring structure, anthocyanins can prevent lipid peroxidation in different lipid environments from those existing in biomembranes and lipids that are bound to human LDLs (Rice-Evans et al. 1996, Wang et al. 1997). Anthocyanins may exist in protonated, deprotonated, hydrated, and isomeric forms, and the relative proportion of these molecules is strongly dependent on pH. The red flavylium cation is the predominant form that exists at very acidic pH (pH 1 to pH 3). In aqueous media, as the pH is increased to a physiological range of 4 to 5, hydration reactions generate the colorless carbinol pseudobase, which may undergo ring fission, generating the light yellow chalcones. The flavylium cation can be transformed to quinone bases through sequential losses of protons and formation of quinone structures. These forms may play an important stabilizing role in the antioxidant action of anthocyanins (Dangles et al. 2000, Lapidot et al. 1999).

The completely conjugated structure of anthocyanins that allows electron delocalization results in very stable radical products that can accommodate extra electrons and still be stable (van Acker et al. 1996). The degree and position of hydroxylation and methoxylation in the B ring affect their stability and reactivity (Pereira et al. 1997) and thereby their antioxidant action (Rice-Evans et al. 1996, Satue-Gracia et al. 1997). The hierarchy of antioxidant activities of fruits and vegetables showed that fruits that are rich in anthocyanins have a higher antioxidant potential than those rich in flavonones, flavanols, and hydroxycinnamates. When antioxidant activity of different phenolic fractions separated from Italian red wine were compared, it was found that anthocyanin fraction was the most effective in scavenging ROS and in inhibiting lipoprotein oxidation and platelet aggregation (Ghisseli et al. 1998, Andersen & Jordheim 2006).

Antioxidant activity of peptides and proteins. Proteins are easily attacked by ROS by virtue of the delocalization of electrons in the peptide bond and the aromatic and heterocyclic side chains that can accommodate extra electrons. Supramolecular electron transport was proposed to exist in biological systems that enabled electron transfer across peptide bonds and hydrogen bonds (Ramasarma 1999, Wang et al. 2009). Bioactive peptides and proteins may also protect unsaturated membrane fatty acids from peroxidation by being proximal to the site of ROS generation (Elias et al. 2008). Proteins and peptides can act as chelators of pro-oxidant metal ions, e.g., iron, through anionic amino acid side groups (e.g., aspartate and glutamate) (Dean et al. 1997, Elias et al. 2008). Soy peptides show increased antioxidant activities when compared with the intact proteins. Peptides derived by enzymatic digestion of soy β-conglycinin and glycinin possessed three to five times higher radical-scavenging activities as compared with the unhydrolyzed proteins (Chen et al. 1998). Amino acids with an imidazole side chain (His) can chelate metal ions strongly and increase antioxidant activity of peptides that contain His (Saito et al. 2003). Soy peptides also prevented the formation of thiobarbituric acid reactive substances and liposome oxidation (Peña-Ramos & Xiong 2002) under in vitro conditions. Soy protein–derived peptides have been observed to bind bile acids and stimulate immune function. Binding and excretion of cholesterol from the intestine is a key step in the reduction of cholesterol levels in the body (Maruyama et al. 2006). Consumption of food containing proteins with similar functionalities is a key step for reducing cardiovascular diseases.

Biochemical and Molecular Mechanism of Action of Functional Food Ingredients

Polyphenols and other nutraceutical ingredients have been proposed to exert their biological actions through various mechanisms. These include direct free radical scavenging; downregulation of radical production through elimination of radical precursors and metal chelation; inhibition of enzymes, such as xanthine oxidase involved in ROS production; and elevation and sparing of other endogenous antioxidants (Di Silvestro 2001). Apart from these, nutraceutical ingredients modulate activity of signal transduction systems and mitochondrial function (Paliyath et al. 2011). Biological effects of nutraceutical ingredients result from all of the above activities.

