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Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review

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ABSTRACT

This review reports on the latest research results and applications of phenolic and polyphenolic compounds. Phenolic compounds, ubiquitous in plants, are an essential part of the human diet and are of considerable interest due to their antioxidant properties and potential beneficial health effects. These compounds range structurally from a simple phenolic molecule to complex high-molecular-weight polymers. There is increasing evidence that consumption of a variety of phenolic compounds present in foods may lower the risk of health disorders because of their antioxidant activity. When added to foods, antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality, and extend the shelf-life of products. Due to safety concerns and limitation on the use of synthetic antioxidants, natural antioxidants obtained from edible materials, edible by-products and residual sources have been of increasing interest. This contribution summarizes both the synthetic and natural phenolic antioxidants, emphasizing their mode of action, health effects, degradation products and toxicology. In addition, sources of phenolic antioxidants are discussed in detail.

1. Introduction

Lipid oxidation is a major cause for food quality deterioration and generation of off odours and off flavours, decreasing shelf-life, altering texture and colour, and decreasing the nutritional value of food (Alamed, Chaiyasit, McClements, & Decker, 2009). Numerous methods have been developed to control the rate and extent of lipid oxidation in foods, but addition of antioxidants is most effective. Antioxidants have become an indispensible group of food additives mainly because of their unique properties of extending the shelf-life of food products without any adverse effect on their sensory or nutritional qualities. Historically, gum guaiac was the first antioxidant approved for stabilization of animal fats especially lard in the 1930s (Nanditha & Prabhasankar, 2009). Halliwell, Aeschbacht, Loligert, and Aruoma (1995) reported that antioxidants are also of interest to biologists and clinicians because they may help to protect the human body against damage by reactive oxygen species (ROS). According to the United States Department of Agriculture (USDA) Code of Federal Regulation, "antioxidants are substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation" (Shahidi & Wanasundara, 1992). Antioxidants for use in food system must be inexpensive, non-toxic and effective at low concentrations; highly stable and capable of surviving processing; have no odour, taste or colour of their own; easy to incorporate and have a good solubility in the product (Kiokias, Varzakas, & Oreopoulou, 2008). One of the

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primary pathways of lipid degradation is that of autoxidation. The process of autoxidation of polyunsaturated lipids in foods involves a free radical chain reaction that is generally initiated by exposure of lipids to light, heat, ionizing radiation, metal ions or metalloprotein catalysts. The enzyme lipoxygenase can also initiate oxidation (Shahidi, 2015; Shahidi & Naczk, 2004). The classic route of autoxidation includes initiation (production of lipid free radicals), propagation and termination (production of non radical products) reactions (Shahidi & Wanasundara, 1992). A general schematic pathway for autoxidation of polyunsaturated lipids is shown in Fig. 1. Antioxidants act at different levels in the oxidative sequence involving lipid molecules. They may decrease oxygen concentration, intercept singlet oxygen ($^1O_2$), prevent first-chain initiation by scavenging initial radicals such as hydroxyl radicals, bind metal ion catalysts, decompose primary products of oxidation to nonradical species and break chain reaction in order to prevent continued hydrogen abstraction from substrates (Shahidi, 2000a, 2000b, 2015; Shahidi & Naczk, 2004).

$$\text{Initiation} \quad RH \rightarrow R^{\bullet} + H^{\bullet} \tag{1}$$

$$\text{Propagation} \quad R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \tag{2}$$

$$ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH \tag{3}$$

$$\text{Termination} \quad \left.\begin{array}{l} R^{\bullet} + R^{\bullet} \\ R^{\bullet} + ROO^{\bullet} \\ ROO^{\bullet} + ROO^{\bullet} \end{array}\right\} \text{Nonradical products} \tag{4}$$

Hydroperoxides are the primary products of lipid oxidation, but hydroperoxides, despite their deleterious effects on health have no effect on flavour quality of foods (Shahidi, 1998). However, these unstable molecules decompose readily to form a myriad of products such as aldehydes, ketones, alcohols and hydrocarbons, amongst others (Shahidi, 1998); these impart unpleasant flavours and odours to fats, oils and lipid containing foods. These aldehydes interact with sulphhydryl and amine groups in proteins and this may alter the functionality of proteins (McClements & Decker, 2007). Faustman, Liebler, McClure, and Sun (1999) reported the ability of unsaturated aldehydes to react with histidine in myoglobin and accelerate the oxidation of oxymyoglobin. Phenolic compounds in foods originate from one of the main classes of secondary metabolites in plants (Naczk & Shahidi, 2004). At a low concentration, phenolics act as an antioxidant and protect food from oxidative rancidity (Karakaya, 2004). Phenolic antioxidants interfere with the oxidation process as free radical terminators and sometimes also as metal chelators. Phenols have been widely studied and confirmed to possess diverse bioactivities which could be beneficial to human health. They are known to reduce the risk of cancer, heart disease, and diabetes; to inhibit plasma platelet aggregation, cyclooxygenase (COX) activity, and histamine release, as well as to exert antibacterial, antiviral, anti-inflammatory, and anti-allergenic activities (Oak, El Bedoui, & Schini-Kerth, 2005; Shetty, 2004; Yang, Landau, Huang, & Newmark, 2001; Yao et al., 2004). The benefits towards many of these conditions arise in part through the antioxidant characteristic of phenolics; therefore, it is important to quantify, identify and evaluate their antioxidant activities (Cevallos-Casals & Cisneros-Zevallos, 2010). This review summarizes both the synthetic and natural phenolic antioxidants, emphasizing their mode of action, health effects, degradation products and toxicology. In addition, sources of phenolic antioxidants are discussed in detail.

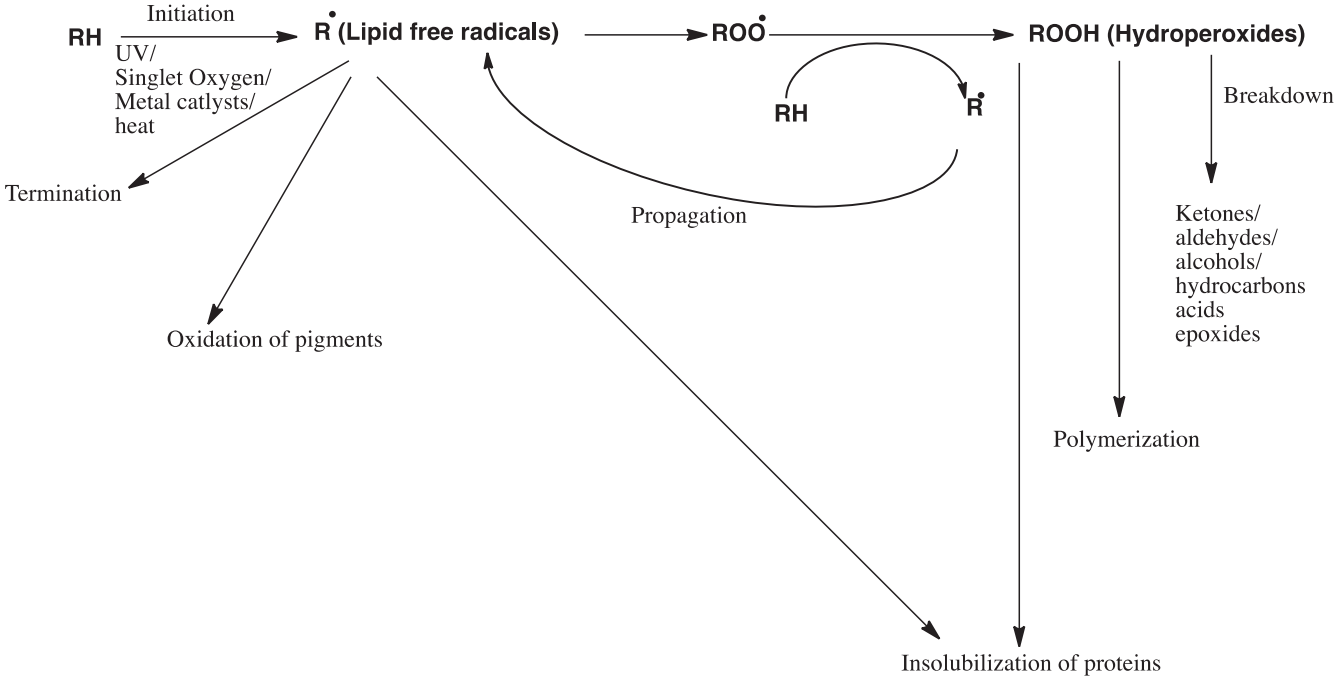


Fig. 1 – General scheme for autoxidation of lipids containing polyunsaturated fatty acids (RH) and their consequences.

2. Phenolic antioxidants

Phenolic compounds are classified as primary antioxidants which are mainly free radical scavengers (FRS) that delay or inhibit the initiation step or interrupt the propagation step of lipid oxidation, thus decreasing the formation of volatile decomposition products (e.g., aldehydes and ketones) that cause rancidity (Alamed et al., 2009; Kiokias et al., 2008; Naczk & Shahidi, 2004; Nanditha & Prabhasankar, 2009; Shahidi & Naczk, 2004; Shahidi, Wanasundara, & Amarowicz, 1994).

3. Mechanism of action of phenolic antioxidants

The antioxidant potential of phenolic compounds depends on the number and arrangement of the hydroxyl groups in the molecules of interest (Cao, Sofic, & Prior, 1997; Sang, Lapsley, Jeong, et al., 2002). Phenolic antioxidants (AH) can donate hydrogen atoms to lipid radicals and produce lipid derivatives and antioxidant radicals (Reaction 5), which are more stable and less readily available to promote autoxidation (Kiokias et al., 2008). The antioxidant free radical may further interfere with the chain-propagation reactions (Reactions 6 and 7).

$$R^{\bullet}/RO^{\bullet}/ROO^{\bullet} + AH \rightarrow A^{\bullet} + RH/ROH/ROOH \quad (5)$$

$$RO^{\bullet}/ROO^{\bullet} + A^{\bullet} \rightarrow ROA/ROOA \quad (6)$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet} \quad (7)$$

As bond energy of hydrogen in a free radical scavenger decreases, the transfer of hydrogen to the free radical is more energetically favourable and thus more rapid (McClements & Decker, 2007). Any compound that has a reduction potential lower than the reduction potential of a free radical (or oxidized species) is capable of donating its hydrogen atom to that of the free radical unless the reaction is kinetically unfeasible. For example, FRS including α-tocopherol ($E^{\circ\prime}$ = 500 mV) which have reduction potential below that of peroxyl radicals ($E^{\circ\prime}$ = 1000 mV) are capable of donating their hydrogen to the peroxyl radical to form a hydroperoxide (McClements & Decker, 2007). The phenoxyl radical is stabilized by delocalization of its unpaired electron around the aromatic ring (Fig. 2), which participates in the termination reaction. Gorden (1990) reported that substitution at the *para* position with an ethyl or n-butyl group rather than a methyl group improves the activity of the antioxidant; however, the presence of chain or branched alkyl groups in this position decreases the antioxidant activity. The stability of the phenoxyl radical is further increased by bulky groups in the 2 and 6 positions as in 2,6-di-t-butyl-4-methylphenol (BHT) (Miller & Quakenbush, 1957), since these substituents increase the steric hindrance in the region of the radical and thereby further reduce the rate of propagation reactions involving the antioxidant radical (Reactions 8, 9, 10).

$$A^{\bullet} + O_2 \rightarrow AOO^{\bullet} \quad (8)$$

$$AOO^{\bullet} + RH \rightarrow R^{\bullet} + AOOH \quad (9)$$

$$A^{\bullet} + RH \rightarrow AH + R^{\bullet} \quad (10)$$

The effect of antioxidant concentration on autoxidation rates depends on many factors, including the structure of the antioxidant, oxidation conditions, and the nature of the sample being oxidized (Shahidi & Naczk, 2004). Often phenolic antioxidants lose their activity at high concentrations and behave as prooxidants (Gorden, 1990) by involvement in initiation reactions (Reactions 11, 12). Phenolic antioxidants are more effective in extending the induction period when added to any oil that has not deteriorated to any great extent. However, they are ineffective in retarding decomposition of already deteriorated lipids (Mabarouk & Dugan, 1961). Thus, antioxidants should be added to foodstuffs as early as possible during processing and storage in order to achieve maximum protection against oxidation (Shahidi & Wanasundara, 1992).

4. Measurement of antioxidant activity

The need to measure antioxidant activity is well documented; these are carried out for meaningful comparison of foods or commercial products and for provision of quality standards for regulatory issues and health claim (Shahidi & Ho, 2007). Lipid oxidation is conventionally studied by determination of peroxide value (PV), thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), or anisidine value (AV) or by assessing volatile compounds (Kristinova, Mozuraityte, Storrø, & Rustad, 2009). There are numerous methods for measuring antioxidant activity; these may be classified into two categories. The first category measures the ability of antioxidants in inhibiting oxidation in a model system by monitoring the

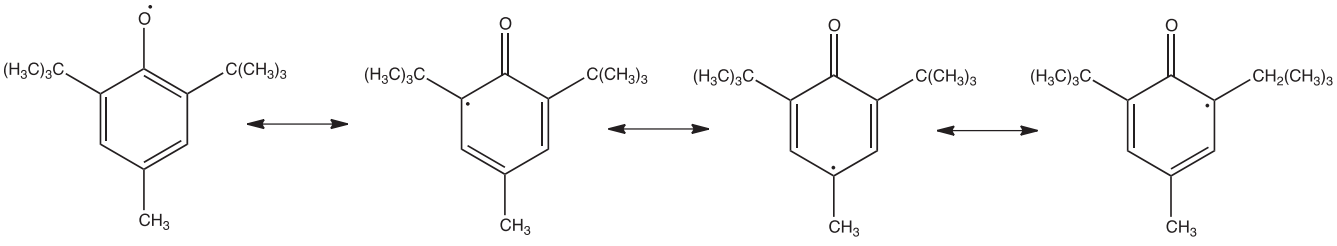


Fig. 2 – Resonance stabilization of phenoxyl radical.

Table 1 – Antioxidant activity measurement methods and units.

Methods	Measurement units
Peroxide value (PV)	Milliequivalents of oxygen per kilogram of sample (meq/kg)
Conjugated dienes and trienes	Conjugable oxidation products (COPs)
Thiobarbituric acid reactive substances (TBARS) assay	Milligrams of malondialdehyde (MDA) equivalents per kilogram sample or micromoles of MDA equivalents per gram of sample (meq/g)
p-Anisidine value (*p*-AnV)	Absorbance of a solution resulting from the reaction of 1 g of fat in isooctane solution (100 ml) with *p*-anisidine
Electrical conductivity method	Oil stability index (OSI) value, which is defined as the point of maximal change of the rate of oxidation
Oxygen radical absorbance capacity (ORAC) assay	μmol of trolox equivalents
Total radical-trapping antioxidant parameter (TRAP) assay	μmol per litre peroxyl radical deactivated
Trolox equivalent antioxidant capacity (TEAC) assay	mM trolox equivalent to 1 mM test substance
2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay	EC_{50} (Concentration to decrease concentration of test free radical by 50%); T_{EC50} (Time to decrease concentration of the test free radical by 50%); AE (Antiradical efficiency ($1/EC_{50}$) T_{EC50})
Ferric reducing antioxidant power (FRAP) assay	Absorbance of Fe^{2+} complex at 593 nm produced by antioxidant reduction of corresponding tripyridyltriazine Fe^{2+} complex

associated changes using physical, chemical or instrumental means. Radical scavenging assays include methods based on hydrogen atom transfer (HAT) or single electron transfer (SET) mechanisms. Oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assays are the major methods that measure HAT whilst trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays represent SET-based methods (Shahidi & Ho, 2007). HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation whilst SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals (Shahidi & Zhong, 2005, 2007). Table 1 summarizes the methods commonly used to measure antioxidant activity and the units they carry. However, a detailed discussion of these methods is beyond the goal of this review and has been communicated elsewhere (Shahidi & Zhong, 2015).

5. Health effect of phenolic antioxidants

Dietary intake of phenolics is greatly affected by the eating habits and preferences of individuals (Shahidi & Naczk, 2004). The average daily intake of dietary polyphenols is approximately 1 g per person; the main sources are beverages, fruits and, to a lesser extent, vegetables and legumes (Scalbert & Williamson, 2000). Simple phenolics such as hydroxycinnamic acid conjugates and flavonoids are important constituents of fruits, vegetables and beverages. These compounds show a wide range of antioxidant activities *in vitro* (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995) and are thought to exert protective effects against major diseases such as cancer and cardiovascular diseases (Boudet, 2007). Oxidative stress imposed by reactive oxygen species (ROS) indeed plays a crucial role in the pathophysiology associated with neoplasia, atherosclerosis and neurodegenerative diseases. The potential mechanism of the protective effects of phenolic compounds, including flavonoids, is thought to be due to their direct scavenging of free radicals (Heim, Tagliaferro, & Bobilya, 2002). Lee and Lee (2006) showed the efficacies of antioxidant therapies, based on antioxidative phenolics, that decreased ROS levels were equivocal at best. Some antioxidants exhibit prooxidant activity under certain conditions and potential carcinogenicity under others, thus dietary supplementation with large amounts of a single antioxidant may be deleterious to the health (Boudet, 2007). One of the most crucial conclusions at the present time is that low risk of cancer is more closely related to a diet rich in multiple antioxidants than one supplemented with an individual antioxidant. A combination of antioxidants with different modes of action is believed to increase efficacy and minimize toxicity (Lee & Lee, 2006). The additive and synergistic effects of dietary phytochemicals obtained from fruits and vegetables are likely responsible for their anticancer activities in a more efficient way than dietary supplements (Liu, 2004). Liu et al. (2000) found that a combination of fruits such as orange, apple, grape and blueberry, renders a synergistic effect on antioxidant activity *in vitro*; the half maximal effective concentration (EC_{50}) of these combined fruits was five times lower than their individual EC_{50}, suggesting a synergistic effect due to the combination of the four fruits (Jaganath & Crozier, 2010). Huang, Liu, Dushenkov, Ho, and Huang (2009) found that oral feeding of orange peel extract, black tea extract and caffeine had anti-obesity effects by suppressing body weight gain and adipose tissue formation, suggesting that orange peel extract acted synergistically with black tea extract and caffeine to render the most effective anti-obesity action. The health implications of dietary phenolic compounds, including flavonoids, are also dependent on the composition of the components of the diet and the bioavailability of the individual compounds under investigation. Increasing evidence shows that hydroxycinnamic acid derivatives and flavonoids can be absorbed into the human body in amounts that are, in principle, sufficient to exert antioxidant or other biological activities *in vivo* (Olthof, Hollman, & Katan, 2001; Scalbert & Williamson, 2000). Dietary polyphenols are substrates for β-glucosidases, UDP-glucuronosyltransferase, or catechol-*O*-methyltransferase in the small intestine as well as for a number of phase I and II enzymes in the liver (Rechner et al., 2002; Scalbert & Williamson, 2000). In addition, ingested polyphenols are subjected to hydrolysis and degradation in the colon due to the activity of enzymes of the colonic microflora (Booth,

Emerson, Jones, & Deeds, 1957). Rechner et al. (2002) demonstrated that intact conjugated polyphenols were present at much lower levels than their degradation products resulting from the action of colonic bacterial enzymes and subsequent metabolism in the liver. Consideration should also be given to these degradation products (Boudet, 2007). Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia, Castillo, Marin, Ortuño, & Del Río, 1997; Manach, Mazur, & Scalbert, 2005; Middleton, Kandaswami, & Theoharides, 2000; Puupponen-Pimiä et al., 2001). Recent research findings indicate that tea polyphenols can protect against different stages of carcinogenesis (Khan & Mukhtar, 2010). EGCG (epigallocatechin-3-gallate), the main catechin in green tea, serves as a cancer chemopreventive agent (lungs, liver, gastrointestinal tract, skin and prostate cancer), as well as anti-obesity and cardiovascular protective compound (Khan & Mukhtar, 2010; Klaus, Pultz, Thone-Reineke, & Wolfram, 2005; Yang & Wang, 1993). The antioxidant activity and beneficial health effects of the main polyphenol of green tea, epigallocatechin gallate (EGCG), was enhanced upon conjugation with docosahexaenoic acid (DHA) and the tetraester so formed was able to arrest colon cancer effectively (Zhong, Chiou, Pan, Ho, & Shahidi, 2012). Polymethoxyflavones, the major components of orange peel, have been found to render health benefits, including anti-inflammatory, anti-carcinogenic, anti-viral, antioxidant, anti-thrombogenic and anti-atherogenic properties (Huang et al., 2009; Lai et al., 2007; Li, Wang, Li, Li, & Wang, 2009; Middleton et al., 2000). Caffeine was also effective in suppressing body weight gain by stimulating thermogenesis, extending sympathetic stimulation, suppressing food intake and reducing adipose tissue mass (Kobayashi-Hattori, Mogi, Matsumoto, & Takita, 2005; Tremblay, Masson, Leduc, Houde, & Despres, 1988). In addition, hydroxytyrosol, one of the major phenolic constituents in olive oil, was reported to reduce the risk of coronary heart disease and atherosclerosis by itself (Salami, Galli, De Angelis, & Visioli, 1995; Tuck & Hayball, 2002). It has also been postulated that hydroxytyrosol inhibits arachidonic acid metabolism (Petroni et al., 1997) and platelet aggregation (Petroni et al., 1994). It is presumed that hydroxytyrosol penetrates into cell membranes and consequently inhibits the production of leukotriene B4 (LTB4) effectively from endogenous arachidonic acid (Kohyama, Nagata, Fujimoto, & Sekiya, 1997; Tuck & Hayball, 2002). Considerable evidence exists from epidemiological and experimental studies for preventive effects of soy or its isoflavones against chronic diseases including cancer (breast, prostate, colorectal, lung), osteoporosis, cardiovascular disorders and menopausal symptoms, but this has not always been consistent (Franke, Halm, Kakazu, & Li, 2010; Levis, Strickman-Stein, Doerge, & Krischer, 2010; Rimbach et al., 2008). Miyake et al. (2005) reported that high intake of soy and isoflavones may be associated with reducing the prevalence of allergic rhinitis. The structural similarity of isoflavones to steroidal oestrogens and the potent binding of genistein, one of the most predominant soy isoflavones, to the oestrogen receptor-beta, including its transactivation, are believed to be the basis for many beneficial effects of soy intake (Cotroneo, Wang, Fritz, Eltoum, & Lamartiniere, 2002; Kuiper et al., 1997). Resveratrol is a polyphenolic compound with potent antioxidant activity. It is found in a number of plants, notably grapes, pistachio, peanuts and berry fruit, and is attracting increased attention due to its health benefits, especially in common age-related diseases such as cancer, type 2 diabetes, cardiovascular disease, and neurological conditions (Marques, Markus, & Morris, 2009). Resveratrol first received the attention of the scientific community in 1990s, when it was shown to be responsible for the cardioprotective effects of red wine (Wu et al., 2001). Rayalam, Yang, Ambati, Della-Fera, and Baile (2008) reported that resveratrol may be more effective in lowering body weight at higher doses, and could be a potential treatment for obesity. It can decrease fat mass by inhibiting adipogenesis and can induce apoptosis in adipocytes by affecting expression of genes that modulate mitochondrial function.

6. Classification of phenolic antioxidants

Phenolic antioxidants can be classified as synthetic and natural (Fig. 3), based on their origin.

6.1. *Synthetic food phenolic antioxidants*

Synthetic phenolic antioxidants currently permitted for use in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ). In addition octyl gallate (OG) and dodecyl gallate (DG) are also used as a synthetic antioxidant (Makahleh, Saad, & Bari, 2015). These synthetic phenolic antioxidants are deliberately added to products in order to prevent or delay the onset of lipid oxidation during processing and storage of fats, oils and lipid-containing foods and have been used by the food industry for some 60 years or so (Saad et al., 2007). Berdahl, Nahas, and Barren (2010) reported that the food and beverage market for antioxidants is a $500 billion industry and is growing at 5–7% per year, and is expected to grow at that rate through the year 2017. However, Verhagen, Schilderman, and Kleinjans (1991) reported that synthetic antioxidants may not be very effective in preventing oxidative deterioration of fats and oils of vegetable origin. Table 2 summarizes the physical and chemical properties of synthetic antioxidants. The use of synthetic phenolic antioxidants in foodstuffs is strictly regulated by governments due to their potential toxicity effects. BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis when used at high levels in laboratory animals (Biparva, Ehsani, & Hadjmohammadi, 2012; Grice, 1986; Rodil, Quintana, Basaglia, Pietrogrande, & Cela, 2010; Wichi, 1988). It has been reported that amongst the various food additives BHA, TBHQ, 2-tert-butyl-4-methylphenol (TBMP) and PG have potential to form molecular complexes with nucleic acid structure and cause damage to double helical structure of DNA (Dolatabadi & Kashanian, 2010). The maximum permitted levels of synthetic antioxidants are given in Table 3. Synthetic phenolics are effective in numerous food systems; however, their use in the food industry has recently declined owing to safety concerns and consumer demand for all natural products (McClements & Decker, 2007). Berdahl et al. (2010) reported that the market is 50% larger for naturals than it is for synthetics, where the market for ascorbates and erythorbates is as large as the entire synthetic market.

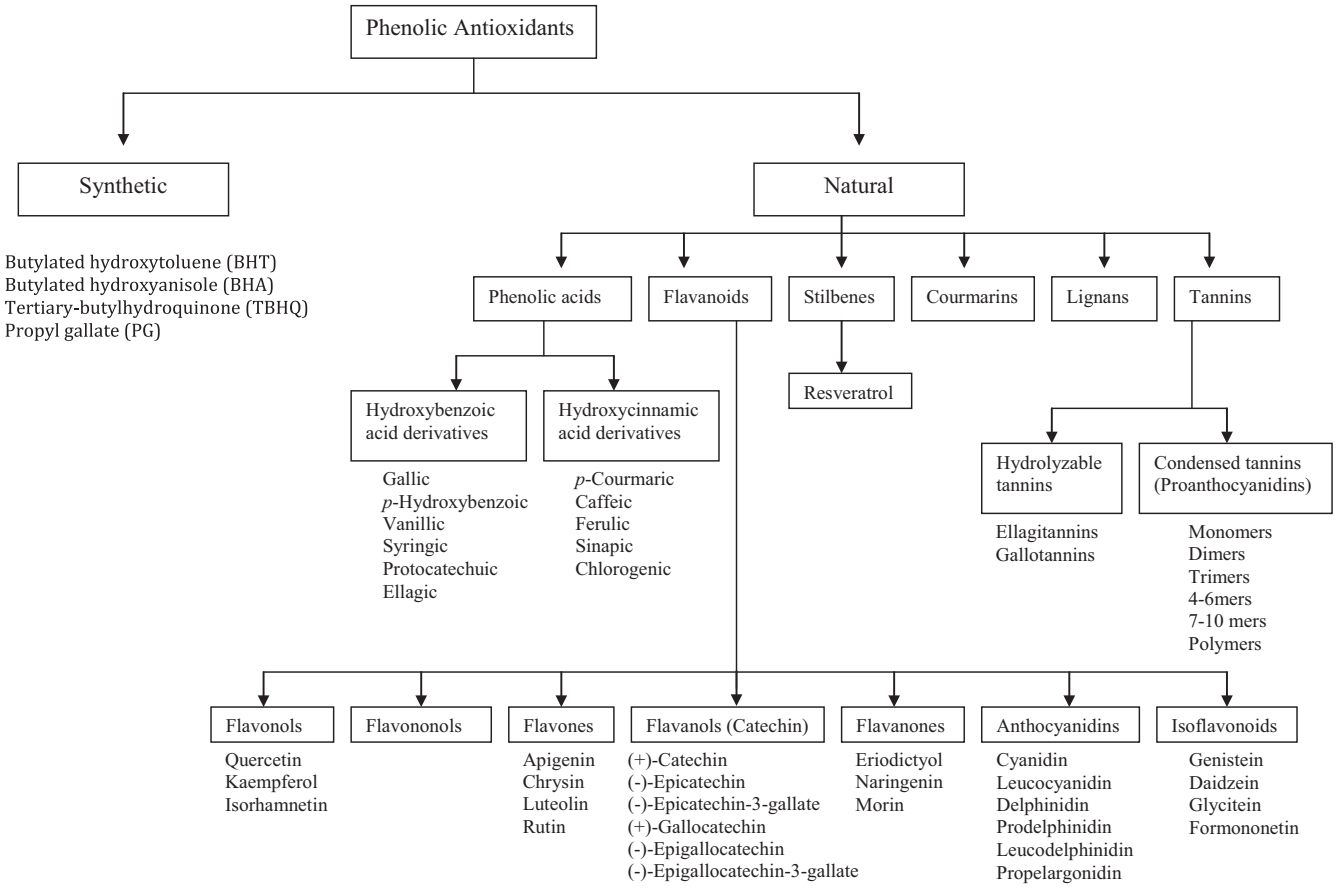


Fig. 3 – Classification of phenolic antioxidants.

Table 2 – Chemical and physical properties of synthetic food antioxidants.

Characteristic	BHA	BHT	Gallate		TBHQ
			Propyl	Dodecyl	
Melting point (°C)	50–52	69–70	146–148	95–98	126–128
Boiling point (°C)	268[a]	265[a]	181 ± 20.8[a]	–	291 ± 20.0[a]
Density (g/cm^3) at 20 °C	–	0.89[a]	1.36 ± 0.06[a]	–	1.09 ± 0.06[a]
Carry-through properties	Very good	Fair-good	Poor	Fair-good	Good
Synergism	BHT and gallates	BHA	BHA	BHA	–
Solubility (w/w%) in					
Water	0	0	<1	<1	<1
Propylene glycol	50	0	6.5	4	30
Lard	30–40	50	1	–	5–10
Corn oil	30	40	0	0	10
Glycerol	1	0	25	–	<1
Methyl linoleate	Verysoluble	Verysoluble	1	1	>10

Source: Adapted from Shahidi and Wanasundara (1992); [a]Andre et al. (2010).

6.1.1. *Butylated hydroxyanisole (BHA)*

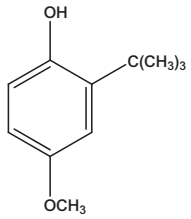
BHA is a monophenolic antioxidant. Commercial BHA is available as white waxy flakes, which is a mixture of two isomers, 3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tetiary-butyl-4-hydroxyanisole (10%) (Fig. 4). BHA is very soluble in fats and oils and insoluble in water and a more effective antioxidant in preventing deterioration of flavour and colour of essential oils (Hettiarachchy & Kalapathy, 2000). BHA is slightly better than BHT in its carry-through properties (Table 2). BHA is particularly effective in controlling the oxidation of short-chain fatty acids such as those found in coconut and palm kernel oils that are used typically in cereal and confectionery products (Shahidi & Wanasundara, 1992). A synergistic effect of BHA with other antioxidants such as BHT, TBHQ or PG provides

Table 3 – Maximum usage levels (Codex General Standards) permitted by Codex Alimentarius Commission for synthetic antioxidants.

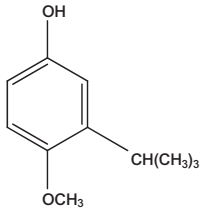
Food category	Maximum usage level (mg/kg)			
	BHA	BHT	PG	TBHQ
Beverage whiteners	100	100	–	100
Milk powder and cream powder (plain)	100	200	200	–
Butter oil, anhydrous milkfat, ghee	175	75	100	–
Vegetable oils and fats	200	200	200	200
Lard, tallow, fish oil, and other animal fats	200	200	200	200
Fat spreads, dairy fat spreads and blended spreads	200	200	200	200
Fat emulsions mainly of type oil-in-water, including mixed and/or flavoured products based on fat emulsions	200	200	200	200
Fat-based desserts excluding dairy-based dessert products	200	200	200	200
Edible ices, including sherbet and sorbet	200	100	–	200
Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	200	200	50	–
Cocoa and chocolate products	200	200	200	200
Imitation chocolate, chocolate substitute products	–	200	200	–
Confectionery including hard and soft candy, nougats, etc.	200	200	200	200
Chewing gum	400	400	1000	400
Decorations (e.g., for fine bakery wares), toppings (nonfruit) and sweet sauces	200	200	200	200
Whole, broken, or flaked grain, including rice	–	–	100	–
Breakfast cereals, including rolled oats	200	100	200	–
Pre-cooked pastas and noodles and like products	200	200	100	200
Bakery wares	200	200	200	–
Processed meat, poultry, and game products in whole pieces or cuts	200	100	200	100
Processed comminuted meat, poultry, and game products	200	100	200	100
Frozen fish, fish fillets, and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Frozen battered fish, fish fillets, and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Smoked, dried, fermented, and/or salted fish and fish products, including molluscs, crustaceans, and echinoderms	200	200	100	–
Semi-preserved fish and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Fully preserved, including canned or fermented fish and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Herbs, spices, seasonings and condiments (e.g., seasoning for instant noodles)	200	200	200	200
Soups and broths	200	100	200	200
Sauces and like products	200	100	200	200
Yeast and like products	200	–	–	–
Food supplements	400	400	400	–
Snacks – potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	200	–	200	–
Processed nuts, including coated nuts and nut mixtures (with e.g., dried fruit)	200	–	200	–
Ready-to-eat savouries	–	200		200
Water-based flavoured drinks, including “sport”, “energy”, or “electrolyte” drinks and particular drinks	–	–	1000	–
Desserts – dairy based/fruit based/cereal and starch based/egg based	–	–	90	–
Mustards	–	–	–	200

Source: Adapted from Codex General Standard for Food Additives (GSFA) online database. Joint FAO/WHO Codex Alimentarius Commission (Anonymous, 2009).

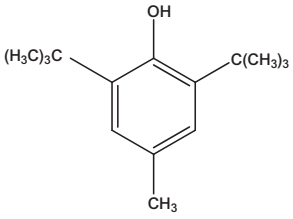
greater antioxidant potency than what might be expected from the contribution of each individual antioxidant. BHA (0.01%) mixed with dodecyl gallate (0.005%) is more effective than BHA alone in stabilizing margarine (Nanditha & Prabhasankar, 2009). An examination of the scientific literature reveals some paradoxical properties that BHA shares with other antioxidants. Thus, BHA may be referred to as an antioxidant, a prooxidant, an anticarcinogen, a carcinogen, and a tumour promoter (Iverson, 1999). It is hypothesized that 1.5–2% BHA give rise to tumour formation in rodent forestomach by inducing heritable changes in DNA. Evidence indicates that reactive oxygen species (ROS), in particular hydroxyl radicals, may play a crucial role (Verhagen et al., 1991). However, dietary BHA at levels of 0.25 and 0.75% was found to protect young female mice against the acute toxicity of monocrotaline (Miranda, Reed, Cheeke, & Buhler, 1981). Williams, Iatropoulos, and Whysner (1999) concluded that BHA is a rodent carcinogen, which is species-specific for all practical purposes, and not relevant to humans. This supports the decisions of authorities that have reviewed these data to recommend continued use of BHA (Iverson, 1995; JECFA, 1996) despite the international agency for research on cancer evaluation that BHA is possibly carcinogenic to humans (IARC, 1987). The potential dietary intake of BHA in Canada was estimated using dietary recall data on food consumption and maximum permitted use levels for this antioxidant. Based on these estimates, it was concluded that the dietary intake of BHA and other permitted phenolic antioxidants (BHT and propyl gallate) is unlikely to



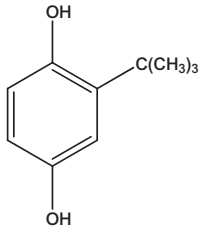
3-tertiary-butyl-4-hydroxyanisole
2-tertiary-butyl-4-methoxyphenol



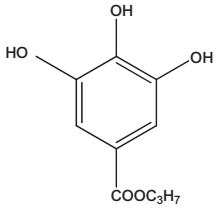
2-tertiary-butyl-4-hydroxyanisole
3-tertiary-butyl-4-methoxyphenol



3,5-di-tertiary-butyl-4-hydroxytoulene
2,6-di-tertiary-butyl-4-methylphenol



tertiary-butylhydroquinone



3,4,5-trihydroxypropylbenzoate (propyl gallate)

Fig. 4 – Chemical structures of synthetic antioxidants.

exceed 1 mg/kg body weight/day and on average is less than 0.4 mg/kg body weight/day (Kirkpatrick & Lauer, 1986). Joint FAO/WHO Expert Committee on Food Additives (JECFA) reported that the acceptable daily intake (ADI) of BHA is 0 to 0.5 mg/kg (Leclercq, Arcella, & Turrini, 2000; Shahidi & Wanasundara, 1992). Furthermore, at concentrations as low as 125 ppm, which is closer to food additive levels, BHA exhibits anticarcinogenic properties (Williams et al., 1999). BHA is used in baked and fried foods as well as in dry cereals, potato products, dessert mixes and beverages (Dolatabadi & Kashanian, 2010; Makahleh et al., 2015).

6.1.2. *Butylated hydroxytoluene (BHT)*

BHT is also a monophenol and commercially available as a white crystalline compound. However, BHT is not as effective as BHA mainly because of the presence of two tert-butyl groups, which offer greater steric hindrance than BHA to the molecule (Nanditha & Prabhasankar, 2009). BHT is soluble in fats and oils and insoluble in water (Table 2) and more effective in suppressing oxidation of animal fats than vegetable oils (Dziezak, 1986). Shahidi and Naczk (2004) reported that BHT can produce radical intermediates with moderate resonance delocalization. The tertiary butyl groups of BHT do not generally allow involvement of the radical formed from it after hydrogen abstraction in other reactions. Thus, a lipid peroxyl radical may join the molecule of BHT in the *para* position to the phenoxy group. The volatile nature of BHA and BHT makes them important additives in packaging materials because they can migrate into foods. For this purpose, these antioxidants are added directly to the wax used in making inner liners or applied to the packaging board as an emulsion (Dziezak, 1986; Shahidi & Wanasundara, 1992). BHT functions synergistically with BHA. A combination of 3-BHA and BHT showed a higher antioxidant activity than either of them used singly in soybean oil, lard, and methyl oleate (Nanditha & Prabhasankar, 2009). Chevillard, Nouhi, Anna, Paquet, and Blank (2010) found the first evidence that BHT exposure not only affects lung function but also leads to impaired adipogenesis in adipocytes. A number of studies have shown that BHT may cause internal and external haemorrhaging at high doses that is severe enough to cause death in some strains of mice and guinea pigs. This effect is due to the ability of BHT to reduce vitamin K dependent blood clotting factors (Ito et al., 1986). Babich (1982) suggested that BHT may exert a positive effect on life span; it exerts a negative effect on the lungs, kidneys, myocardial cells, liver metabolism of lipids, and clotting factors,

and is a potential behavioural and developmental teratogen. Williams et al. (1999) reported that BHT is not genotoxic or reproducibly carcinogenic, although at high doses of 250 mg/kg/day or greater, it was associated with some unconfirmed increases in spontaneous neoplasms and, like BHA, has some tumour-promoting activity. The overall evaluation of IARC (International Agency for Research on Cancer) was that BHT is not carcinogenic to humans (IARC, 1987). According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake (ADI) for BHT is 0–0.3 mg/kg body weight (Leclercq et al., 2000).