The direct antioxidant action of polyphenols is a result of their peroxidation chain breaking ability. Chain breakers donate a proton to the free radical or accept an electron, and the whole molecule can be stabilized through a delocalized electron system. Vitamins C and E and nutraceuticals, e.g., polyphenols and carotenoids, can act as chain breakers during free radical–mediated chain reactions (Guo et al. 2009, Pietta 2000, Rice-Evans et al. 1996). Upregulation of the expression of antioxidant enzymes, GPX, CAT, and SOD, as well as the inhibition of xanthine oxidase, which is involved in the generation of ROS, can help in lowering the production of ROS (Du et al. 2007). Curcumin and flavonoids enhance the in vivo synthesis of GSH (Biswas et al. 2005, Li et al. 2006, Molina et al. 2003, Myhrstad et al. 2002). The increase in GSH levels in various cell types subjected to polyphenol treatment results from an increased transcription of the γ-GSS (glutathione synthase) gene (Moskaug et al. 2005, Myhrstad et al. 2002). GSH has multifunctional roles, including being a reducing agent and a cofactor in enzyme function, as well as playing a role in the chemical antioxidant defense and the detoxification of xenobiotics.

Dinkova-Kostova & Talalay (2008) categorizes antioxidants into two types, the direct antioxidants, which are short-lived redox-active molecules and are either replenished through diet or regenerated via innate enzyme systems, and indirect antioxidants, which may or may not show redox activity. Sulphoraphane found in broccoli, and curcumin in turmeric, show multiple modes of action. These molecules contain Michael acceptor functionalities (olefin or acetylene structure conjugated to electron withdrawing groups) as well as phenolic hydroxyl groups (with higher efficiency if located in ortho positions) that can scavenge ROS or nitrogen-centered free radicals (Dinkova-Kostova et al. 2007). In addition to being direct antioxidants, such molecules also act as inducers of cytoprotective enzymes involved in detoxification of xenobiotics. They can activate the Keap1/Nrf2/ARE (Kelch ECH-associating protein 1/NF-E2-related factor 2/antioxidant response elements) pathway (Dinkova-Kostova & Wang 2011, Kensler et al. 2007, Wang et al. 2010), resulting in increased expression of phase 2 enzymes (enzymes involved in glucuronylation and sulfation of xenobiotics hydroxylated by phase 1 enzymes, resulting in highly water soluble molecules targeted for secretion outside the body). This is a key pathway involved in cytoprotection from ROS and inflammation and helps to prevent the development of chronic degenerative diseases. The mechanism of action of sulphoraphane potentially involves modification of key cysteine residues on Keap1, which enables the dissociation of Nrf-2 from the activated complex, and its migration into the nucleus, where it activates AREs (Young-Hoon et al. 2010). However, the dithiocarbamate group formed during the reaction between the isothiocyanate group of sulphoraphane and the thiol groups in Keap1 is not stable, and the validity of the hypothesis was not substantiated. Synthesis of a number of sulphoraphane analogs by replacing the reactive isothiocyanate group with sulfoxythiocarbamate enabled the formation of stable thiocarbamate adducts with the cysteine thiol groups of Keap1. Evaluation of 24 sulphoraphane analogs that retained key structural features using various cell lines (hepatoma, retinal pigment epithelial cells, and keratinocytes) and mouse skin under stress conditions resulted in the efficient induction of

phase 2 enzymes proving the efficiency of sulfothiocarbamate analogs as ingredients as efficient as sulphoraphane (Young-Hoon et al. 2010). Other structures, such as an acetylenic tricyclic bis (cyano enone), having two highly electrophilic Michael acceptors and synthetic triterpenoids have also been found to be potent activators of phase 2 enzymes (Benasson et al. 2010, Dinkova-Kostova et al. 2010).