6.1.3. *Tertiary-butylhydroquinone (TBHQ)*

TBHQ (Fig. 4) is a highly effective preservative for unsaturated vegetable oils, many edible animal fats and meat products. It does not cause discolouration even in the presence of iron, and does not change the flavour or odour of the material to which it is added (Kashanian & Dolatabadi, 2009). Metabolically, TBHQ is formed from 3-*tert*-butyl-4-hydroxyanisole (BHA), another widely used food additive, by *O*-demethylation (Okubo, Yokoyama, Kano, & Kano, 2003). TBHQ may be considered as an alternative to hydrogenation for increasing oxidative stability (Dziezak, 1986; Shahidi & Wanasundara, 1992). TBHQ is commercially available as a beige coloured powder and may be used alone or in combination with BHA or BHT at a maximum concentration of 0.02% or 200 ppm, based on the fat content of foods, including essential oils (Shahidi & Naczk, 2004). Chelating agents such as citric acid and monoacylglycerol citrate can further enhance lipid stabilizing properties of TBHQ, which in combination is primarily used in vegetable oils and shortenings but not extensively for animal fats (Shahidi & Wanasundara, 1992). Khan and Shahidi (2001) reported that amongst synthetic antioxidants, TBHQ was more effective than BHA and BHT and served as the strongest antioxidant in borage and evening primrose oil triacylglycerols (TAG). The two *para*-hydroxyl groups in TBHQ are responsible for its antioxidant activity (Nanditha & Prabhasankar, 2009). TBHQ reacts with peroxyl radicals to form a semiquinone resonance hybrid. The semiquinone radical intermediate may undergo different reactions to form more stable products. They can also react with one another to produce a dimer or react with one another to produce a quinone and a hydroquinone molecule or add to a lipid peroxyl radical to produce a semiquinone (Shahidi & Naczk, 2004). In high doses, TBHQ has some negative health effects in laboratory animals, such as precursor to stomach tumours and damage to DNA. A number of studies have shown that TBHQ causes DNA cleavage *in vitro* and the formation of 8-hydroxydeoxyguanosine in calf thymus DNA due to the generation of ROS such as superoxide radical anion and hydrogen peroxide (Kashanian & Dolatabadi, 2009). TBHQ and its metabolite 2-*tert*-butyl-1,4-benzoquinone (TBQ) were both cytotoxic in human monocytic leukaemia U937 cells, TBQ being more strongly cytotoxic (Okubo et al., 2003). Increase of antioxidative potential by TBHQ protects against cell death associated with 6-hydroxydopamine-induced oxidative stress in neuroblastoma SH-SY5Y cells (Hara, Ohta, Ohta, Kuno, & Adachi, 2003). The acceptable daily intake (ADI) of TBHQ is 0–0.2 mg/kg body weight (Nanditha & Prabhasankar, 2009; Shahidi & Wanasundara, 1992). Recent studies suggest that addition of TBHQ could prevent biodiesel oxidation and increase the storage time (De Araujo, Barbosa, Viana, & Ferreira, 2011; Goulart, Teixeira, Ramalho, Terezo, & Castilho, 2014).

6.1.4. *Propyl gallate (PG)*

Propyl gallate (PG, Fig. 4) has been used since 1948 to stabilize cosmetic, and food-packaging materials, and foods containing fats, and as an additive in edible fats, oils, mayonnaise, shortening, baked products, pressure-sensitive adhesives, lubricating oil additives and transforming oils (Zurita et al., 2007). Propyl gallate is commercially prepared by esterification of gallic acid with propyl alcohol followed by distillation to remove the excess alcohol and is available as a white crystalline powder. It is soluble in ethanol but practically insoluble in water (Shahidi & Naczk, 2004). With a melting point of 148 °C, PG loses its effectiveness during heat processing and is therefore not suitable in frying applications that involve temperatures exceeding 190 °C. PG chelates iron ions and forms an unappealing blue-black coloured complex. Hence, PG is always used with chelators such as citric acid to eliminate the pro-oxidative iron and copper catalysts (Shahidi & Naczk, 2004). Good synergism between PG and BHA and BHT is obtained; however, their coapplication with TBHQ is not permitted (Buck, 1984; Shahidi & Wanasundara, 1992). Propyl gallate and its metabolite, gallic acid, have been shown to exhibit liver toxicity and enhance carcinogenesis (Eler, Peralta, & Bracht, 2009; Kim, Kang, Lee, Lee, & Lee, 2008). PG is cytotoxic to isolated rat hepatocytes because it impairs mitochondria, leading to ATP depletion (Nakagawa, Nakajima, Tayama, & Moldeus, 1995). PG inhibits the growth of microorganisms by inhibiting respiration and nucleic acid synthesis; it also decreases hepatic microsomal hydrolase and demethylase activities and inhibits the activity of some redox enzymes (Han & Park, 2009). A number of studies have demonstrated that the antioxidative and cytoprotective properties of propyl gallate may change to prooxidative, cytotoxic and genotoxic in the presence of Cu(II) (Jacobi, Eicke, & Witte, 1998; Jacobi, Hinrichsen, Weß, & Witte, 1999). The acceptable daily intake (ADI) of PG is 0–0.25 mg/kg body weight (Nanditha & Prabhasankar, 2009).

6.2. *Natural phenolic antioxidants*

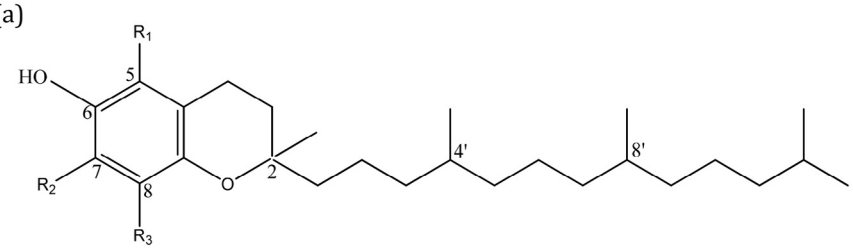
In recent years, the possible toxicity of synthetic antioxidants has been considered. Thus, the potential of plant products to serve as antioxidants to protect against various diseases induced by free radicals has been explored (Hou et al., 2003). In addition to scavenging free radicals, the multiple activities of antioxidants include inactivating metal catalysts by chelation, reducing hydroperoxides into stable hydroxyl derivatives, and interacting synergistically with other reducing compounds (Frankel & Finley, 2008). Increased popularity of natural food additives may prompt more food manufacturers to replace synthetic antioxidants with ingredients containing natural antioxidative compounds. Therefore, research on natural additives has gained momentum as they are perceived as posing no health risk to the consumers (Shahidi & Wanasundara, 1995). Naturally-occurring antioxidant compounds are flavonoids, phenolic acids, lignans, terpenes, tocopherols, phospholipids and polyfunctional organic acids, amongst others. Sources of natural antioxidants are primarily plant phenolics that may occur in

all parts of plants. They can be found in fruits, vegetables, nuts, seeds, leaves, flours, roots and barks (Wanasundara, Amarowicz, & Shahidi, 1996). There have been numerous studies on the biological activities of phenolics, which are potent antioxidants and free radical scavengers (Naczk & Shahidi, 2004, 2006; Tung, Wu, Kuo, & Chang, 2007).

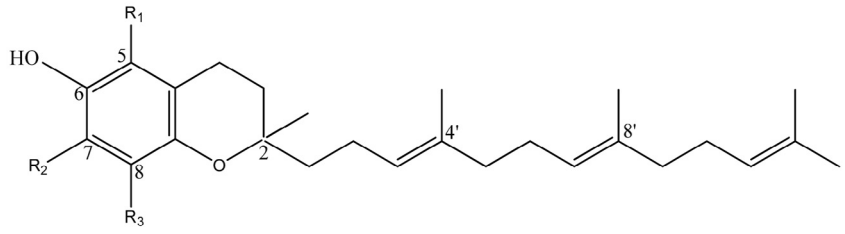
6.2.1. *Tocopherols and tocotrienols*

Tocopherols and tocotrienols (collectively known as tocols) are monophenolic compounds, and comprise a group of eight chromanol homologues that possess vitamin E activity in the diet (Blekas, Tsimidu, & Bosku, 1995). Tocopherols consist of a 6-chromanol group and an apolar phytal chain, having the prefix α-, β-, γ-, or δ-, depending on the number and position of methyl groups attached to the chromane rings (Dziezak, 1986; Kiokias et al., 2008; Shahidi & Wanasundara, 1992). The tocotrienols differ from tocopherols in having unsaturated side chains at positions 3′, 7′, and 11′ (Seppanen, Song, & Csallany, 2010). The chemical structures and vitamin E activity related compounds are given in Fig. 5. They may retard the development

(a)



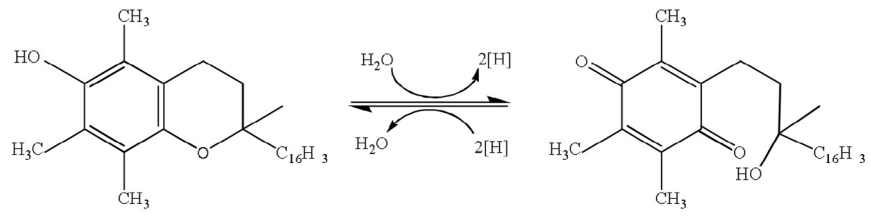
Tocopherol



Tocotrienol

Compound	R_1	R_2	R_3
5,7,8-Trimethyl tocopherol (α-tocopherol) 5,7,8-Trimethyl tocotrienol (α-tocotrienol)	CH_3	CH_3	CH_3
7,8-Dimethyl tocopherol (β-tocopherol) 7,8-Dimethyl tocotrienol (β-tocotrienol)	H	CH_3	CH_3
5,8-Dimethyl tocopherol (γ-tocopherol) 5,8-Dimethyl tocotrienol (γ-tocopherol)	CH_3	H	CH_3
8-Methyl tocopherol (δ-tocopherol) 8-Methyl tocotrienol (δ-tocotrienol)	H	H	CH_3

(b)



α – tocopherol

α – tocopherylquinone (stable)

Fig. 5 – (a) Chemical structures of tocopherols and tocotrienols, (b) α-Tocopherol/α-tocopherylquinone redox system (adapted from Shahidi & Naczk, 2004).

of precancer lesions and tumours (Lee et al., 2009) and combat free radical reactions that can cause DNA mutations (Gester, 1993). Tocopherols are present in oilseeds, leaves, and other green parts of higher plants. α-Tocopherol is present mainly in the chloroplasts of plant cells, whilst β-, γ- and δ-homologues are usually found outside these organelles. In contrast, tocotrienols are not found in the green parts of the plants but, rather, in the bran and germ fractions of certain seeds and cereals (Kamal-Eldin & Appelqvist, 1996). Tocopherols and tocotrienols play two main roles as antioxidants, firstly as scavengers of lipid peroxyl radicals, and secondly as singlet oxygen quenchers and chemical scavengers (Munne-Bosch & Alegre, 2002). The antioxidant activity of tocols is mainly due to their ability to donate their phenolic hydrogen to lipid free-radicals (Kamal-Eldin & Budilarto, 2015). With regard to vitamin E activity, α-tocopherol is the most potent member of this family (Shahidi & Naczk, 2004) whilst the other tocols are less effective, being 15–40% for β-tocopherol, 1–20% for γ-tocopherol and 15–30% for δ-tocopherol. Meanwhile, the corresponding values for tocotrienols are 1% for α-tocotrienol, 15–30% for β-tocotrienol, 1–5% for γ-tocotrienol and 1% for δ-tocotrienol (Shahidi, 2000a). Although it is generally agreed that the relative antioxidant activity of tocopherols *in vivo* is in the order of $\alpha > \beta > \gamma > \delta$, there is a widespread confusion concerning their relative potency *in vitro* (Burton & Ingold, 1981). Hamam and Shahidi (2006) reported that γ- and δ-tocopherols were stronger antioxidants *in vitro*. The chemical structures of the tocopherols and tocotrienols support a hydrogen-donating power in the order $\alpha > \beta > \gamma > \delta$. This order was also obtained when the activity of the four tocopherols was compared in a homogeneous solution of dichlorobenzene (Burton & Ingold, 1981), but a reverse order ($\alpha < \beta \approx \gamma < \delta$) was obtained when relative antioxidant potencies were compared in fats, oils, and lipoproteins *in vitro* (Gottstein & Grosch, 1990; Hamam & Shahidi, 2006; Kamal-Eldin & Appelqvist, 1996; Kamal-Eldin & Budilarto, 2015; Lea & Ward, 1959; Olcott & Van Der Ven, 1968). The reason behind this reverse order is not yet clearly understood. However, it is now known that the "absolute" and "relative" *in vitro* activities of the tocopherols are not only dependent on their absolute chemical reactivities towards hydroperoxyl and other free radicals, but also on many other possible factors such as temperature and light, type of substrate and solvent, and by other chemical species acting as prooxidants and synergists in the system (Kamal-Eldin & Budilarto, 2015). The antioxidant activity of α-tocopherol is based mainly on the tocopherol–tocopherylquinone system (Fig. 5) (Shahidi & Wanasundara, 1992). Tocopherols (AH_2) are radical scavengers and quench lipid radicals ($R^{\bullet}$), thus regenerating RH molecules as well as producing a tocopheryl semiquinone radical. Two tocopheryl semiquinone radicals (AH) may form a molecule of tocopheryl quinone (A) and a regenerated tocopherol molecule (Reactions 11 and 12) (Schuler, 1990).

$$R^{\bullet} + AH_2 \rightarrow RH + {}^{\bullet}AH \quad (11)$$

$$2\,{}^{\bullet}AH \rightarrow A^{\bullet} + AH_2 \quad (12)$$

However, in the absence of antioxidants such as ascorbic acid and glutathione, which recycle tocopheroxyl and tocotrienoxyl radicals (Munne-Bosch & Alegre, 2002), the radicals may undergo radical–radical coupling with other lipid peroxyl radicals to form adducts, may disproportionate to form quinones or may undergo self-coupling with other tocopheroxyl and tocotrienoxyl radicals to form dimers and/or trimers (Kamal-Eldin & Appelqvist, 1996; Kamal-Eldin & Budilarto, 2015; Munne-Bosch & Alegre, 2002). Several authors have claimed that adding tocopherols to vegetable oils (even to the refined oils, where some tocopherols are removed during deodourization) hardly shows any improvement in oxidative stability due to the fact that an optimum concentration of these compounds is still present (Abuzaytoun & Shahidi, 2006; Hamam & Shahidi, 2006; Kiokias et al., 2008). Tocotrienols are present especially in palm oil and in cereal grain oils, especially rice bran oil (Cerretani, Lerma-Garcia, Herrero-Martinez, Gallina-Toschi, & Simo-Alfonso, 2010). The relative concentrations of tocopherols and tocotrienols vary widely from one oil to another, which can be used to distinguish them according to their botanical origin. Thus, α-tocopherol is the most representative antioxidant in olive oil (Tasioula-Margari & Okogeri, 2001) whilst γ- and δ-tocopherol contents are high in soybean and sunflower oils, with soybean oil particularly rich in γ-tocopherol (Cerretani et al., 2010). Tocopherol and/or tocotrienol concentrations have been used to detect the adulteration of olive oil with red palm and hazelnut oils and soybean oil with linseed oil (Dionisi, Prodolliet, & Tagliaferri, 1995; Manandhar, Nagao, & Yamazaki, 1986). Approximate content of tocopherols and tocotrienols found in vegetable oil are given in Table 4. In food manufacturing practice, it is recommended to keep the amount of total tocopherol (natural or added) at levels between 50 and 500 ppm (Kiokias et al., 2008). Kamal-Eldin and Appelqvist (1996) reported the synergistic effect of tocols with other antioxidants. Kancheva et al. (2014) observed synergism for the binary mixtures of α-tocopherol with ascorbyl palmitate and dehydrozingerone, which is one half of the molecule of curcumin. α-Tocopherol and β-carotene may play complementary roles or even show synergism in systems of low oxygen pressure. Parkhurst, Skinner, and Strum (1968) found that mixed tocopherols (α-, γ-, and δ-) gave better protection to lard than individual tocopherols and suggested some synergistic interaction. Synergism between tocopherols and phospholipids has also been reported in the literature (Hudson & Mahgoub, 1981; Kamal-Eldin & Appelqvist, 1996; Shahidi, 2000a), which are mainly due to the metal-chelating properties of the latter (Linow & Mieth, 1976). Kago and Terao (1995) suggested that phospholipids enhance the antioxidant potency of tocopherols through a physical rather than a chemical action. It was postulated that the phospholipids form reverse micelles (or microemulsions) when dissolved in organic solvents or bulk oils and that tocopherols are solubilized and positioned in these microemulsions with their active phenolic group near the polar region where peroxyl radicals are concentrated (Kamal-Eldin & Appelqvist, 1996). Maillard reaction products (called melanoidins) produced by reactions of reducing sugars with amino acids are good inhibitors of oxidation, and act as synergists with tocopherols (Kamal-Eldin & Appelqvist, 1996; Yamagushi & Fujimaki, 1974). Ascorbic acid was mentioned to regenerate α-tocopherol from its tocopheroxyl radical *in vivo* and *in vitro* and thereby restoring its antioxidant activity (Han, Yi, & Shin, 1991; Iglesias, Pazos, Torres, & Medina, 2012;

Table 4 – Tocopherol and tocotrienol contents (mg/kg) of vegetable oils.

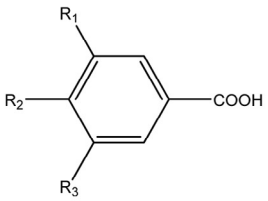
Oil	Tocopherol				Tocotrienol			
	α	β	γ	δ	α	β	γ	δ
Borage[c]	–	–	150	1350	–	–	–	–
Camelina[a]	38	0.9	720	15	–	–	–	–
Coconut[b]	5–10	–	5	5	5	Trace	1–20	–
Corn[a]	180	11	440	22	9.4	–	13	2.6
Cottonseed[b]	40–560	–	270–410	0	–	–	–	–
Evening primrose[c]	160	–	420	65	–	–	–	–
Linseed[a]	12	Trace	520	9.5	–	–	–	–
Maize, grain[b]	60–260	0	400–900	1–50	–	0	0–240	0
Maize, germ[b]	300–430	1–20	450–790	5–60	–	–	–	–
Olive[b]	1–240	0	0	0	–	–	–	–
Palm[b]	180–260	Trace	320	70	120–150	20–40	260–300	70
Peanut[b]	80–330	–	130–590	10–20	–	–	–	–
Rapeseed/canola[b]	180–280	–	380	10–20	–	–	–	–
Safflower[b]	340–450	–	70–190	230–240	–	–	–	–
Sesame[a]	79	4.1	360	12	Trace	–	3.4	–
Soybean[b]	30–120	0–20	250–930	50–450	0	0	0	–
Sunflower[b]	350–700	–	10–50	1–10	–	–	–	–
Walnut[a]	560	20–40	590	450	–	–	–	–
Wheat grain[b]	560–1200	660–810	260	270	20–90	80–190	–	–
Wheat germ[c]	1100	440	330	110	–	110	–	–

Sources: Adapted from [a] Schwartz, Ollilainen, Piironen and Lampi (2008); [b] Shahidi and Naczk (2004); [c] Shahidi (2004).

Kamal-Eldin & Appelqvist, 1996; Kamal-Eldin & Budilarto, 2015; Shahidi, 2000a; Sharma & Buettner, 1993). Glutathione is also capable of regenerating α-tocopherol from its radical (Niki, Tsuchiya, Tanimura, & Kamiya, 1982). The commercial synthesis of α-tocopherol involves the condensation of 2,3,5-trimethylhydroquinone with phytol, isophytol, or phytal halogenides (Shahidi & Wanasundara, 1992). Extraction of vitamin E from natural sources has received increasing interest due to the high antioxidant activity associated with this family of compounds. Besides its well-known antioxidant activity, studies have demonstrated that synthetic vitamin E is less effective than natural vitamin E (Hadolin, Skerget, Knez, & Bauman, 2001). Tocopherols are now obtained mainly by vacuum distillation of deodourizing-step residues generated in the refining of vegetable oils. Throughout this process, that includes several steps such as solvent recovery and purification, copious amounts of organic solvents and energy are required, and thermal degradation of tocopherols is commonly encountered (de Lucas, Martinez de la Ossa, Rincón, Blanco, & Gracia, 2002). Interest in the application of supercritical fluid extraction using carbon dioxide (SFE-CO_2) has grown continuously because SFE-CO_2 shows several advantages over classical extraction processes with organic solvents. Under the supercritical fluid conditions, supercritical fluid extraction (SFE) technology is suitable to decrease volatility and thermal degradation during compounds extraction (de Lucas et al., 2002; Gelmez, Kıncal, & Yener, 2009; Nyam et al., 2010). The total tocopherol content of the extracts is usually between 30 and 80%, but higher in γ- and δ-tocopherols. To obtain stable α-tocopheryl acetate, the mixture is methylated and subsequently acetylated (Shahidi & Naczk, 2004). The α-tocopheryl acetate is the commercially available form of vitamin E, which is not an antioxidant because its active —OH group is esterified. However, under acidic aqueous conditions, tocopherol is released by hydrolysis and the released tocopherol may then act as an antioxidant (Schuler, 1990).