Polyphenols, including flavonoids and anthocyanins, that contain orthodihydroxy groups have also been found to stimulate the transcription of genes encoding antioxidant enzymes through the Keap1/Nrf2/ARE pathway and thereby enhance detoxification (Chen et al. 2000, Dinkova-Kostova & Talalay 1999). Polyphenols may influence these pathways mediated through ARE binding, either by modifying the ability of Keap1 in sequestering Nrf2 and/or by its activation of MAPK proteins (ERK, JNK, and p38) that are involved in the stabilization of Nrf2 (Masella et al. 2005). The activated form of Nrf2 migrates to the nucleus and binds to the ARE-containing promoters of genes, encoding antioxidant enzymes SOD, CAT, γ-GSS, glutathione-S-transferase (GST), glutathione reductase, and GPX. The green tea polyphenol EGCG (epigallocatechin gallate) has been proposed to conjugate with GSH, causing the disruption of the cell's redox status. This in turn can activate protein kinase pathways that trigger Nrf2 phosphorylation on serine/threonine residues and enable enhanced nuclear migration as well as binding to the ARE promoting transcriptional activity of antioxidant and phase II enzymes (Andreadi et al. 2006, Na et al 2008, Wu et al. 2006). EGCG induction of antioxidant enzymes was mediated through Akt/ERK1/2 (non-specific serine/threonine kinase of the Akt family, protein kinase B/extracellular signal-regulated kinase) signaling pathways in mammary cells. EGCGs that possess orthodihydroxy structure may also directly interact with Cys residues of Keap1, promoting the dissociation and nuclear migration of Nrf2. (Na et al. 2008).

Structural features of polyphenols that enable these mechanisms are intriguing (Benasson et al. 2008). The expression of cytoprotective enzymes, GST or NADP(H)-quinone oxidoreductase (NQO1), is a common feature of cancer-preventive molecules, diphenols and phenylpropanoids, including flavonoids. The efficiency of induction of NQO1 by these agents was considered to be linearly correlated with their relative ability to release an electron. It was proposed that the induction of NQO1 can be considered as a two-step process; the first step involves the oxidation of diphenols into quinones, and the second involves the oxidation of thiol groups in Keap1 involved in Nrf activation. Through quantum mechanical calculations, one can predict the efficiency of oxidation of diphenols and the reducing capacity (electron affinity) of the quinones. This is an ideal method to validate the functional properties of ingredients.

The Keap1/Nrf2/ARE pathway is also a prime target for cancer chemoprevention. The generation of ROS is a critical initiating factor in many cancers. The transcription factor Nrf2 controls the expression of several genes associated with cytoprotection, including antioxidant enzymes, xenobiotic-metabolizing enzymes (phase 2 enzymes), metal-chelating enzymes, drug transporters, and molecular chaperones (Hayes et al. 2010). Under homeostatic conditions, the Nrf2 action is restricted by channeling Nrf2 through proteasomal degradation controlled through ubiquitination by CUL3-Rbx1. However, under oxidative stress conditions, Nrf2 binds to Keap1 and escapes ubiquitination, and accumulates in the nucleus, activating different classes of AREs. Several phytochemicals, including polyphenols, can activate this pathway.

Stress and ROS are key factors that influence the development of neural disorders and aging. Consequently, stress-mediated pathways, e.g., the Keap1/Nrf2/ARE pathway, serve as a potential target for the prevention of neurodegenerative diseases and enhancement of longevity.

Neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, are thought to result from abnormalities in protein conformation resulting from mitochondrial dysfunction, causing oxidative stress. Resveratrol, a stilbene produced through the flavonoid biosynthetic pathway and

commonly found in grapes, has been found to increase the expression levels of cytoprotective heat shock protein HSP70 and NAD:NADH ratio, regulating sirtuins, which are related to aging, regulation of metabolism, and stress tolerance (Calabrese et al. 2008). Thus, molecules with stilbene functionalities may be useful in designing functional ingredients targeted to the prevention of neurodegenerative disorders and aging, or in general, to target diseases caused by protein misfolding. Pterostilbene, a structural analog of resveratrol, has been found to be a strong inhibitor of cyclooxygenase 2, an enzyme involved in the inflammatory pathway, showing their ability to directly target enzymes (Hougee et al. 2005). Curcumin is also a strong inhibitor of this enzyme. Pterostilbenes have been found to provide cardioprotection through lowering blood cholesterol levels by activating peroxisome proliferator–activated receptor (PPAR) α-isoform (Pan et al. 2008, Rimando et al. 2005). PPARs are transcription factors that are activated by different dietary components; fatty acids activate PPARα and prostaglandins activate PPARγ. Activated PPARs heterodimerize with the retinoid X receptor (RXR) and bind to hormone response elements causing the expression of target genes. This may be a common pathway given that several studies have shown the carotenoids β-carotene and lycopene to be ligands of the RXR, and have been implicated in the inhibition of biochemical pathways that lead to the development of inflammatory diseases (Zhang et al. 2011). Therefore, functional ingredients with these structures can have multifunctional roles in disease prevention.

Polyphenols from various sources (fruits, vegetables, processed products, juice, and wine) have been widely studied for their ability to prevent/arrest the development of various forms of cancer (Singletary et al. 2003). Multiple modes of action on key targets have been proposed for the observed beneficial effects, including antioxidant function, anti-inflammatory function, modulation of signal transduction, and gene expression, leading to the induction of apoptosis and necrosis (Ahn et al. 2003, Eng et al. 2001, O'Prey et al. 2003, Pietta et al. 1996). Treatment of several types of cancer cells with EGCG induced changes in the expression of a large number of genes that are involved in the control of cell division, proliferation, and apoptosis (Guo et al. 2005). Similar conclusions were made in relation to the mechanism of cancer prevention by green- and black-tea polyphenols (Beltz et al. 2006). Tea polyphenols were able to modulate the activities of key enzymes that are involved in cell function, such as MAP kinases and protein kinases. Alteration of gene expression by polyphenol treatment resulted in changes in the levels of transcripts for cyclins, several oncogenes, and tumor suppressor genes. Enzymes associated with metastasis, urokinase and matrix metalloproteinases, and angiogenesis and VEGF (vascular endothelial growth factor) were reduced by tea polyphenols.

The suppression of dimethyl benzantracene (DMBA)-induced mammary carcinogenesis by resveratrol was correlated with the downregulation in the expression of NFκB, COX-2, and matrix metalloprotease-9 (MMP-9) expression (Banerjee et al. 2002). The flavonol quercetin altered the expression levels of over 4,000 human genes in Caco-2 cells (van Erk et al. 2005), some of which, including CDC6 (cyclin-dependent kinase 6), CDK4 (cyclin-dependent kinase 4), and cyclin D1, that have key functions in cell proliferation were downregulated, whereas the expression of several tumor suppressor genes was promoted. These changes were correlated with the induction of cell-cycle arrest and the inhibition of cell proliferation. This treatment also downregulated the signal transduction pathways, such as the β-catenin/T-cell factor signaling and MAPK signaling.

Polyphenols from grape, wine, and several other sources have been tested for their effect on inhibiting breast cancer cell proliferation. Polyphenol fraction from red grape wine strongly and specifically inhibited the proliferation of estrogen receptor positive MCF-7 breast cancer cells (Hakimuddin et al. 2004). Polyphenol action was potentially induced through the disruption of calcium homeostasis within the cell that resulted in the necrosis of cancer cells. Immortalized normal cells (MCF-10A) were not affected by the polyphenol treatment (Hakimuddin et al. 2006).