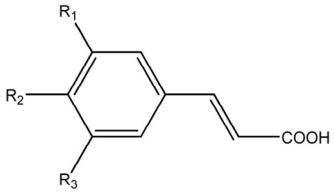
6.2.2. *Phenolic acids (hydroxybenzoic and hydroxycinnamic acids)*

Phenolic acids, known to serve as multipurpose bioactive compounds, are widely spread throughout the plant kingdom. Most of them are an integral part of the human diet and are also consumed as medicinal preparations. Many of the health protective effects of phenolic compounds have been ascribed to their antioxidant, antimutagenic, anticarcinogenic, antiinflammatory, antimicrobial, and other biological properties (Xu, Ye, Liu, Ma, & Chen, 2008). Substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids in plants, with hydroxycinnamic acids being the more common (Fig. 6). These derivatives differ in the pattern of the hydroxylation and methoxylation in their aromatic rings (Mattila & Hellström, 2007; Shahidi & Naczk, 2004). Technically speaking, only benzoic acid derivatives are phenolic acids and cinnamic acid derivatives are phenylpropanoids. The basic pathway for synthesis of phenolic acids in plants begins from sugars through to aromatic amino acids – phenylalanine, and, in some rare cases, tyrosine. The formation of *trans*-cinnamic acid from phenylalanine and *p*-hydroxycinnamic acid from tyrosine is catalysed by phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL), respectively (Amarowicz, Carle, et al., 2009; Amarowicz, Zegarska, et al., 2009; Shahidi, 2015). Phenolic acids are present in some plant foods mostly in the bound form. The most common hydroxycinnamic acids are caffeic, *p*-coumaric and ferulic acids, which frequently occur in foods as simple esters with quinic acid or glucose. Probably the most well-known bound hydroxycinnamic acid is chlorogenic acid, which is combined caffeic and quinic acids. Unlike hydroxycinnamates, hydroxybenzoic acid derivatives



Acid	R_1	R_2	R_3
p-Hydroxybenzoic	H	OH	H
Protocatechuic	OH	OH	H
Vanillic	OCH_3	OH	H
Syringic	OCH_3	OH	OCH_3
Gallic	OH	OH	H

Hydroxybenzoic acid



Acid	R_1	R_2	R_3
p-Courmaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	OCH_3	OH	H
Sinapic	OCH_3	OH	OCH_3

Hydroxycinnamic acid

Fig. 6 – Chemical structures of naturally occurring phenolic acids and related compounds.

are mainly present in foods in the form of glucosides; *p*-hydroxybenzoic, vanillic and protocatechuic acids are the most common forms (Herrmann, 1989; Mattila & Hellström, 2007; Shahidi & Chandrasekara, 2010; Shahidi, McDonald, Chandrasekara, & Zhong, 2008; Shahidi & Naczk, 2004; Yeo & Shahidi, 2015). Phenolic acids behave as antioxidants, due to the reactivity of their phenol moiety (hydroxyl substituent on the aromatic ring). Although there are several mechanisms, the predominant mode of antioxidant activity is believed to be radical scavenging via hydrogen atom donation. Other established antioxidant, radical quenching mechanisms are through electron donation and singlet oxygen quenching (Shahidi & Wanasundara, 1992). Substituents on the aromatic ring affect the stabilization and therefore affect the radical-quenching ability of these phenolic acids. Different acids therefore have different antioxidant activities (Rice-Evans, Miller, & Paganga, 1996). The antioxidant behaviour of the free, esterified and glycosylated phenolics has been reported (Robbins, 2003). There is an awareness and interest in the antioxidant behaviour and potential health benefits associated with these simple phenolic acids. It is their role as dietary antioxidants that has received the most attention in recent literature (Rice-Evans et al., 1996; Robbins, 2003). Because of their ubiquitous presence in plant-based foods, humans consume phenolic acids on a daily basis. The estimated range of consumption is 25 mg–1 g per day depending on the diet consumed (fruit, vegetables, grains, teas, coffees, spices) (Clifford, 1999). Caffeic acid, one of the most prominent naturally occurring cinnamic acids, is known to selectively block the biosynthesis of leukotrienes, components involved in immunoregulation diseases, asthma and allergic reactions (Koshihara et al., 1984). Other studies have reported that caffeic acid and some of its esters might possess antitumour activity against colon carcinogenesis (Olthof et al., 2001; Robbins, 2003).

6.2.3. *Flavonoids*

Typically, when discussing phenolics in plant foods, flavonoids are the predominant class described, because they account for approximately two-third of the dietary phenols (Robbins, 2003). Flavonoids are cyclized diphenylpropanes that commonly occur in plants and particularly plant foods (Cao et al., 1997). More than 6000 flavonoids have been identified (Harborne & Williams, 2000). The immediate family members of flavonoids include flavones, flavonol, isoflavones, flavanones, flavanonol, flavanol and anthocyanidin. Flavanones undergo a series of transformations affecting the heterocyclic carbon ring to give rise to anthocyanins and catechins (Das, 1994). Flavonoid derivatives vary in their structure around the heterocyclic oxygen ring, but all have the characteristic C_6—C_3—C_6 carbon skeleton (Fig. 7) (Shahidi & Naczk, 2004; Yao et al., 2004). In general, all flavonoids are derivatives of the 2-phenylchromone parent compound composed of three phenolic rings (A, B and C in Fig. 7), all of which exhibit various levels of hydroxylation and methoxylation (Yao et al., 2004). The biochemical activities of flavonoids and their metabolites depend on their chemical structures and the relative orientation of various moieties in the molecules. Isoflavonoids are found mainly in the Leguminosae family, consisting of a phenyl ring (the A-ring, Fig. 7) fused with the six-membered heterocyclic C-ring and another phenyl ring (the B-ring) at the C-3 position, whereas for flavonoids, the B-ring is substituted to the C-2 position. Despite the subtle structural differences, some isoflavonoids are more active as antioxidants than their corresponding flavonoids (Han et al., 2009). Flavones and flavonols occur as

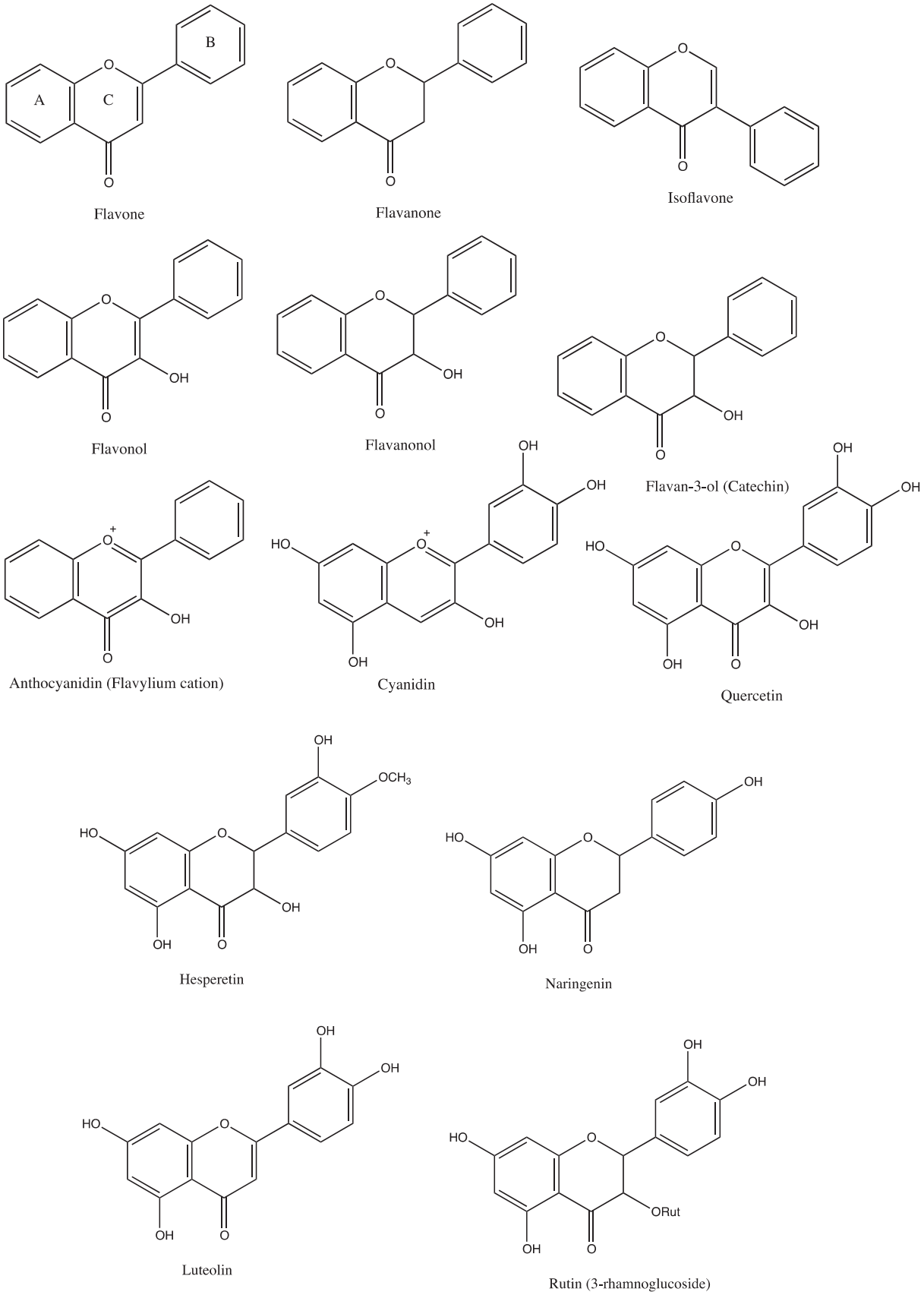


Fig. 7 – Chemical structures of major classes of flavonoids and some selected flavonoids and flavonoid related compounds.

aglycones in foods; approximately 200 flavonols and some 100 flavones have been identified in plants. These compounds possess a double bond between C-2 and C-3. Flavonols are different from flavones in that they possess a hydroxyl group in the 3-position and can be regarded as 3-deoxyflavonols (Fig. 7) (Shahidi & Naczk, 2004). Meanwhile, flavonones and flavononols are characterized by the presence of a saturated C_2—C_3 bond and an oxygen atom (carbonyl group) in the 4-position. Thus, flavonones may be referred to as dihydroflavones. Flavononols differ from flavonones by having a hydroxyl group in the 3-position and are often referred to as 3-hydroxyflavonones or dihydroflavonols (Shahidi & Naczk, 2004). Amongst flavonoids, anthocyanins and catechins, known collectively as flavans because of lack of the carbonyl group in the 3-position, are important; flavan-3-ols and flavan-3,4-diols belong to this category (Shahidi & Naczk, 2004). Examples of some naturally occurring flavonoids in foods and food components are provided in Table 5. Flavonols are represented mainly by quercetin, kaempferol and myricetin, of these, quercetin is most ubiquitous. In a recent study by Sultana and Anwar (2008), the highest concentrations of flavonol were detected in the medicinal plant, moringa (*Moringa oleifera*), followed by strawberry, peepal, spinach and cauliflower amongst 22 plant materials (Jaganath & Crozier, 2010). Unlike flavonols, flavones are not widely distributed with significant concentrations being reported in only celery, parsley and artichoke (Jaganath & Crozier, 2010). Flavan-3-ols are found abundantly in fruits such as apricots, sour cherries, grapes and blackberries whereas flavanones are exclusively found in citrus fruits in their glycosidic forms (Jaganath & Crozier, 2010). The most widespread anthocyanin in fruits is cyanidin-3-glucoside (Kong, Chia, Goh, Chia, & Brouillard, 2003). Anthocyanins occur in abundance in berries where they provide the fruits with their distinctive and vibrant palate of colours. Cranberry, blackberry and elderberry contain derivatives of only one type of anthocyanin (i.e., cyanidin), whilst a wide array of anthocyanins occur in blueberry and blackcurrant (Jaganath & Crozier, 2010). Meanwhile, soybeans are almost the sole dietary source of isoflavones. Common isoflavones such as genistein, daidzein and glycitein, also occur, albeit in low levels, in black beans and green peas (Jaganath & Crozier, 2010). Delmonte, Perry, and Rader (2006) reported the presence of isoflavones in red clover and a perennial vine kudzu (*Pueraria lobata*).

Table 5 – Different classes of flavonoids and dietary sources.

Class	Name	Dietary source
Flavone	Chrysin	Fruit skins
	Apigenin	Parsley, celery
Flavonone	Naringin	Citrus, grapefruit
	Naringenin	Citrus
	Taxifolin	Citrus
	Eriodictyol	Lemons
	Hesperidin	Oranges
	Isosakuranetin	Citrus
Flavonol	Kaempferol	Leek, broccoli, endives, grapefruit, black tea
	Quercetin	Onion, lettuce, broccoli, tomato, tea, berries, apples, olive oil, cranberry
	Rutin	Buckwheat, citrus, red pepper, red wine, tomato skin
Flavononol	Engeletin	White grapeskin
	Astilbin	White grapeskin
	Genistin	Soybean
	Taxifolin	Fruits
Isoflavone	Genistein	Soybean
	Daidzin	Soybean
	Daidzein	Soybean
Flavanol	(+)-Catechin	Tea
	(+)-Gallocatechin	Tea
	(–)-Epicatechin	Tea
	(–)-Epigallocatechin	Tea
	(–)-Epicatechin gallate	Tea
	(–)-Epigallocatechin gallate	Tea
Anthocyanidin	Epigenidin	Stored fruits
	Cyanidin	Cherry, raspberry, strawberry, grapes
	Delphinium	Dark fruits
	Pelargonidin	Dark fruits

Source: Adapted from Shahidi and Naczk (2004).

In general, the ability of flavonoids to be effective antioxidants depends on three factors: (i) the metal-chelating potential that is strongly dependent on the arrangement of hydroxyls and carbonyl group around the molecule, (ii) the presence of hydrogen-/electron-donating substituents able to reduce free radicals, and (iii) the ability of the flavonoid to delocalize the unpaired electron leading to the formation of a stable phenoxyl radical. Both known modes of the antioxidant action, i.e., preventive mechanism and chain-breaking mechanism, are postulated to be responsible for the high activity of flavonoids (Musialik, Kuzmicz, Pawłowski, & Litwinienko, 2009). There are numerous studies devoted to the importance of flavonoid structure for their antiradical activity as chain-breaking antioxidants (Cao et al., 1997; Rice-Evans et al., 1996; Zhou, Miao, Yang, & Liu, 2005). It is generally accepted that the excellent antioxidant properties of flavonoids are due to the presence of catechol hydroxyl groups in the B ring (Musialik et al., 2009; Rice-Evans et al., 1996; Zhou et al., 2005). Shahidi and Wanasundara (1992) reported that the *o*-dihydroxylation of the B ring contributes to the antioxidant activity. The *p*-quinol structure of the B ring has been shown to impart an even greater activity than *o*-quinol; however, *p*- and *m*-hydroxylation of the B ring does not occur naturally. All flavonoids with 3′,4′-dihydroxy configuration possess antioxidant activity. The TEAC (trolox equivalent antioxidant activity) assay, originally described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993), is based on scavenging of long-lived radical anions (Scott, Chen, Bakac, & Espenson, 1993). In this assay radicals are generated through the peroxidase activity of metmyoglobin in the presence of hydrogen peroxide and can easily be detected spectrophotometrically at 734 nm or by electron paramagnetic resonance (Shahidi & Zhong, 2007; van den Berg, Haenen, van den Berg, & Bas, 1999). Trolox equivalent antioxidant activity of flavonoids is given in Fig. 8. Quercetin has an identical number of hydroxyl groups in the same positions as catechin, but also contains the 2,3-double bond in the C ring and the 4-oxo group which allows delocalization between the A and B rings stabilizing the aryloxyl radical after hydrogen donation (Fig. 7). This structural advantage confers an enhancement

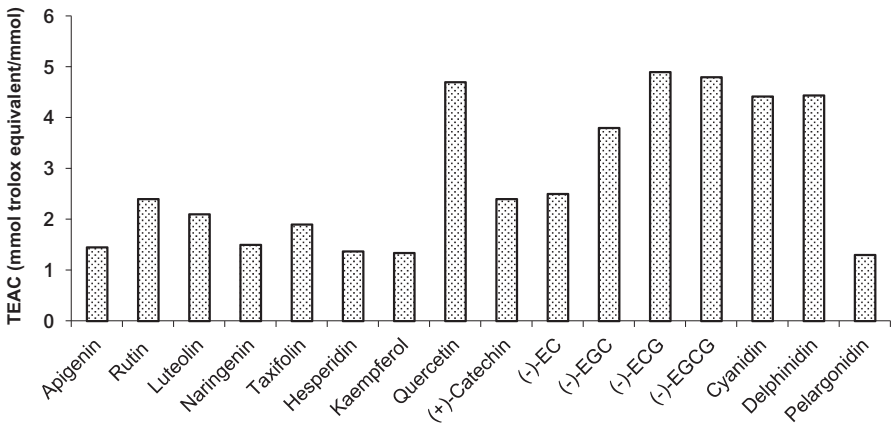


Fig. 8 – Trolox equivalent antioxidant activity of flavonoids (adapted from Rice-Evans et al., 1996).

of the TEAC value to 4.7 ± 0.10 mM (Rice-Evans et al., 1996). Cyanidin has o-dihydroxy structure in the B ring as quercetin, and the same number and arrangement of the five hydroxyl groups. Thus, the same TEAC value (4.44 ± 0.01 mM) as quercetin. Dehydroxylation to a monophenol in the B ring as in pelargonidin (1.3 ± 0.1 mM) gives much the same value as the equivalent flavan-3-ol, kaempferol. Two flavanones, naringenin and hesperitin (Fig. 7), have only one hydroxyl group on the B ring and possess little antioxidant activity. The glycosylation of flavonoids reduces their activity when compared to the corresponding aglycones (Shahidi & Wanasundara, 1992). Blocking the 3-hydroxyl group in the C ring of quercetin as a glycoside (whilst retaining the 3′,4′-dihydroxy structure in the B ring) as in rutin or removing the 3—OH group in the C ring as in luteolin decreases the antioxidant activity to a value of 2.40 ± 0.12 and 2.10 ± 0.05 mM, respectively (Fig. 7). Thus, the maximum effectiveness for radical scavenging apparently requires the 3—OH group attached to the 2,3-double bond and adjacent to the 4-carbonyl in the C ring (Rice-Evans et al., 1996). Han et al. (2009) reported that the isoflavonoid genistein was a better antioxidant than the isomeric flavonoid apigenin in phosphatidyl liposomes at pH 7.4. In the isoflavone, it is clear that both hydroxyl groups in 4′ and 5′ positions are needed for significant antioxidant activity as in genistein. Even 6,7,4′-trihydroxyisoflavone is marginally active, when compared to analogous flavone apigenin, which is inactive as an antioxidant. Genistein is particularly active. The resonance-stabilized quinoid structures show that for isoflavone the carbonyl group at position four remains intact and can interact with the 5-hydroxy group, if present; however, in flavone, the carbonyl group at position four loses its functionality. This may explain the superior antioxidant activity of genistein compared with that of apigenin (Shahidi & Naczk, 2004). The current data on the antioxidant properties of naturally occurring isoflavones and their corresponding metabolites indicate that the metabolism of genistein and daidzein to the oxidative metabolites 3′-hydroxygenistein and 3′-, 6-, and 8-hydroxyldaidzein as well as to the bacterial metabolite equol enhances their antioxidant properties. These compounds are more effective antioxidants than the known antioxidants, quercetin and ascorbic acid (Rufer & Kulling, 2006).