The grape and wine polyphenols also altered the gene expression in tumors derived from estrogen receptor negative MDA-MB231 cells as xenografts in athymic nude mice (Hakimuddin et al. 2008). Inhibition of phospholipase C- and NFκB-signaling pathways may be involved in polyphenol-mediated tumor growth inhibition in MDA-MB231 breast cancer cells. In addition, modulation of the expression of several genes involved in cell cycle, e.g., cyclins and cyclin-dependent kinases, and those involved in cell-cycle arrest [GADD45A, (growth arrest and DNA-damage-inducible, alpha), CDKN1A (cyclin-dependent kinase inhibitor 1A) (p21, Cip1), TP53, (tumor protein p53) (Li-Fraumeni syndrome)] and so on, was noticed (Hakimuddin & Paliyath 2011). Thus, it is becoming increasingly clear that phytochemicals, including polyphenols, carotenoids, and several others, as well as their metabolites formed after dietary intake, may exert their health beneficial action through multiple functional modes, among which their antioxidant function is only one. Phytochemicals can also modulate the signal transduction cascades affecting the receptor and enzyme (protein kinase) functions (Williams et al. 2004). Flavonoids are also very strong inhibitors of calcium-calmodulin function through the inhibition of target enzymes, e.g., cAMP-phosphodiesterase (Paliyath & Poovaiah 1984, 1985), which can potentially alter cellular cAMP levels and signal transduction. In terms of their efficacy, catechin (flavan-3-ol) was more efficient than quercetin (flavonol), and naringenin (flavone) showed the lowest inhibition.

Monoterpenes have been shown to be very effective in cancer prevention. Various studies (Crowell et al. 1994, Crowell & Gould 1994, Elegbede et al. 1984, Gould 1997, Stark et al. 1995) show their effectiveness against breast, colon, liver, lung, and pancreatic cancers (Gould 1997). D-limonene was observed to be a strong inhibitor of DMBA-induced mammary tumors in rats with no adverse effects observed, even when the diet contained 10,000 ppm of the compound (Elegbede et al. 1984). At a molecular level, D-limonene inhibited the posttranslational isoprenylation and activation of p21ras and other G proteins, affecting signal transduction and gene expression. Uncontrolled activation of ras has been observed to enhance cancer development. Many structural analogs, some of them formed as metabolites within the body, were observed to be better inhibitors of isoprenylation (Crowell et al. 1994). Perillyl alcohol, derived from limonene, was the most potent inhibitor of isoprenylation of small G-proteins (21–26 kDa). Perillyl alcohol, provided through diet at 16%, reduced the growth of pancreatic tumors to less than half and also caused tumor regression (Stark et al. 1995). All these results suggest that natural functional ingredients act through multiple mechanisms that enhance their in vivo efficacy, and achieving this should be a prime goal in the design of any ingredients.

BIOACCESSIBILITY AND BIOAVAILABILITY OF FUNCTIONAL INGREDIENTS

A fundamental property of any functional ingredient is its chemical structure that determines its physicochemical properties as well as the suitability and ease of absorption. Functional ingredients can be absorbed by an active process or through passive diffusion through the membrane. Other factors, such as food matrix interactions, also influence the absorption of ingredients because functional ingredients may become bound and form complexes with macromolecules in food during food processing or consumption (Holst & Williamson 2008). Such complexes have to be broken as they occur during digestion, before the active ingredients can be absorbed. The terms bioaccessibility and bioavailability refer to such conditions. The amount of the potentially absorbable form of an active ingredient in the food can be termed as the bioaccessible form of the ingredient, whereas bioavailability refers to the amount of active ingredient that is actually absorbed by the body. The absorption of functional ingredients is tremendously influenced by the food matrix interactions. The uptake of flavonoids was influenced by the chemical structure (e.g.,

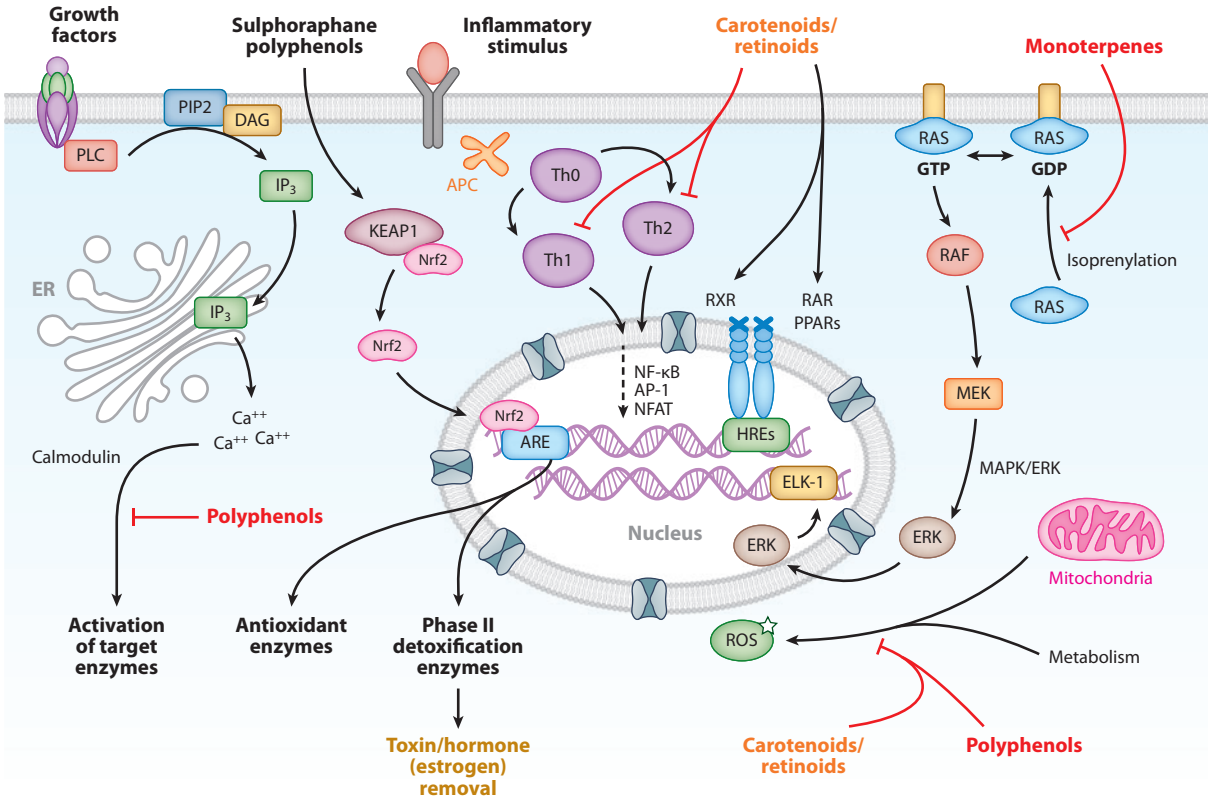

Figure 2

Schematic of cellular targets of various types of functional ingredients found in fruits and vegetables. Flavonoids are very strong inhibitors of calcium- and calmodulin-promoted proteins/enzymes (protein kinases). Sulphoraphane and polyphenols are activators of the Keap-1/Nrf2 pathway, resulting in the overexpression of antioxidant enzymes and phase II detoxification enzymes. Phase II enzymes help remove xenobiotics (toxins) entering the cell or induce metabolic removal of steroid hormones. Carotenoids and retinoids are inhibitors of inflammatory responses mediated through T-helper cells. They can bind to receptors and transcription factors, resulting in the downregulation of pro-inflammatory cytokines. Monoterpenes are strong inhibitors of RAS isoprenylation and membrane localization. Activation of the Ras pathway is a key step in the development of some forms of cancers. Carotenoids, retinoids, and polyphenols themselves are very strong scavengers of reactive oxygen species and other activated radicals. Abbreviations: AP1, activator protein 1; APC, antigen presenting cell; ARE, antioxidant response elements; DAG, diacylglycerol; ELK-1 [E Twenty-six (ETS)-like Transcription Factor 1]; ER- Endoplasmic reticulum; ERK, extracellular signal-regulated kinases; GDP/GTP, guanosine di-/triphosphate; HRE, hormone response elements; IP_3, inositol trisphosphate; MAPK, mitogen activated protein kinase; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa-B; PIP2, phosphatidylinositol bisphosphate; PLC, phospholipase C; PPAR, peroxisome proliferator activated receptor; RAF, rapidly activated fibrosarcoma oncogene analog MAPKKK; RAR, retinoic acid receptor; RXR, retinoid X receptor; ras, rat sarcoma subfamily of small GTPases; ROS, reactive oxygen species; Th0, Th1, Th2, T-helper cells.