Flavonoids with high antioxidant activity have the following characteristic structures: (1) a 3′,4′-dihydroxyl group in the B ring, (2) the 3—OH moiety in the C ring, (3) the C2—C3 double bond in the C ring conjugated with a 4-keto group causing electron delocalization from the B ring, and (4) both 3—OH group in C ring and 5—OH group in A ring combined with a 4-carbonyl group and C2—C3 double bond (Sun & Powers, 2007). In addition to antiradical efficiency, flavonoids act as metal chelators. The chelating capacity of flavonoids for Cu and Fe ions has been investigated (Brown, Khodr, Hider, & Rice-Evans, 1998; Engelmann, Hutcheson, & Cheng, 2005; Mira et al., 2002). The ortho-hydroxyl groups on the B ring, the 3-hydroxyl combined with the 4-carbonyl group or the 5-hydroxyl combined with the 4-carbonyl group are the metal-complexing sites. However, Cao et al. (1997) observed that flavonoids efficiently scavenged peroxyl or hydroxyl radicals but were prooxidant with Cu^{2+}. Shahidi and Naczk (2004) reported that such complexations with Cu may contribute to the antioxidative action of flavonoids. Chelation of metal ions renders them catalytically inactive. The antioxidant mechanisms of flavonoids might include synergistic effects. The antioxidant activity of flavonoids usually increases with an increase in the number of hydroxyl groups and a decrease in glycosylation. EC and ECG (epicatechin and epicatechin gallate), respectively with a vicinal diphenol structure in the B ring and a saturated C ring exhibit the strongest effects (Yao et al., 2004). Salah et al. (1995) showed that the total antioxidative activity and the order of effectiveness of green tea polyphenols as radical scavengers is ECG > EGCG (epigallocatechin gallate) > EGC > gallic acid > EC = catechin. In addition, lyophilized derivatives of green tea catechins exhibited strong antioxidant acitivities (Zhong et al., 2012; Zhong & Shahidi, 2011). However, the oxidation of low density lipoproteins is inhibited by catechin, EC, ECG, and EGCG to a similar degree, not as much as in the presence of EGC or gallic acid. Pekkarinen, Heinonen, and Hopia (1999) reported that myricetin and rutin exhibit a synergistic effect with α-tocopherol. Myricetin, quercetin and rutin protect α-tocopherol from decomposition, where myricetin is more protective than quercetin and rutin (Pekkarinen et al., 1999). The interaction between flavonoids and α-tocopherol might be resulting from their different localization in methyl linoleate. The synergistic

effect of (–)-epicatechin and quercetin with α-tocopherol in egg yolk phosphatidylcholine was partly explained by the different localization of antioxidants in the substrate (Terao, Piskula, & Yao, 1994). The remarkable synergistic effect of quercetin with α-tocopherol in a fish oil-bile salt emulsion, has been shown to be due to different localization of these antioxidants (Pekkarinen et al., 1999). The daily intake of flavonoids (reported at above 100 mg) is almost at the same level as the sum of the daily doses of other antioxidants including β-carotene (2–3 mg), vitamin C (70–100 mg), and vitamin E (7–10 mg) (Musialik et al., 2009). These data correspond to the reports of many health benefits originating from dietary intake of flavonoids. Some flavonoids have been found to possess antilipoperoxidant (Terao et al., 1994), antitumoural (Deschner, Ruperto, Wong, & Newmark, 1991), antiplatelet (Tzeng, Ko, Ko, & Teng, 1991), anti-ischaemic, anti-allergic, and anti-inflammatory (Middleton & Kandaswami, 1992) activities (Cao et al., 1997). One interesting example of flavonoid activity is the so-called "French paradox", that is, despite high fat intake, mortality from coronary heart disease is lower in some regions of France, a fact attributed to the regular drinking of red wine which contains high levels of flavonoids (approximately 200 mg per glass) and resveratrol (0.1–15 mg/l) (Zhou et al., 2005). In contrast to their beneficial effects, some flavonoids have also been found *in vitro* to be mutagenic (Ahmad, Fazal, Rahman, Hadi, & Parish, 1992; Cao et al., 1997; Popp & Schimmer, 1991; Rahman et al., 1992; Sahu & Gray, 1993, 1994). Flavonoids, such as quercetin and kaempferol, have been shown to induce nuclear DNA damage and lipid peroxidation in the presence of transition metals (Ahmad et al., 1992; Rahman et al., 1992; Sahu & Gray, 1993, 1994). These harmful effects were suspected to result from the prooxidant rather than antioxidant action of the related flavonoids (Hanasaki, Ogawa, & Fukui, 1994; Sahu & Gray, 1994). The rapid metabolic inactivation of mutagenic flavonoids catalysed by catechol-*O*-methyltransferase has been demonstrated by Zhu, Ezell, and Liehr (1994) *in vivo*. Cao et al. (1997) suggested that the action of catechol-*O*-methyltransferase could be a major reason for the lack of carcinogenic activities of some flavonoids *in vivo*. Transition metal-initiated prooxidant action of a flavonoid may also be responsible for changes in regulation of enzyme activities by the flavonoid (Cao et al., 1997).

6.2.4. *Stilbenes*

Stilbenes, in particular *trans*-resveratrol and its glucoside, are beneficial to health, having antioxidative, anticarcinogenic, and antitumour properties (Burns, Yokota, Ashihara, Lean, & Crozier, 2002; Jung et al., 2009; Torres, Poveda, Jimenez-Barbero, Ballesteros, & Plou, 2010). In plants a major form of resveratrol is *trans*-resveratrol-3-*O*-β-D-glucoside, often referred to as piceid or polydatin (Fig. 9) (Jensen, Wertz, & O'Neill, 2010). Resveratrol (3,5,4′-trihydroxystilbene) is produced by plants in response to damage, particularly in grapevines (Langcake & Pryce, 1976), pines, and legumes (Soleas, Diamandis, & Goldberg, 1997). The main dietary stilbene is resveratrol from red wine and peanuts (Burns et al., 2002) with lesser amounts found in pistachio, berries, red cabbage, spinach and certain herbs (Jaganath & Crozier, 2010). *trans*-Resveratrol has gained significant worldwide attention because of its ability to inhibit or retard a wide variety of animal diseases, including cardiovascular disease and cancer (Jaganath & Crozier, 2010). Gülçin (2010) reported that resveratrol is an effective antioxidant in different *in vitro* assays including total antioxidant activity, reducing power, DPPH•, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$ and $O_2^{\bullet-}$ scavenging, hydrogen peroxide scavenging, and metal chelating activities, when compared to standard antioxidant compounds such as BHA, BHT and α-tocopherol (Gülçin, 2010). Resveratrol may also be used in minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality, and prolonging the shelf life of pharmaceuticals. Hubbard et al. (2013) confirmed that resveratrol is an effective allosteric activator of SIRT1, an enzyme that belongs to the sirtuin family of nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases as well as responsible for gene silencing, DNA repair, and metabolic regulation, which are all related to ageing. Pterostilbene, a derivative of resveratrol with only one hydroxyl group, is found to be more effective in rendering health benefits than resveratrol itself (Chang et al., 2012; Larrosa, Tomás-Barberán, & Espín, 2003; Stivala et al., 2001). McCormack and McFadden

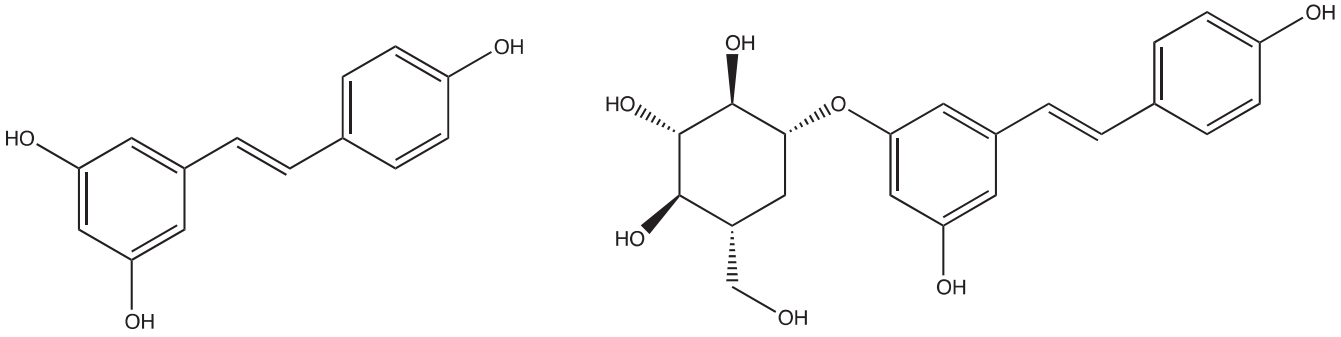


Fig. 9 – Chemical structures of resveratrol and its glucoside (adapted from Jensen et al., 2010).

(2012) reported that pterostilbene inhibits cancer growth through alteration of the cell cycle, induction of apoptosis, and inhibition of metastasis *invitro*. Furthermore, Pterostilbene has been shown to inhibits vascular endothelial growth factor (VEGF) production in human breast cancer [MDA-MB-231] cells (Sanghani, Kandra, Bavadekar, & Vansal, 2013). More recently, Siddalingappa, Benson, Brown, Batty, and Chen (2015) synthesized and evaluated the ester and ether-based poly-(ethylene glycol) succinylamide resveratrol and polylactide succinyl ester resveratrol polymer conjugates (soluble and polymeric micelles) and demonstrated that polymeric conjugates could be an effective approach to improve the pharmacokinetic profile of resveratrol as well as stability *in vitro* and *in vivo*.

6.2.5. *Tannins*

Tannins are defined as hydrolysable or condensed (proanthocyanidins), depending on their chemical structures (Fig. 10) (Shahidi & Naczk, 2004). The condensed tannins are oligomers and polymers of flavonoids, specifically flavan-3-ols, whilst hydrolysable tannins are glycosylated gallic acid (Ferreira & Li, 2000; Khanbabaee & van Ree, 2001). The phenolic groups of tannins bind very tightly with the —NH groups of peptides and proteins, they prevent their hydrolysis and digestion in the stomach and therefore are known to be anti-nutritional in nature (Shahidi & Naczk, 2004). In general terms, proanthocyanidins are principally found in fruits, especially berries, cocoa and some beverages like wine, beer and tea. Berries, legumes and leafy vegetables are the major sources of hydrolysable tannins (Serrano, Puupponen-Pimi, Dauer, Aura, & Saura-Calixto, 2009). Tannins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and are able to scavenge radicals such as hydroxyl, superoxide, and peroxyl, which are known to be important in cellular prooxidant state (Gyamfi & Aniya, 2002). Systemic effects related to mechanism of antioxidation have been debated since verification *in vivo* has not been successful due to inadequate biomarkers of effects (Halliwell, Rafter, & Jenner, 2005; Huang, Boxin, & Prior, 2005; Prior, Wu, & Schaich, 2005). Riedl, Carando, Alessio, McCarthy, and Hagerman (2002) have demonstrated that both hydrolysable and condensed tannins scavenge free radicals in a kinetically complex fashion involving both a fast and a slow scavenging step. Using the structure–activity studies for monomeric and polymeric phenolic compounds shows that 4 moles of radical are scavenged per *ortho*-substituted diphenol group (Riedl et al., 2002). The fast scavenging reaction is inhibited by complexation of the tannin with protein, but the overall capacity of the tannin–protein complex for scavenging is similar to that of the free tannin (Riedl et al., 2002). Scalbert (1991) reported that tannins seem to affect bacterial growth in several mechanisms, such as inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. In addition, several hydrolysable tannins have been shown to significantly inhibit the cytopathic effects of human immunodeficiency virus, HIV, and the expression of HIV-antigen in human lymphotropic virus type I-positive MT-4 cells (Nakashima et al., 1992). Amarowicz and Pegg (2013) reported antiproliferative activities in a concentration-dependent manner of hydrolysable tannins against five carcinoma cell lines, namely MCF-7 (oestrogen receptor-positive breast carcinoma), DU-145 (androgen receptor-negative prostate carcinoma), HT-29 (colon carcinoma), SK-MEL-5 and MDA-MB-435 (melanoma; skin carcinoma). Yoshida, Nakata, and Okuda (1999) reported that raspberry extracts containing ellagitannins sanguiin H6 and lambertianin exhibits antitumour activities.

7. Degradation of phenolic antioxidants

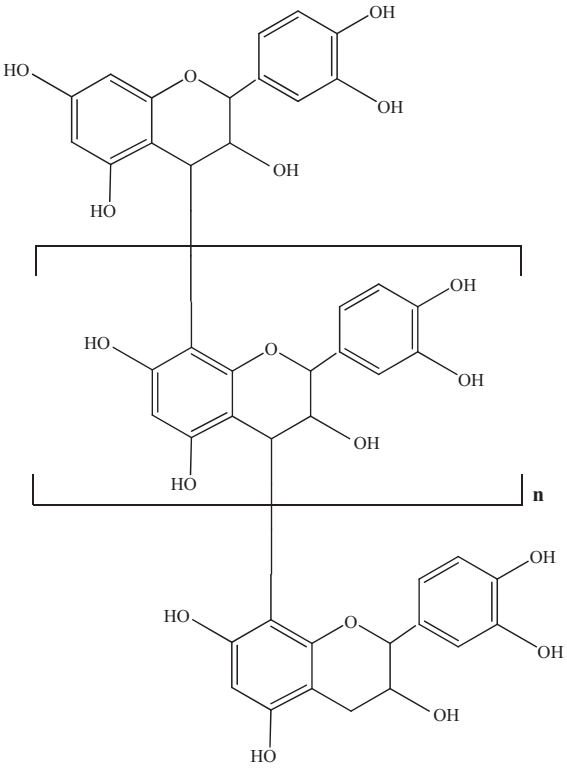
Phenolic antioxidants generally undergo degradation during oxidative processes such as those of fats and oils and produce a range of products, especially dimers of the antioxidants (Shahidi & Naczk, 2004). These dimers may be produced by the formation of phenoxyl radicals followed by radical rearrangement and a coupling reaction with another radical (Kikugawa, Kunugi, & Kurechi, 1990; Shahidi & Wanasundara, 1992). Moreover, most oxidation products of antioxidants retain some antioxidant activity, which may influence the potential of the parent antioxidant during the course of its degradation (Kikugawa et al., 1990). Amongst the breakdown products of BHT shown in Fig. 11a, products (2) through (4) possess antioxidant properties whilst products (1) and (5) are not effective as antioxidants (Kikugawa et al., 1990; Shahidi & Naczk, 2004). The antioxidant activities of these breakdown products were determined as the ratio of the induction period of the oil containing these compounds to that of the oil containing the parent antioxidant (Kikugawa et al., 1990). The degradation products of BHA shown in Fig. 11b are less effective than BHA itself, in the order of BHA > (7) > (6) (Shahidi & Naczk, 2004). All oxidation products of TBHQ retained some antioxidant effect, but compounds (9) and (11) exhibited a greater antioxidant activity than that of TBHQ (Fig. 11c). As shown in Fig. 12a, irradiation of propyl gallate in ethanol produces ellagic acid with excellent antioxidant activity (Shahidi & Naczk, 2004). Degradation of mixtures of BHA, BHT and PG produced heterodimers between different antioxidant components (Fig. 12b). Although product (14) was new, the heterodimer (15) and heterodimer (16) exhibited activities comparable to that of PG (Shahidi & Naczk, 2004). Studies have shown that extended exposure to certain temperatures, several other factors such as oxygen, alkali, light, minerals, and hydroperoxides degrade α-tocopherol (Sabliov et al., 2009). It has been shown that UV light initiates formation of α-tocopherol oxy radical (breaking O—H bond) and can be dissociated to make a semiquinone anion-radical. The new semiquinone anion-radical could interact with oxygen and convert into a di-radical (Sabliov et al., 2009).

8. Natural sources of phenolic antioxidants

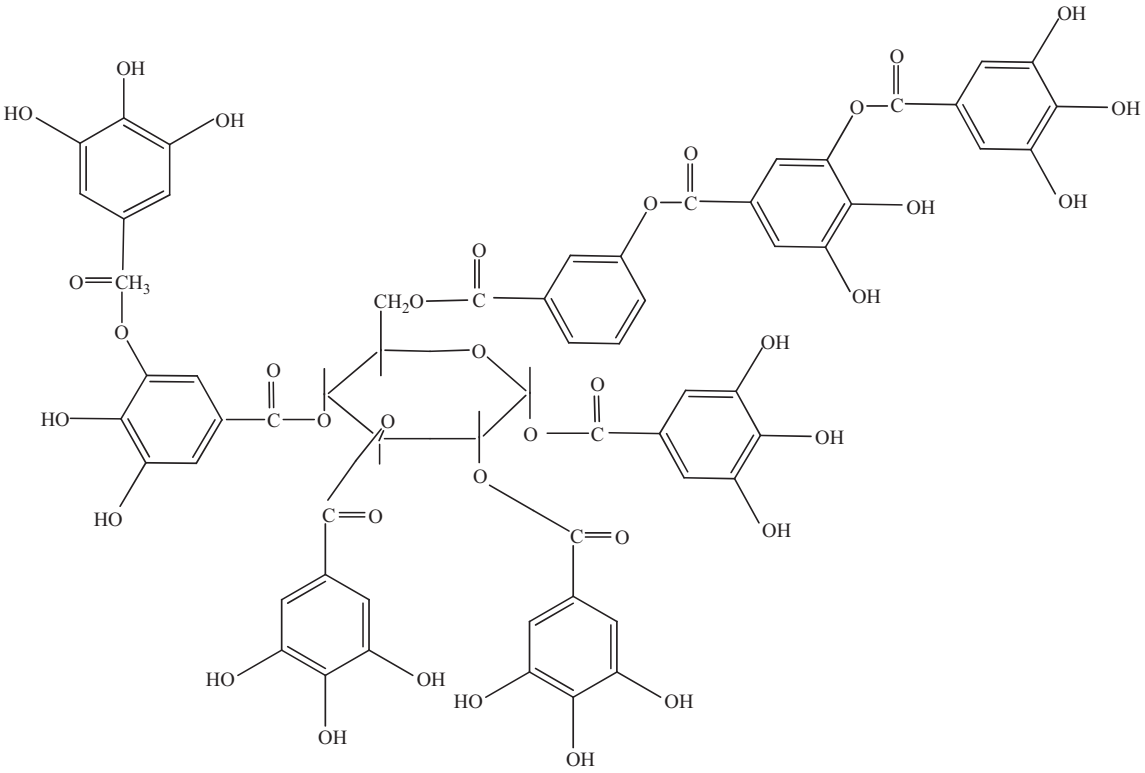
8.1. *Cereals and legumes*

8.1.1. *Cereals*

Whole grains are recommended for healthy diets and are recognized sources of dietary fibre and antioxidant compounds (Ragaee, Abdel-Aal, & Noaman, 2006). Phenolic acids and flavonoids are present in cereals in the free and conjugated forms.

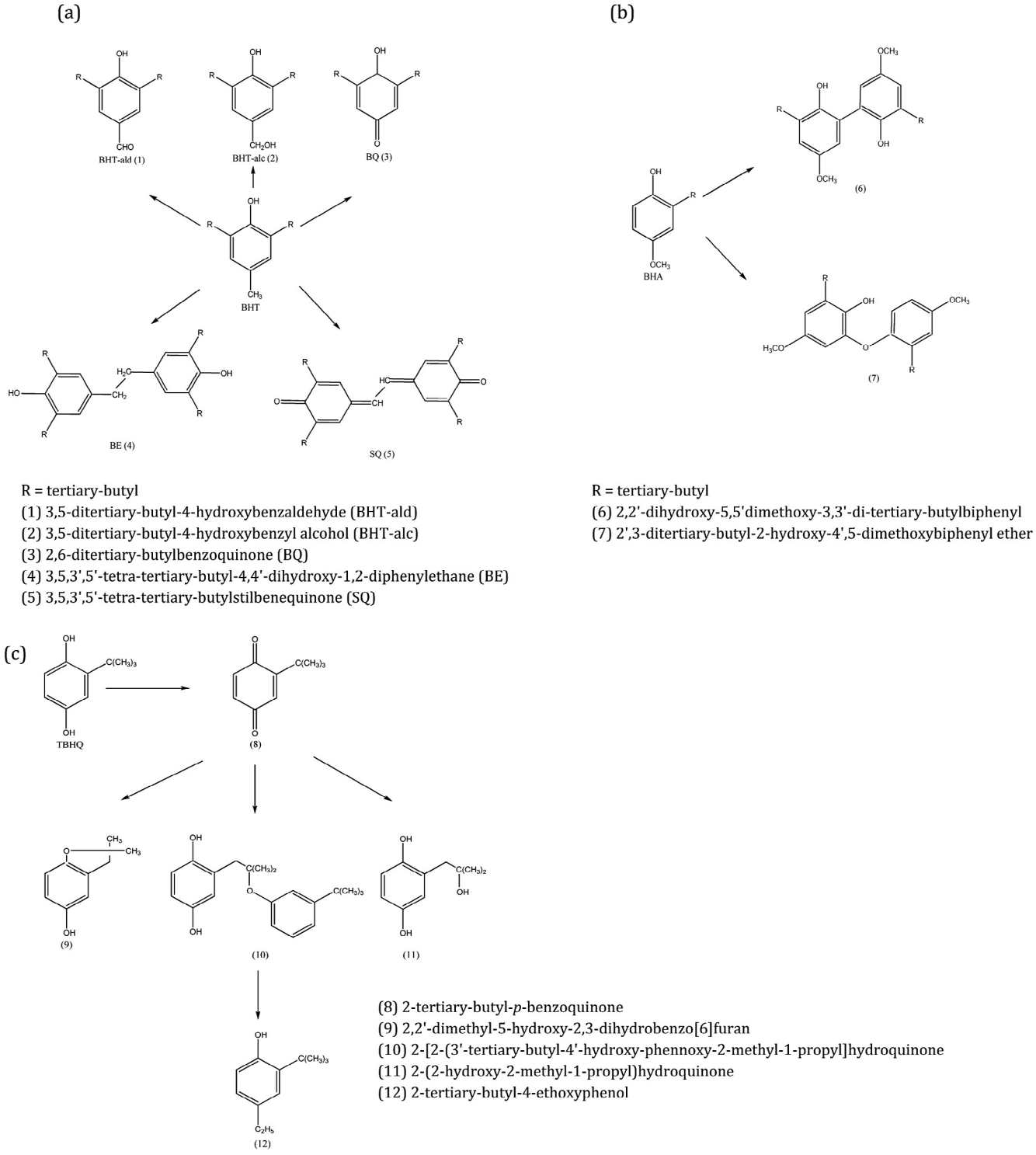


Condensed Tannin



Hydrolyzable Tannin

Fig. 10 – Chemical structures of condensed and hydrolysable tannins.