sugar attached to the flavonoid moiety), the presence of hydrophobic molecules, (e.g., lipids in the diet), and the presence of emulsifying agents, alcohol, and food matrix components, including carbohydrates, proteins, and fiber (Scholz & Williamson 2007). The uptake of lycopene from the diet is highly favored from a lipid-enriched environment in the product or food. During fruit ripening, there are several physiological changes that enhance the catabolic processes in the cell, and these changes enhance complex formation within the tissue or during the processing of the

tissue into juice and pulp (Jacob & Paliyath 2008, 2011, Oke et al. 2010). Thus, the bioaccessibility and bioavailability of active ingredients are influenced by several physicochemical factors.

The molecular characteristics of nutraceuticals have great influence on their absorption, and this may be one reason for the limited uptake and extremely low physiological levels observed in the case of flavonoids. Lipinski's rule of five (Lipinski et al. 2001) discusses the influence of physicochemical characteristics of small molecular weight functional ingredients in the diet. Components with relative molecular masses of >500, having more than five hydrogen bond donors and 10 hydrogen bond acceptors, and also showing high hydrophilicity are likely to be absorbed poorly. Flavonoids, especially anthocyanins, are highly hydrophilic molecules and are usually present as their glycosides. This enables the formation of hydrogen bonds with macromolecular components in food, such as carbohydrates (pectin) and proteins. Digestion of the food matrix can release bound components, which can be absorbed by the body as observed in carotenoids (Granado-Lorencio et al. 2007). Enzymatic processes, including catabolic breakdown, methylation, and deglycosylation, that transform the functional ingredient may also enhance bioavailability (Yang et al. 2008). O-methylated epigallocatechin gallates were observed to be better inhibitors of mast cell–dependent allergic reactions than their nonmethylated natural forms of tea polyphenols (Maeda-Yamamoto et al. 2004). Esterification of EGCG, which is highly water soluble using long chain fatty acids, enhanced its lipophilicity and increased octanol:water partition coefficient, suggesting that the esterified form may provide better bioavailability (Zhong & Shahidi 2011). Therefore, structural modifications induced during biosynthesis, chemical synthesis, or processing (Oke & Paliyath 2006, Oke et al. 2010) can greatly influence the bioavailability of components that are poorly absorbed by the body.

Several reviews summarize the studies on the bioaccessibility and bioavailability of polyphenolic components in biological systems (Holst & Williamson 2008; Manach et al. 2004, 2005; Scholz & Williamson 2007; Williamson & Clifford 2010; Vitaglione et al. 2008; Yang et al. 2008).

CONCLUSION

There have been wide-ranging systematic studies on the role of food and food ingredients that were traditionally classified as non-nutritional ingredients and their impact on health in the past three decades. Most of these studies have been focused on efficacy and mechanisms of action. Through these studies, we have a wide-ranging understanding of how the functional food ingredients exert their beneficial effects within the body. The studies that focus on structure and function are relatively few. A major issue with regard to functional food ingredients is their bioavailability. Therefore, any attempts to modify the structure of ingredients must also look into the changes in bioavailability. Also, several functional ingredients are metabolized within the colon, and the metabolites may also provide health benefits not only to the colon but to other tissues after their absorption. Soluble and insoluble fiber (e.g, cellulose, pectin, inulin) components can provide indirect benefits as prebiotics. Synthetic low molecular weight ingredients, such as fructooligosaccharides and galactooligosaccharides, can provide similar and added benefits, and are being increasingly used in the industry. Future studies will involve the use of structural analogs of functional food ingredients with better efficacy, as well as improved delivery strategies to target the components to desired tissues. An important property of functional food ingredients (e.g., anthocyanins and monoterpenes) is their specificity toward inducing apoptosis/necrosis of cancer cells. Designer functional foods with such properties can be developed using these ingredients, and these may serve to reduce the severity and augment the efficiency of chemotherapy in cancer treatment. Thus, functional food ingredients will continue to have a major role in the future of food industry.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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