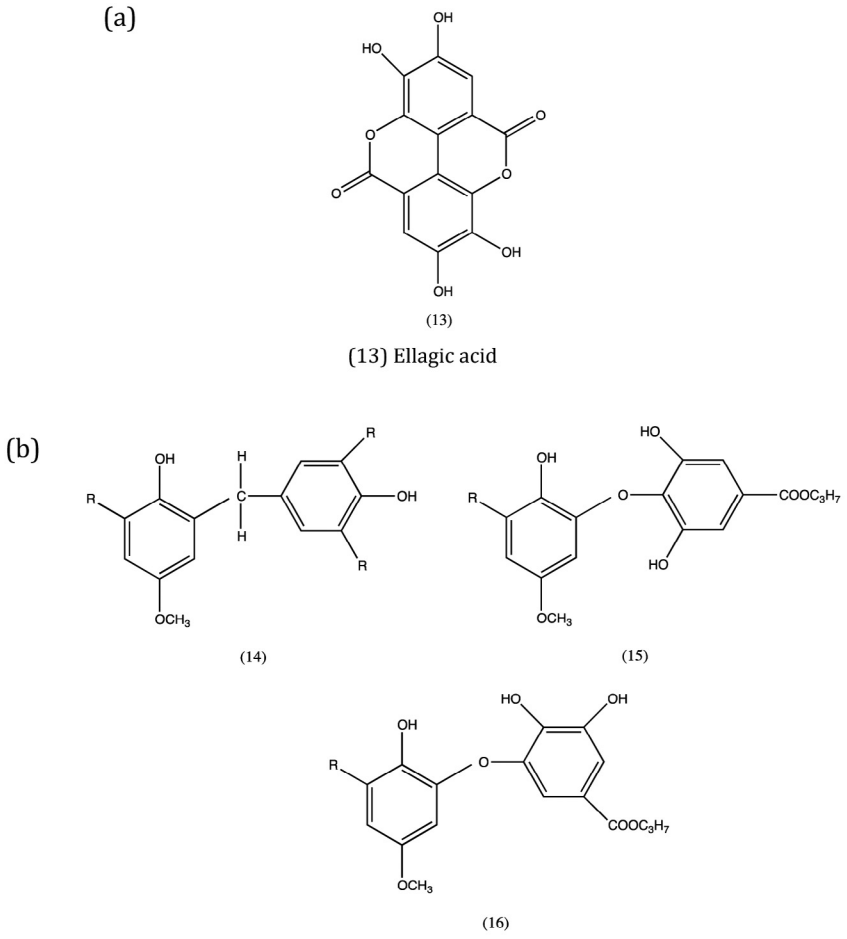
R = tertiary-butyl
(1) 3,5-ditertiary-butyl-4-hydroxybenzaldehyde (BHT-ald)
(2) 3,5-ditertiary-butyl-4-hydroxybenzyl alcohol (BHT-alc)
(3) 2,6-ditertiary-butylbenzoquinone (BQ)
(4) 3,5,3',5'-tetra-tertiary-butyl-4,4'-dihydroxy-1,2-diphenylethane (BE)
(5) 3,5,3',5'-tetra-tertiary-butylstilbenequinone (SQ)

R = tertiary-butyl
(6) 2,2'-dihydroxy-5,5'dimethoxy-3,3'-di-tertiary-butylbiphenyl
(7) 2',3-ditertiary-butyl-2-hydroxy-4',5-dimethoxybiphenyl ether

(8) 2-tertiary-butyl-*p*-benzoquinone
(9) 2,2'-dimethyl-5-hydroxy-2,3-dihydrobenzo[6]furan
(10) 2-[2-(3'-tertiary-butyl-4'-hydroxy-phennoxy-2-methyl-1-propyl]hydroquinone
(11) 2-(2-hydroxy-2-methyl-1-propyl)hydroquinone
(12) 2-tertiary-butyl-4-ethoxyphenol

Fig. 11 – (a) Degradation products of BHT, (b) BHA, and (c) TBHQ.

The outer layers of cereal grains (husk, pericarp, testa, and aleurone cells) contain the greatest concentrations of total phenolics, whereas their concentration is considerably lower in the endosperm layers (Kähkönen et al., 1999; Liyana-Pathirana & Shahidi, 2005, 2006; Madhujith & Shahidi, 2006, 2007a, 2007b). For instance, free and esterified phenolic acids were found in wheat, corn, rice, and oat (Sosulski, Krygier, & Hogge, 1982). Some researchers have reported that phenolic acids are concentrated in the cell walls of the outer layers, where they are mainly esterified to the arabinose side groups of arabinoxylans. However, others have indicated that phenolic acids are present mainly in the aleurone layer and endosperm (Yu, Vasanthan, & Temelli, 2001). Naczk and Shahidi (2006) reported that the highest concentration of phenolic acids



(13) Ellagic acid

(14) 3,3',5'-tri-tertiary-butyl-5-methoxy-2,4'-dihydroxydiphenylmethane
(15) propyl-3,5-dihydroxy-4-(2'-hydroxy-5-methoxy-3'-tertiary-butyl phenoxy) benzoate
(16) propyl-3,4-dihydroxy-5-(2'-hydroxy-5'-methoxy-3'-tertiary-butyl phenoxy) benzoate

Fig. 12 – (a) Degradation product of PG and (b) mixture of BHA and PG.

and flavonoids is in the aleurone layer of cereal grains, but these compounds are also found in embryos and seed coat of grains. Total antioxidant capacities of cereals are shown in Fig. 13. Water and ethanol extract of buckwheat showed the highest TEAC value (93.6 ± 1.6) and millet and corn had the lowest TEAC values (9.1 ± 3.0) (Serpen, Gökmen, Pellegrini, & Fogliano, 2008). Wheat extracts have shown potential antioxidant properties as wheat phenolics appear to serve as powerful antioxidants

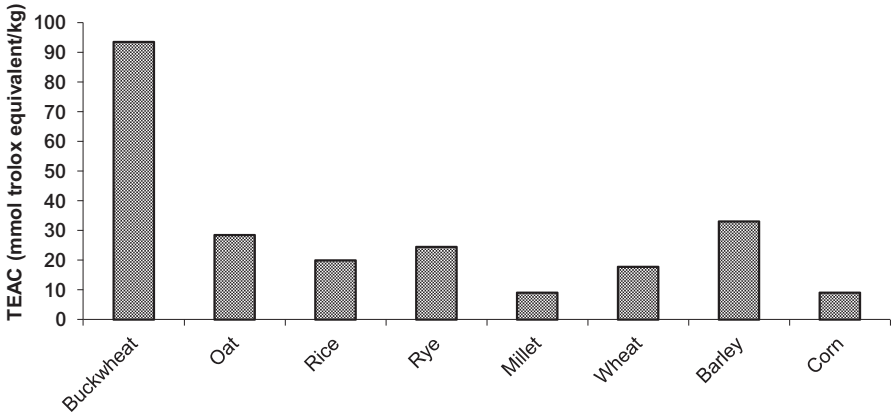


Fig. 13 – Total antioxidant capacities, expressed as mmol of Trolox equivalent antioxidant capacity (TEAC) per kg of cereals (adapted from Serpen et al., 2008).

Table 6 – Phenolic content and antioxidant activity of cereals.

Cereal	Total phenolic content[a]	DPPH scavenging (IC_{50}) capacity[b]	FRAP[c]
Barley	16.4	>200	15.56
Buckwheat	50.7	76.7	49.43
Wheat	16.2	>200	12.15
Rye	13.2	>200	8.94

Source: Adapted from Đorđević, Šiler-Marinković, and Dimitrijević-Branković (2010).
[a] mg gallic acid equivalents/g dried extract.
[b] μg/ml.
[c] nmol Fe^{2+}/mg dried extract.

through radical scavenging and/or metal chelation (Liyana-Pathirana, Dexter, & Shahidi, 2006; Liyana-Pathirana & Shahidi, 2006), whilst barley contains substantial amounts of phenolic antioxidants that effectively scavenge peroxyl, DPPH, and hydroxyl radicals, and control oxidation of LDL cholesterol, thereby having a great potential in the development of nutraceuticals rich in antioxidants (Madhujith & Shahidi, 2006, 2007a). The antioxidant activities and total phenolics of some cereals are shown in Table 6. Adom and Liu (2002) reported that corn had the highest total phenolic content (15.55 ± 0.60 μmol of gallic acid eq/g of grain) of the grains tested, followed by wheat (7.99 ± 0.39 μmol of gallic acid eq/g of grain), oats (6.53 ± 0.19 μmol of gallic acid eq/g of grain), and rice (5.56 ± 0.17 μmol of gallic acid eq/g of grain). Recently, Ndolo and Beta (2014) showed that sinapic acid is more concentrated in the pericarp and germ of wheat, whereas isoferulic acid is concentrated in the germ of purple barley. Moreover, syringic and vanillic acids have been shown to be concentrated in the pericarp and sinapic acid in the aleurone layer of yellow corn (Ndolo & Beta, 2014).

8.1.1.1. Wheat. Wheat kernels contain a number of phenolic compounds, namely ferulic, vanillic, gentisic, caffeic, salicylic, syringic, *p*-coumaric and sinapic acids as well as vanillin and syringaldehyde, none of which contribute to the antioxidant activity of the products (Liyana-Pathirana & Shahidi, 2005, 2006). Of these, ferulic acid was the primary phenolic acid in the grain, accounting for up to 90% of total phenolic acids (Liyana-Pathirana et al., 2006; Liyana-Pathirana & Shahidi, 2005, 2006; Okarter, Liu, Sorrells, & Liu, 2010). A study on six varieties of whole wheat grain reported no vanillic acid in the free or bound fractions. Further, no syringic acid or *p*-hydroxybenzoic acid were detected in the free fraction, which is consistent with the results reported by Moore et al. (2005), but not consistent with that reported by Mattila, Pihlava, and Hellstrom (2005) and Okarter et al. (2010). Whole wheat varieties tested contained mainly α- and β-tocopherols and α- and β-tocotrienols. β-Tocotrienol was the predominant form of vitamin E found in all varieties of whole wheat (Okarter et al., 2010). Tricin (5,7,4-trihydroxy 3,5-dimethoxyflavone) was found to be the dominant flavone pigment in wheat. In addition, two C-glycosylflavones, namely 6-C-pentosyl-8-C-hexosylapigenin and 6-C-hexosyl-8-C-pentosylapigenin were isolated from wheat bran (Naczk & Shahidi, 2006). Liyana-Pathirana and Shahidi (2007a) reported that phenolic compounds were concentrated in the outermost layers, namely the bran. Thus, consumption of wheat with bran in the form of whole grain may provide beneficial health effects. Asymmetrical distribution of antioxidative components in the wheat grain was prominent. The concentration of bioactive constituents was greater in the external layers; thus the bran fraction alone demonstrated a higher antioxidant activity than that of other milling fractions (Liyana-Pathirana & Shahidi, 2007b). Campestanyl ferulate and sitostanyl ferulate were the main components of steryl ferulates present in wheat grain and that rye and wheat and especially their bran fractions are comparable to corn as sources of steryl ferulate (Hakala et al., 2002). Wheat bran also contained ferulic acid dehydrodimers (DiFA), which strengthen the aleurone walls during the maturation of wheat grain by the formation of bridges between two arabinoxylan chains. These dehydrodimers are products of oxidative coupling of ferulic acid catalysed by peroxidase (Naczk & Shahidi, 2006).

8.1.1.2. Barley. Barley phenolics include tyrosine, tyramine and its derivatives, phenolic acids, their esters and glycosides, anthocyanins, proanthocyanidins, lignans and substances related to lignin (Naczk & Shahidi, 2006). The free forms of salicylic, *p*-hydroxybenzoic, vanillic, protocatechuic, *o*-, *m*- and *p*-coumaric, syringic, ferulic and sinapic acids have been identified in barley grains (Madhujith, Izydorczyk, & Shahidi, 2006; Shahidi, 2009; Shahidi & Naczk, 2004). Yu et al. (2001) reported the presence of chlorogenic and protocatechuic acids in barley. Ferulic acid was the predominant free phenolic acid in barley seeds (Nordkvist, Salomonsson, & Aman, 1984) and barley bran (Renger & Steinhart, 2000). On the other hand, *p*-hydroxybenzoic acid was the major bound phenolic acid detected in barley extracts obtained by sequential treatment of grain with acid, α-amylase and cellulase (Yu et al., 2001). Madhujith and Shahidi (2009) found that the antioxidant and antiradical activities of insoluble-bound phenolic fraction, in general, were higher than those of soluble conjugates and free phenolic fractions. Madhujith and Shahidi (2006) reported total phenolic content ranging from 0.81 to 1.38 mg of ferulic acid eq/g in defatted barley flour. The coloured barley grains contained at least eight pigmented compounds that were identified as derivatives of cyanidin, delphinidin and pelargonidin, as well as cyanidin-3-arabinoside and, possibly, cyanidin-3-glucoside (Madhujith & Shahidi, 2009; Shahidi & Naczk, 2004). Barley grains also contain a range of flavanols such as (+)-catechin, (–)-epicatechin, dimeric prodelphinidin B3 and procyanidin B3, as well as trimeric procyanidin C2 and three trimeric prodelphinidins (Goupy, Hugues, Boivin, & Amiot, 1999). Osawa, Katsuzaki, Hagiwara, Hagiwara, and Shibamoto (1992) and Kitta, Hagiwara, and Shibamoto (1992) isolated a novel flavone C-glycoside, 2″-*O*-glycosylisovitexin, from green barley leaves and reported that its antioxidative activity was similar to that exhibited by α-tocopherol. Sharma and Gujral (2010) reported that the antioxidant activity of barley significantly increased with duration of germination up to 24 hours and also a strong positive correlation existed between total phenolic content and antioxidant activity of barley. Phenolic extracts from whole barley kernel possessed high antioxidant, antiradical, and antiproliferative potentials. Therefore, inclusion of whole barley

into the daily diet may render beneficial health benefits (Madhujith & Shahidi, 2007a, 2007b). Another study showed that breads made by replacing 40% of wheat flour with barley flour increased the antioxidant properties of the breads (Holtekjølen, Bævre, Rødbotten, Berg, & Knutsen, 2008).

8.1.1.3. Buckwheat. Buckwheat is categorized as a pseudo cereal and its seed serves as a rich source of flavonoids. Flavonols such as rutin, hyperin, quercitrin and quercetin and flavones such as vitexin, isovitexin, orientin, and isoorientin have been identified in immature buckwheat seeds (Guo et al., 2012; Luo, Peng, Fei, Yang, & Fan, 2014; Shahidi & Naczk, 2004). Only six flavonoids have been identified in mature seeds and their hulls. Rutin and isovitexin were the only flavonoids of seeds, although the hulls contained rutin, quercetin, orientin, vitexin, isovitexin and isoorientin (Dietrych-Szostak & Oleszek, 1999). Four catechins, namely (–)-epicatechin, (+)-catechin 7-*O*-β-D-glucopyranoside, (–)-epicatechin 3-*O*-*p*-hydroxybenzoate and (–)-epicatechin 3-*O*-(3,4-di-*O*-methyl)gallate were identified in the ethanolic extracts of buckwheat groats (Dietrych-Szostak & Oleszek, 1999). Of the isolated flavonoids, vitexin and isovitexin revealed almost no peroxyl radical-scavenging activity (Watanabe, Ohshita, & Tsushida, 1997). However, the isolation of isovitexin from long-life rice seeds (Ramarathnam, Osawa, Namiki, & Kawakishi, 1989) suggests that this compound probably acts as a protectant in the seeds against oxidative damage. In buckwheat the content of ferulic and hydroxycinnamic acids is low. Bran–aleurone fraction of buckwheat contained bound syringic, *p*-hydroxybenzoic, vanillic and *p*-coumaric acids (Shahidi & Naczk, 2004). Watanabe et al. (1997) identified protocatechuic acid and 3,4-dihydroxybenzaldehyde from buckwheat hulls. Presence of soluble condensed tannins based on pelargonidin and cyanidin structures has been confirmed (Shahidi & Naczk, 2004). Watanabe et al. (1997) found that condensed tannins of buckwheat were composed of a mixture of proanthocyanidins with various degrees of polymerization. Antioxidant activity of buckwheat seeds and leaves proved to be higher when compared with those of oats, barley, buckwheat straws and hulls (Holasova et al., 2002). The antioxidant effects of two types of buckwheat sprouts on human hepatoma HepG2 cells revealed that both tartary buckwheat (TBS) and common buckwheat (CBS) sprout could decrease the production of intracellular peroxide and remove the intracellular superoxide anions in HepG2 cells, but TBS reduced the cellular oxidative stress more effectively than CBS, possibly because of its higher rutin (and quercetin) content (Liu, Chen, Yang, & Chiang, 2008). Substituting 15% of wheat flour in the bread formula with common buckwheat flour enhanced functional components, namely rutin and quercetin and its antioxidant activity (Lin, Liu, Yu, Lin, & Mau, 2009). Recently, Chitarrini et al. (2014) showed that the healthy antioxidant compounds present in buckwheat possess antimicrobial activity. Thus, the authors suggested that buckwheat antioxidants could be considered as markers for tolerance against mycotoxigenic pathogens and used for improving food safety (Chitarrini et al., 2014). Phenolic extracts from the sprouts of buckwheat also have been shown to exhibit strong antioxidant activity (Brajdes & Vizireanu, 2012; Ren & Sun, 2014). A study has shown that buckwheat honeys could protect non-site-specific hydroxyl radical-mediated DNA damage and site-specific hydroxyl radical-mediated DNA strand breaks under *in vitro* conditions (Zhou et al., 2012).

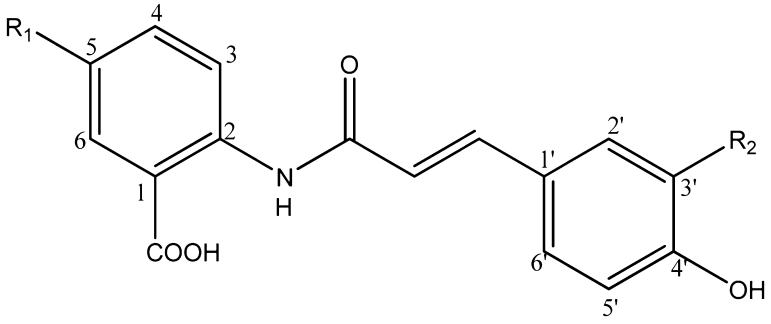
8.1.1.4. Corn. In corn, insoluble bound phenolic acids constitute the predominant fraction of phenolic acids present (Naczk & Shahidi, 2006). The embryo and aleurone layer of corn contain phenolic acids linked covalently to amine functionalities, such as feruoylputrescine, *p*-coumarylputrescine, diferuloylputrescine, di-*p*-coumarylputrescine, *p*-coumarylspermidine, diferuloylspermidine and diferuloylspermine (Sen et al., 1994). Kurilich and Juvik (1999) reported the presence of tocopherols in corn kernel tissue. Monomeric flavan-3,4-diols, namely leucopelargonidin and lecocyanidin and two favonols, kaempferol and quercetin, have also been reported in hydrolysates of aleurone tissue of corn (Shahidi & Naczk, 2004). Anthocyanins cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside were found in Chinese purple corn (Yang & Zhai, 2010). Mohsen and Ammar (2009) reported that the ethanolic extract of corn tassels was successfully utilized to retard the oxidation of sunflower oil. The present investigation depicted that corncob is a good source of potent antioxidants that can be used in the nutraceutical and functional food applications, and to protect vegetable oil (Sultana, Anwar, & Przybylski, 2007). These findings suggest that *p*-coumaric acid derivatives, which might play a beneficial role against oxidative damage, exist in corn steep liquor, thus this byproduct might be a useful source of phenolic antioxidants (Niwa, Doi, Kato, & Osawa, 2001).

8.1.1.5. Millets. Millets are rich sources of phenolics and tannins, which can act as antioxidants (Hegde, Rajasekaran, & Chandra, 2005). Protocatechuic, gallic, and caffeic acids were the predominant free phenolic acids, whilst ferulic, caffeic and coumaric acids were the major bound phenolic acids found in finger millets (Subba Rao & Muralikrishna, 2002). Chandrasekara and Shahidi (2010) have shown that ferulic acid and *p*-coumaric acids are the major bound phenolics found in several varieties of millets (kodo, finger, foxtail, proso, pearl, and little millets). Viswanath, Urooj, and Malleshi (2009) identified diadzine, gallic, coumaric, syringic and vanillic acids as major phenolic acids from the extracted phenolics of finger millets. The only millet flavonoids reported were flavones, eight of which were identified in the leaves of finger millet, namely orientin, isoorientin, vitexin, isovitexin, saponarin, violanthin, lucenin-1 and tricin (Dykes & Rooney, 2006). The tiny millet grain has different colours, usually a dark brown seed coat, richer in polyphenols compared to other continental cereals such as barley, rice, maize and wheat. Finger millet seed coat is a good source of polyphenols with significantly higher antioxidant activity compared to the whole flour (Viswanath et al., 2009). Watanabe (1999) identified three antioxidative phenolic compounds, one serotonin derivative and two flavonoids, were isolated from an ethanolic extract of Japanese barnyard millet (cv. Kurohie) grain by Sephadex LH-20 chromatography and preparative high-performance liquid chromatography. Their structures were established to be *N*-(*p*-coumaroyl) serotonin, luteolin, and tricin. Inclusion of millet in multigrain breads is now practiced in North America. Shahidi and Chandrasekara (2013) suggested that millet grain phenolics are bioaccessible, possess bioactivities against several pathophysiological conditions, as well

as could serve as potential natural source of antioxidant in food and biological systems.

8.1.1.6. Sorghum. Sorghum is a good source of phenolic compounds (Hahn, Faubion, & Rooney, 1983). Protocatechuic, *p*-hydroxybenzoic, caffeic, *p*-coumaric and ferulic acids are found in the free and bound forms. However, cinnamic and vanillic acids are found in the free and/or bound forms only in some sorghum varieties. Gallic acid is present only in the bound form (Hahn et al., 1983). Recently, four phenolic acids (caffeic, *p*-coumaric, ferulic and sinapic acids) were detected in all pearling fine and pearled kernel fractions of two sorghum genotypes namely white (PR6E6) and red (PR6E14) (Luthria & Liu, 2013). Sorghum tannins readily associate with sorghum proteins (Shahidi & Naczk, 2004). Only varieties with a pigmented testa have condensed tannins. Sorghum tannins are excellent antioxidants, which slow hydrolysis in foods, produce naturally dark-coloured products and increase the dietary fibre levels of food products (Dykes & Rooney, 2006). The proanthocyanidins in sorghums are the B-type with (–)-epicatechin as extension units and catechin as terminal units (Gu et al., 2002, 2003; Gupta & Haslam, 1978). The anthocyanins are the major class of flavonoids studied in sorghum. Unlike common anthocyanins, sorghum anthocyanins are unique since they do not contain the hydroxyl group in the 3-position of the C-ring and thus are called 3-deoxyanthocyanins (Dykes & Rooney, 2006). The antioxidant properties of the 3-deoxyanthocyanidins are similar to those of the anthocyanins (Awika, Rooney, & Waniska, 2004a). The two common 3-deoxyanthocyanidins in sorghum are the yellow, apigeninidin, and the orange, luteolinidin (Awika, Rooney, & Waniska, 2004b). Other 3-deoxyanthocyanins identified in sorghum grains include apigeninidin 5-glucoside, luteolinidin 5-glucoside, 5-methoxyluteolinidin, 5-methoxyluteolinidin 7-glucoside, 7-methoxyapigeninidin, 7-methoxyapigeninidin 5-glucoside, 5-methoxyapigeninidin and 7-methoxyluteolinidin (Dykes & Rooney, 2006). Red pericarp sorghums have flavan-4-ol compounds, such as luteoforol and apiforol, which are produced from flavanones (i.e., naringenin and eriodictyol) and may be precursors of sorghum anthocyanidins (Dykes & Rooney, 2006). Other flavonoids isolated and identified in sorghum grains include the flavones apigenin and luteolin, which are predominant in tan-pigmented sorghums. Flavanones, eriodictyol and eriodictyol 5-glucoside have been reported. The flavonol, kaempferol 3-rutinoside-7-glucuronide and the dihydroflavonols taxifolin, and taxifolin 7-glucoside have also been isolated (Dykes & Rooney, 2006). Kil et al. (2009) suggested that sorghum could be used as a natural ingredient with biological function for its antioxidant and antimicrobial properties in the food industry. Fernandez et al. (2009) reported that some sorghum varieties indigenous to West Africa (Nigeria and Niger) have a yellow endosperm, which is pigmented by carotenoids. The most abundant carotenoids identified are lutein, zeaxanthin and β-carotene (Taylor, Belton, Beta, & Duodu, 2014).

8.1.1.7. Oat. Oat is a source of a number of compounds that exhibit antioxidant activity. Vitamin E (tocols), phytic acid, phenolic compounds and avenanthramides are amongst the most abundant antioxidants in oat along with flavonoids and sterols. These antioxidants are concentrated in the outer layers of the kernel (Peterson, 2001). The distribution of antioxidants within oat kernels is important in implementing technologies to develop oat products with enhanced antioxidant capacity. Oat germ, the high-lipid compartment of kernels has concentrated levels of tocopherols (α- and γ-homologues), whereas tocotrienols are concentrated in endosperm and absent in the germ (Peterson, 1995). Oat bran is less effective in scavenging free-radicals compared with other cereal brans such as wheat, barley and rye (Martínez-Tomé et al., 2004). However, Lehtinen and Laakso (1997) reported that oat itself has greater antioxidant capacity than the same three cereals, with highest activity observed for its soluble fibre fraction. Avenanthramides (Fig. 14) are a group of alkaloids in oat grain that consist of an anthranilic acid derivative linked to a hydroxycinnamic acid derivative by a pseudo peptide bond and exhibit antioxidant activity (Peterson, Hahn, & Emmons, 2002). The three most abundant avenanthramides are *N*-(4′-hydroxy-3′-methoxycinnamoyl)-5-hydroxyanthranilic acid, *N*-(4′-hydroxycinnamoyl)-5-hydroxyanthranilic acid, and *N*-(3′,4′-dihydroxycinnamoyl)-5-hydroxyanthranilic acid (Peterson et al., 2002). In general, avenanthramides show a higher antioxidant activity than ferulic acid, gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, vanillic acid, vanillin, and phytic acid which are typical in cereals (Martínez-Tomé et al., 2004). *p*-Hydroxybenzoic acid, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, and ferulic acid were identified in oat milling fractions (Emmons, Peterson, & Paul, 1999). Oat flour has been shown to consist of three flavones, apigenin, luteolin and tricin (Emmons et al., 1999; Shahidi & Naczk, 2004). Oat phenolics, including avenanthramides, have been shown to interact synergistically with vitamin C to protect LDL during oxidation (Chen et al., 2004). In addition, oat phenolics have been shown to possess anti-inflammatory and anti-hypertensive activity (Chu



	R_1	R_2
A	OH	H
B	OH	HCH_3
C	OH	OH
D	H	H

Fig. 14 – Chemical structures of oat avenanthramides.

et al., 2013). Recently, Bindu and Krishnaveni (2013) showed that supplementation of oats porridge significantly decreased the low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and blood pressure.

8.1.1.8. Rice. Ferulic acid was found as the most abundant phenolic acid (up to 355 mg/kg) followed by sinapic acid in wild rice (Irakli, Samanidou, Biliaderis, & Papadoyannis, 2012; Qiu, Liu, & Beta, 2010). They both occur mainly in the insoluble form. Other monomeric phenolic acids present in wild rice consisted of *p*-coumaric, vanillic, syringic, and *p*-hydroxybenzoic acids, along with two phenolic aldehydes, *p*-hydroxy benzaldehyde and vanillin, present in both soluble and insoluble forms. Phenolic acid dehydrodimers are cell wall bound and only appear in the insoluble fractions featured by diferulic acids (DiFA) and disinapic acids (DiSA) (Qiu et al., 2010). The antioxidant activity of wild rice was 30 times greater than that of the white rice (Qiu, Liu, & Beta, 2009). Meanwhile, rice bran contained a higher phenolic content (2.51–3.59 mg/g) than wheat bran (Iqbal, Bhanger, & Anwar, 2005; Lai, Li, Lu, & Chen, 2009). Furthermore, rice bran contained 100 different antioxidants, amongst the most powerful of which were oryzanols, tocopherols and tocotrienols (Iqbal et al., 2005). Black rice is an especially economically important rice species and derives its name from its rich natural anthocyanin compounds, such as cyanidin 3-glucoside and peonidin 3-glucoside, which possess antioxidative and anti-inflammatory activities (Kong & Lee, 2010). Isovitexin, a flavonoid also known as *C*-glucosylflavone, with good antioxidant activity, has been reported in rice hulls (Osawa, Narasimhan, Kawakishi, Namdci, & Tashiro, 1985). Moreover, anisole, *m*-hydroxybenzaldehyde, vanillin (4-hydroxy-3-methoxybenzaldehyde), and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) have been identified in methanolic extracts of wild rice hulls (Asamarai, Addis, Epley, & Krick, 1996). Cycloartenyl ferulate and 24-methylenecycloartanyl ferulate are major steryl ferulates in rice bran. Rice steryl ferulates (γ-oryzanol) have been shown to possess good antioxidant activity (Nyström, Mäkinen, Lampi, & Piironen, 2005). Butsat and Siriamornpun (2010) demonstrated that rice bran and husk can be considered as valuable sources of bioactive components with high antioxidant properties.

8.1.1.9. Rye. Ferulic acid is the most abundant hydroxycinnamic acid in rye, followed by sinapic acid and *para*-coumaric acid. Amongst the benzoic acid derivatives only small amounts of vanillic acid, syringic acid and *para*-hydroxybenzoic acid have been reported in rye grain and products (Andreasen, Christensen, Meyer, & Hansen, 2000). The antioxidant activities of purified monomeric and dimeric hydroxycinnamates and of phenolic extracts from rye (whole grain, bran, and flour) were investigated using an *in vitro* copper-catalysed human LDL oxidation assay. The most abundant ferulic acid dehydrodimer (diFA) found in rye, 8-O-4-diFA, was a slightly better antioxidant than ferulic acid and *p*-coumaric acid. The antioxidant activity of the monomeric hydroxycinnamates decreased in the order of caffeic acid > sinapic acid > ferulic acid > *p*-coumaric acid (Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001). Steryl ferulate extracts from wheat or rye bran were studied for their capability to inhibit hydroperoxide formation in bulk methyl linoleate and methyl linoleate emulsion and found to possess good antioxidant activity (Karamac, Amarowicz, Weinder, Abe, & Shahidi, 2002; Nyström et al., 2005). The key difference in the steryl ferulates of rice from those of wheat and rye is the sterol structure. In rice, the sterols are 4,4-dimethylsterols with two methyl groups in carbon C4, whereas the sterols in other cereals are principally desmethylsterols that have no methyl groups in their C4 position (Nyström et al., 2005).

8.1.2. *Legumes*

Amarowicz and Pegg (2008) reviewed legumes as a source of natural antioxidants. The major polyphenolic compounds of legumes consist mainly of tannins, phenolic acids and flavonoids (Campos-Vega, Loarca-Piña, & Oomah, 2010). Flavonol glycosides, tannins and anthocyanins are responsible for the colour of seed coat in dry beans (Madhujith, Naczk, & Shahidi, 2006; Madhujith & Shahidi, 2005; Shahidi & Naczk, 2004). Legumes with the highest polyphenolic content are the dark, highly pigmented varieties, such as red kidney beans (*Phaseolus vulgaris*) and black gram (*Vigna mungo*) (Campos-Vega et al., 2010). The distribution of phenolic compounds differs in the cotyledon and the seed coat, with non-flavonoid phenolic compounds, such as free and combined hydroxybenzoic and hydroxycinnamic acids, being located mainly in the cotyledon of lentils (Duenas, Hernandez, & Estrella, 2002). Flavonoids, such as glycosides of flavonols and flavones, were identified in the seed coat of lentils, together with *trans*-resveratrol-3-*O*-glucoside, and higher concentrations of proanthocyanidins (Duenas et al., 2002; Duenas, Sun, Hernandez, Estrella, & Spranger, 2003). According to the literature, total phenolic content is directly associated with antioxidant activity (Amarowicz, Troszynska, Barylko-Pikielna, & Shahidi, 2004; Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003; Shahidi & Naczk, 2004). Total phenolic contents (TPC) of the extracts (using acidic 70% acetone) from selected legumes are presented in Table 7. Lentils have the highest phenolic, flavonoid and condensed tannin contents (7.53 mg gallic acid equivalents/g, 2.21 and 8.70 mg catechin equivalents/g, respectively), followed by black beans and red kidney beans (Xu & Chang, 2007). Pulses with the highest total phenolic content (lentil, black, and red kidney beans) exert the highest antioxidant capacity as assessed by DPPH radical scavenging, FRAP, and ORAC (Xu & Chang, 2007; Yeo & Shahidi, 2015). Condensed tannins (proanthocyanidins) have been quantified in hulls of several varieties of field beans (*Vicia faba*) (Amarowicz et al., 2004; Beninger & Hosfield, 2003; Campos-Vega et al., 2010; Smulikowska et al., 2001; Troszyńska, Estrella, López-Amóres, & Hernández, 2002). Tannins are located mainly in the testa and play an important role in the defence system of seeds that are exposed to oxidative damage by many environmental factors (Troszyńska et al., 2002). Beach pea hulls were found to contain very high amounts of condensed tannins compared to those reported for other legumes such as pigeon pea, chickpea, cowpea and green pea (Shahidi, Chavan, Naczk, & Amarowicz, 2001). Tannins in beans are linear polymers of flavan-3-ol (catechin and gallocatechin) and flavan-3,4-diol (leucocyanidin and leucodelphinidin) units (Martin-Tanguy, Guillaume, & Kossa, 1977). Oomah, Luc, et al. (2011) have shown that phenolic content linearly related to tannin content is the best predictor of antioxidant activity in low-tannin faba bean genotypes. More recently, Yeo and Shahidi (2015) introduced a new indicator,

Table 7 – Phenolic content and antioxidant activity of legumes.

Legumes	Total phenolic[a]	Total flavonoid[b]	Condensed tannin[c]	DPPH scavenging capacity[d]	FRAP value[e]	ORAC value[f]
Green pea	1.13	0.26	0.91	0.59	1.03	6.17
Yellow pea	1.28	0.29	1.02	1.36	1.34	13.30
Chickpea	1.57	2.87	1.21	0.94	0.81	7.41
Lentil	7.53	2.21	8.70	19.09	10.65	38.60
Yellow soybean	2.23	0.41	0.85	1.40	0.34	44.08
Black soybean	6.18	2.57	4.09	17.58	9.93	122.75
Red kidney	5.90	2.93	5.37	18.94	9.22	23.26
Black bean	6.89	3.21	6.74	18.33	11.03	51.54

Source: Adapted from Xu and Chang (2007).
[a] mg gallic acid eq/g.
[b] mg catechin eq/g.
[c] mg catechin eq/g.
[d] μmol Trolox eq/g.
[e] mmol Fe^{2+} eq/100 g.
[f] μmol Trolox eq/g.

the ratio of insoluble bound phenolics to soluble phenolics, and suggested it as an effective means to monitor changes in the antioxidant activity of lentils during processing as exemplified for the germination process. There are, however, only a few reports on identification and quantification of flavonoids in food legumes (Xu & Chang, 2007). For example, only several reports on common beans (Beninger, Hosfield, & Bassett, 1999; Beninger, Hosfield, & Nair, 1998; Hempel & Bohm, 1996; Madhujith, Naczk, et al., 2006; Romani et al., 2004) and peas (Troszyńska et al., 2002) are available. Hempel and Bohm (1996) reported that 3-*O*-glucuronides and 3-*O*-rutinosides of quercetin and kaempferol were the main flavonoid glycosides in six varieties of yellow and green French beans. Ferulic acid was the most abundant phenolic acid in common beans and *p*-coumaric and sinapic acids were also present (Luthria & Pastor-Corrales, 2006; Oomah, Cardador-Martinez, & Loarca-Pina, 2005; Shahidi & Naczk, 2004). Legumes, namely mung bean, field pea, faba bean, lentil and pigeon pea contained 18 to 31 mg of total phenolic acids (*trans*-ferulic, *trans*-p-coumaric and syringic acids) per kilogram, whilst in navy bean, lupin, lima bean, chickpea and cowpea, the total phenolics content ranged from 55 to 163 mg/kg (Sosulski & Dabrowski, 1984). Oomah, Caspar, Malcolmson, and Bellido (2011) reported that green and red lentil hulls are excellent sources of potent phenolic antioxidants. Long-Ze, Harnly, Pastor-Corrales, and Luthria (2008) analysed twenty four common bean samples and reported that common beans tested contained the same hydroxycinnamic acids, whereas they had different flavonoid components. Black beans contained primarily the 3-*O*-glucosides of delphinidin, petunidin, and malvidin, whilst kaempferol and its 3-*O*-glycosides were present in pinto beans. Light red kidney beans had traces of quercetin 3-*O*-glucoside and its malonates, but pink and dark red kidney beans contained the diglycosides of quercetin and kaempferol. Small red beans contained kaempferol 3-*O*-glucoside and pelargonidin 3-*O*-glucoside, whilst flavonoids were undetected in great northern and navy beans. According to the USDA (2002) survey on isoflavone content, lentils do not contain any significant amount of isoflavones. Chickpeas contained daidzein, genistein, and formononetin (0.04, 0.06, and 0.14 mg/100 g, respectively), and approximately 1.7 mg/100 g biochanin (Campos-Vega et al., 2010). Dini Schettino and Dini (1998) identified two new isoflavonoid derivatives (Fig. 15), mutabilin (3′-methoxy-5-hydroxy-7-*O*-β-D-glucosylisoflavone) and mutabilein (3′-methoxy-5, 7-dihydroxyisoflavone) in seeds of *Lupinus mutabilis* (Fabaceae) (Shahidi & Naczk, 2004). Furthermore, formononetin, genistein and the phytoestrogen secoisolariciresinol were found in seeds of *L. mutabilis* (23, 2420, and 3.1 μg/100 g, respectively) (Mazur, Duke, Wahala, Rasku, & Adlercreutz, 1998). Delphinidin 3-glucoside is the major anthocyanin in beans of Canadian Wonder cultivar, whilst cyanidin 3-diglucoside, 3,5-diglucoside, pelargonidin 3-glucoside and 3,5-diglucoside are the minor anthocyanins (Stanton & Francis, 1966). The anthocyanins in seed coats of beans were identified as delphinidin 3-glucoside 65.7%, petunidin 3-glucoside 24.3%, and maldivin 3-glucoside 8.7% (Salinas-Moreno, Rojas-Herrera, Sosa-Montes, & Pérez-Herrera, 2005) which are mainly responsible for the colour of black and purple seed coat beans (Shahidi & Naczk, 2004). Chickpeas contain a wide range of polyphenolic compounds, including flavonols, flavone glycosides,

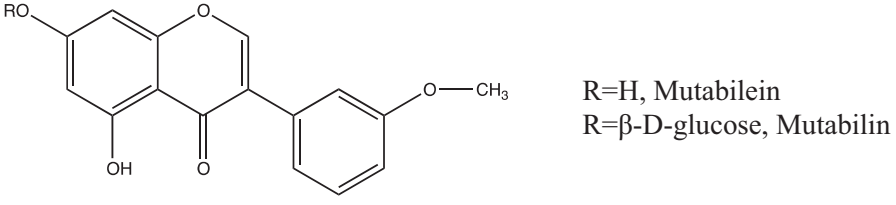


Fig. 15 – Chemical structures of isoflavonoid derivatives found in *Lupinus mutabilis*.

flavonols, and oligomeric and polymeric proanthocyanidins (Sarma, Singh, Mehta, Singh, & Singh, 2002; Zia-Ul-Haq et al., 2008). Amarowicz et al. (2010) found catechin and epicatechin glucosides, procyanidin dimers, quercetin diglycoside, and *trans*-*p*-coumaric acids as the dominant phenolics in green lentils. Madhujith, Amarowicz, and Shahidi (2004) studied the phenolic constituents of bean varieties with different hull colours (white, brown, red, and black) and identified delphinidin, cyanidin, procyanidin, and phenolic acids as the major phenolic compounds present in the bean hulls. Bean phenolics exhibited strong protection against *in vitro* 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) radical-induced DNA strand scission. Beans, especially the coloured ones, can be considered an important source of natural antioxidants, hence, a food with potential health benefits (Madhujith & Shahidi, 2005). Siah, Konczak, Agboola, Wood, and Blanchard (2012) found that the crude phenolic extracts obtained from raw and roasted faba beans exhibited potential health-beneficial properties, including potent antioxidant activities (based on both reagent- and cell-based assays), chemopreventative effects (through induction of cancer cell apoptosis), protection against reactive oxygen species, H_2O_2 and inhibitory effect on angiotensin converting enzyme (ACE), α-glucosidase and lipase (based on *in vitro* methods). Xu and Chang (2009) investigated the effects of boiling and steaming under atmospheric and high pressure on the phenolic components and antioxidant properties of pinto and black beans. In comparison with the original raw beans, both processing methods caused significant ($p < 0.05$) decreases in total phenolic content, total flavonoid content, condensed tannin content, monomeric anthocyanin content, DPPH radical scavenging activity, FRAP and ORAC values in both pinto and black beans. Recently, two anthocyanin compounds, peonidin-3-rutinoside and malvidin-3-O-glucoside were newly identified in the adzuki bean extract (Han et al., 2015).

8.2. *Fruits and vegetables*

8.2.1. *Fruits*

Fruits are rich in antioxidants that help in lowering the incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich et al., 2000; Gordon, 1996; Halliwell, 1996). The most abundant antioxidants in fruits are polyphenols; vitamins A, B, C and E and carotenoids are present to a lesser extent in some fruits. These polyphenols, most of which are flavonoids, are present mainly in the ester and glycoside forms (Lim, Lim, & Tee, 2007). In tropical fruits guava, papaya and star fruit have high antioxidant potential when compared to orange, as measured by scavenging of DPPH radical and iron (III) reducing assays (Lim et al., 2007). Banana, star fruit, water apple, langsat and papaya are though weaker than orange as primary antioxidants, however, they exhibit powerful secondary antioxidant potential as measured by the iron (II) chelating experiment (Lim et al., 2007). García-Alonso, Pascual-Teresa, Santos-Buelga, and Rivas-Gonzalo (2004) reported the highest content of flavanols in plum (*var*. Claudia), apples, custard apple, peach, strawberry and cherry, with concentrations of total flavanols between 70 and 370 mg/100 g of dry weight. On the other hand, the lowest contents were noted in avocado, banana and pear (*var*. blanquilla), with values lower than 4 mg/100 g of dry weight. The total antioxidant activities of 12 fruits and 5 commercial fruit juices were evaluated by Wang, Cao, and Prior (1996) using an automated ORAC assay and a peroxyl radical generator. On the basis of the wet weight of fruits (edible portion), strawberry had the highest ORAC activity followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon. On a dry weight basis, strawberry had the highest ORAC followed by plum, orange, pink grapefruit, tomato, kiwi fruit, red grape, white grape, apple, honeydew melon, pear, and banana. Amongst the commercial fruit and vegetable juices, grape juice had the highest antioxidant activity, followed by tomato juice, orange juice, and apple juice. According to Vinson, Su, Zubik, and Bose (2001), cranberry had the highest total phenols, followed by red grape, on a fresh weight basis (Table 8). Citrus fruits have a very low phenolic concentration. Contribution of ascorbic acid to the antioxidants in fruits is minor, except for melon, nectarine, orange, white grape and strawberry (Vinson, Teufel, & Wu, 2001). The fruit extracts' antioxidant quality (measured by IC_{50}) was better than the vitamin antioxidants and most pure phenols, suggesting a synergism amongst the antioxidants in the extract mixture. The quantity/quality index, PAOXI, is one comprehensive parameters for comparing food antioxidants which is the ratio of phenol concentration (μmol/kg) to the IC_{50} value (μM) (Vinson & Hontz, 1995). According to PAOXI (Table 8), cherry is most effective followed by red grape, blueberry, strawberry, white grape, cranberry, banana and apple. Thus, small fruits such as berries are amongst the best sources of polyphenol antioxidants (Sun, Chu, Wu, & Liu, 2002; Vinson, Teufel, et al., 2001). Cherry had the highest

Table 8 – Total phenol content and total phenol antioxidant index (PAOXI) of fruits.

Fruit	Total phenols (μmol/g)		Total PAOXI × 10^{-3}[a]	
	Dry weight	Wet weight	Dry weight	Wet weight
Apple	34.1	6.4	110	20.6
Avocado	12.7	3.5	60.5	16.7
Banana	42.3	11.2	108	28.7
Blueberry	62.0	8.9	273	40.5
Cantaloupe	8.1	0.9	32.4	3.6
Cherry	52.3	10.9	523	109
Cranberry	158.8	22.7	212	31.2
Grape (white)	52.3	6.7	262	33.5
Grape (red)	63.7	13.6	351	50.3
Grape fruit	7.5	0.9	39.5	4.7
Lemon	19.6	2.4	67.6	8.28
Melon (honeydew)	11.4	1.3	36.8	4.2
Nectarine	12.3	1.5	64.7	7.89
Orange	18.9	1.4	55.6	4.1
Peach	27.9	2.4	60.7	5.22
Pear	41.4	6.6	81.2	12.9
Pineapple	11.9	2.3	44.1	8.52
Plum	58.2	7.8	116	15.6
Strawberry	72.3	4.6	603	38.3
Watermelon	19.5	2.2	44.3	5.0

Source: Adapted from Vinson, Su, et al. (2001).

[a] Ratio of phenol concentration (μmol/kg) to the IC_{50} value (μM).

wet weight PAOXI of any fruit or vegetable studied. Finally, it was concluded that fruits had significantly better quantity and quality of phenolic antioxidants than vegetables. Fruits, especially apples and cranberries, have phenolic antioxidants that can bind to lower density lipoproteins and protect them from oxidation (Vinson, Teufel, et al., 2001). Wolfe et al. (2008) found that pomegranate and berries (wild blueberry, blackberry, raspberry, and blueberry) had the highest cellular antioxidant activity values, whereas banana and melons had the lowest. It is evident that berry fruits are consistently ranked amongst the top sources of total phenolics and antioxidant activity, containing levels up to 4 times greater than other fruits, 10 times higher than vegetables, and 40 times higher than cereals (Halvorsen et al., 2002). Recent review by Alasalvar and Shahidi (2013) reveals that individuals who regularly consume generous amounts of dried fruits (dates, raisins, prunes, figs, acai berries, apples, bananas, black currants, blackberries, cherries, citrus fruits, cranberries, gingers, goji berries, guavas, kiwis, mangoes, mulberries, nectarines, papayas, passion fruits, peaches, pears, pineapples, raspberries, star apples, and strawberries, amongst others) have a lower rate of CVD, obesity, various types of cancer, type 2 diabetes, and other chronic diseases.

8.2.1.1. Bilberries. Bilberry contains the highest amount of anthocyanins amongst different berry varieties, with a ratio of 30:36:13 for cyanidin-, delphinidin-, and malvidin-3-*O*-glycosides (Du, Jerz, & Winterhalter, 2004; Wang, Cao, & Prior, 1997). Bilberry anthocyanosides representing all possible combinations of 5 anthocyanidins (cyanidin, delphinidin, peonidin, petunidin, malvidin) with 3 sugar moieties (3-*O*-arabinosides, 3-*O*-glucosides, 3-*O*-galactosides) were separated by HPLC of bilberry extract (Madhavi, Bomser, Smith, & Singletary, 1998; Martinelli, Baj, & Bombardelli, 1986). Du et al. (2004) identified delphinidin-3-*O*-sambubioside, and cyanidin-3-*O*-sambubioside in bilberry. Presence of resveratrol in bilberries was reported by Lyons et al. (2003). Fresh bilberries were also a source of flavonols. The flavonol content of fresh bilberries was around 41 mg/kg (Jamison, 2003). Presence of caffeic, chlorogenic, *p*-coumaric, ferulic and syringic acids and derivative of *p*-hydroxybenzoic acid, *o*- and *m*-coumaric, gallic, *m*- and *p*-hydroxybenzoic, protocatechuic and vanillic acids in bilberry juice has been reported (Brenneisen & Steingger, 1981). Potent antioxidant properties of *Vaccinium* fruits have been documented (Wang et al., 1997). A commercial extract of *Vaccinium myrtillus* (bilberry), called *V. myrtillus* anthocyanin (VMA), containing largely glycosides of delphinidin and cyanidin (Baj, Bombardelli, Gabetta, & Martinelli, 1983) has been used to treat various microcirculation diseases resulting from capillary fragility and has been used to maintain normal vascular permeability (Wang et al., 1997). A recent study supported the use of bilberry and bilberry extracts in functional foods and food supplements designed for the prevention of chronic diseases associated with oxidative stress (Valentová, Ulrichová, Cvak, & Šimánek, 2007).

8.2.1.2. Blackberries. Blackberries are a rich source of anthocyanins and other polyphenolic antioxidants (Siriwoharn, Wrolstad, Finn, & Pereira, 2004). Extensive studies have been carried out on blackberry anthocyanins, and their identities have been well-characterized as being solely cyanidin-based compounds. Five anthocyanins were detected in blackberries and they were identified as cyanidin 3-glucoside (major anthocyanin), cyanidin 3-rutinoside, malonic acid acylated cyanidin 3-glucoside and xylose-cyanidin derivative (Siriwoharn et al., 2004). Stintzing, Stintzing, Carle, and Wrolstad (2002) isolated cyanidin 3-dioxalyl-glucoside, a novel zwitterionic anthocyanin, from evergreen blackberry. Wada and Ou (2002) also reported the presence of cyanidin 3-(6′-*p*-coumaryl)glucoside in Marion berries and cyanidin 3-arabinoside in Evergreen blackberries, and Dugo, Mondello, Errante, Zappia, and Dugo (2001) reported the presence of cyanidin 3-galactoside, cyanidin 3-arabinoside, pelargonidin 3-glucoside, and malvidin 3-glucoside in a commercial Italian blackberry extract (Siriwoharn et al., 2004). Several phenolic acids have been detected in blackberries, namely gallic, caffeic, ferulic, *p*-coumaric and ellagic acids. Of these, ellagic acid was the major phenolic acid present (Sellappan, Akoh, & Krewer, 2002). Compared with blackberries grown in temperate climates, the tropical highland blackberry (*Rubus adenotrichus*) presents high contents of ellagitannins and low contents of anthocyanins (Acosta-Montoya et al., 2010). Elisia, Hu, Popovich, and Kitts (2010) suggested that the anthocyanins, and more specifically cyanidin-3-glucoside contribute mainly to the antioxidant ability of blackberry to suppress both peroxyl radical-induced chemical and intracellular oxidation.

8.2.1.3. Blackcurrants. Blackcurrant (*Ribes nigrum*) berries are widely cultivated for their use in beverages reputed to be excellent for health due to their high content of antioxidant phenolics (Lu & Foo, 2003). Black currants were named as "superfruits" due to the presence of one of the important sources of potential health promoting phytochemicals, with potent immunomodulatory, antimicrobial and anti-inflammatory actions, inhibition of low-density lipoprotein as well as reduction of cardiovascular diseases (Nour, Stampar, Veberic, & Jakopic, 2013). The fruit serves as a rich source of phenolic compounds such as anthocyanins, flavonoids, phenolic acids and proanthocyanidins (Costantino, Albasini, Rastelli, & Benvenuti, 1992; Foo & Porter, 1981; Koeppen & Herrmann, 1977; Shahidi & Naczk, 2004). Flavonoids in blackcurrant, unlike other berries, are dominated by myricetin, followed by quercetin and kaempferol (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999; Mikkonen et al., 2001; Vuorinen, Määttä, & Törrönen, 2000). Eleven delphinidin-, cyanidin-, malvidin-, petunidin-, and peonidin-based anthocyanins were detected in blackcurrant, with the main components being delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside and cyanidin-3-*O*-rutinose (Borges, Degeneve, Mullen, & Crozier, 2009). Recently, Nour et al. (2013) detected and quantified nine individual anthocyanins using HPLC-MS in ethanolic extracts of black currants, i.e. delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, petunidin 3-rutinoside, pelargonidin 3-rutinoside, peonidin 3-rutinoside, petunidin 3-(6-coumaroyl)-glucoside and cyanidin 3-(6-coumaroyl)-glucoside. Blackcurrant seeds are a rich source of potent antioxidants, the seeds also contain high levels of polyunsaturated fatty acids, especially γ-linolenic acid (Traitler, Winter, Richli, & Ingenbleek, 1984), which is stable in the intact seed (Lu & Foo, 2003; Lu, Foo, & Wong, 2002; Shahidi & Naczk, 2004). Srivastava et al. (2010) reported the antioxidant and anti-inflammatory activities of polyphenolics from Southeastern U.S. range blackberry cultivars. Flavon(ol)

glycosides such as myricetin-3-rutinoside, myricetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-glucoside and kaempferol-3-glucoside have been identified in blackcurrant seeds (Lu & Foo, 2003). Aureusidin, like myricetin, quercetin or kaempferol, could also be a hydrolysis product found in blackcurrant, and its presence was first reported by Lu and Foo (2003). Two novel noncyanogenic nitrile-containing compounds, nigrumin-5-*p*-coumarate and nigrumin-5-ferulate were also isolated from seed residues (Lu et al., 2002). Lu et al. (2002) identified a number of phenolic acids and their derivatives in blackcurrant seeds, namely caffeic, ferulic, *p*-coumaric, gallic, protocatechuic and *p*-hydroxybenzoic acids as well as 1-cinnamoyl-β-D-glucoside and 1-*p*-coumaroyl-β-D-glucoside. A recent study on antioxidant activity of berries suggests that blackcurrants had the highest antioxidant capacity in the FRAP assay followed by blueberries, raspberries, and red currants, and the lowest was noted for cranberries (Borges et al., 2009). Furthermore, researchers have provided convincing evidence that these small and soft-fleshed berry fruits may have an enormous potential for cancer prevention (Bishayee et al., 2011; Gopalan et al., 2012).

8.2.1.4. *Blueberries*. Several studies have confirmed that blueberries contain high antioxidant activity compared with other fruits (Cao, Sofic, & Prior, 1996; Prior et al., 1998; Vinson, Teufel, et al., 2001; Wang et al., 1996). The high antioxidant capacity of whole blueberries has been highly correlated to their anthocyanin and total phenolic contents (Kalt & Dufour, 1997; Kalt, McDonald, & Donner, 2000; Prior et al., 1998). The anthocyanins in blueberries exist almost exclusively in the skin, whereas phenolics, in general, and antioxidant properties are mostly in the skin (Lee & Wrolstad, 2004). Fifteen anthocyanins were detected in blueberries, many of which exhibited substantial antioxidant activity. Most antioxidant activity was attributed to delphinidin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside, petunidin-3-*O*-galactoside, malvidin-3-*O*-galactoside, malvidin-3-*O*-arabinoside, 5-*O*-feruloylquinic acid and traces of a quercetin-*O*-diglucoside (Borges et al., 2009). Rabbiteye blueberries also contain a higher level of catechin than southern highbush blueberries (Ballington, Ballinger, & Maness, 1987; Francis, Harborne, & Barker, 1966; Prior, Lazarus, Cao, Muccitelli, & Hammerstone, 2001). The skins of blueberries consist mainly of cinnamic acids and flavonol glycosides, with minor amount of gallic acid and syringic acid. The flesh consists of only cinnamic acids. Chlorogenic acid (5-caffeoylquinic acid) is the main phenolic compound present in the flesh. The seed fraction also has cinnamic acids and flavonol glycosides (Lee & Wrolstad, 2004). Oligomeric B-type procyanidins from dimers to octamers have been identified in blueberry (Gu et al., 2002; Prior et al., 2001). Blueberry processing waste is high in antioxidant activity and total phenols and has the potential to be a good source of natural colourant and nutraceutical (Lee & Wrolstad, 2004). Blueberry leaves were found to serve as a good source of phenolics that possess high antioxidant activity. The antioxidant activity of crude phenolic extracts of blueberry leaves in β-carotene–linoleate model system was comparable to that displayed by BHA. The DPPH radical scavenging effect of crude phenolic extracts, at 100 μg/assay, was over 90% (Naczk, Amarowicz, Zadernowski, Pegg, & Shahidi, 2003). A recent study has shown that phenolic compounds such as chlorogenic acid, quercetin, ellagic acid and quercetin-3-galactoside present in the blueberry extract could be used to control pathogenic microorganisms due to its antimicrobial activity (Shen et al., 2014).

8.2.1.5. *Cranberries*. Cranberries have the highest amount of phenolic antioxidants of any commonly consumed fruit (Vinson, Teufel, et al., 2001) and this is the world's first government-approved use for a fruit to treat urinary tract infection (Vinson, Bose, Proch, Al Kharrat, & Samman, 2008). Cranberry phenolics include simple phenolic acids and flavonoids that include anthocyanins, proanthocyanidins, as well as flavonols (Gregoire, Singh, Vorsa, & Koo, 2007). Anthocyanins of cranberry consist largely of galactoside and arabinoside conjugates of cyanidin and peonidin (Singh, Wilson, Kalk, Cheong, & Vorsa, 2009). Proanthocyanidin isolates contain epicatechin (monomer), dimer (epicatechin-(4β → 8, 2β → O → 7)-epicatechin), and trimer (epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → O → 7)-epicatechin) (Singh et al., 2009). Isolated cranberry flavonols included myricetin-3-β-galactoside, myricetin-3-α-arabinofuranoside, quercetin-3-β-galactoside, quercetin-3-β-glucoside, quercetin-3-rhamnospyranoside, and quercetin-3-*O*-(6″-*p*-benzoyl)-β-galactoside (Singh et al., 2009; Yan, Murphy, Hammond, Vinson, & Neto, 2002). Only a small percentage of the total flavonol content in cranberry or cranberry juice exist as aglycones such as free myricetin, quercetin, and kaempferol (Haekkinen, Kaerenlampi, Heinonen, Mykkaenen, & Toerroenen, 1999). Yan et al. (2002) suggested that compared to a standard dietary antioxidant such as vitamin E, both the flavonol glycosides and cyanidin 3-galactoside from whole cranberry are effective in scavenging free radicals and preventing LDL oxidation, cyanidin 3-galactoside being superior to flavonols present in this regard. Fifteen phenolic acids, namely benzoic, *o*-hydroxybenzoic, cinnamic, *m*-hydroxybenzoic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, phthalic, 2,3-dihydroxybenzoic, vanillic, *o*-hydroxycinnamic, 2,4-dihydroxybenzoic, *p*-coumaric, ferulic, caffeic, and sinapic acids were identified in cranberry fruit in the free and bound forms (Zuo, Wang, & Zhan, 2002). Sinapic, caffeic and *p*-coumaric acids were the major bound phenolic acids whilst *p*-coumaric, 2,4-dihydroxybenzoic and vanillic acids were the predominant free phenolic acids (Naczk & Shahidi, 2006; Zuo et al., 2002). Cranberry and cranberry juice phenolics are also known to provide a rich source of antioxidants that can protect LDL from oxidative injury (Wilson, Porcari, & Harbin, 1998; Wilson, Porcari, & Maher, 1999). The order of total polyphenols on a fresh weight basis follows the order of dried cranberries > frozen cranberries > cranberry sauce > jellied sauce. Cranberry powder is not usually considered a food, but it had the most polyphenols of the cranberry products (Vinson et al., 2008). The frozen cranberries, 100% juice, and dried cranberries provide the most antioxidants distantly followed by mixed and 27% cranberry juice and other cranberry products (Vinson et al., 2008).

8.2.1.6. *Raspberries*. Red raspberries (*Rubus idaeus* L.) are known to possess strong antioxidant capacity, mainly as a result of their high levels of anthocyanins and other phenolic compounds (Kafkas, Özgen, Özoğul, & Türemiş, 2008; Kähkönen, Hopia, & Heinonen, 2001). Raspberries contain a unique phytochemical profile rich in hydrolysable tannins (ellagitannins) and anthocyanins (more specifically cyanidin glycosides) that

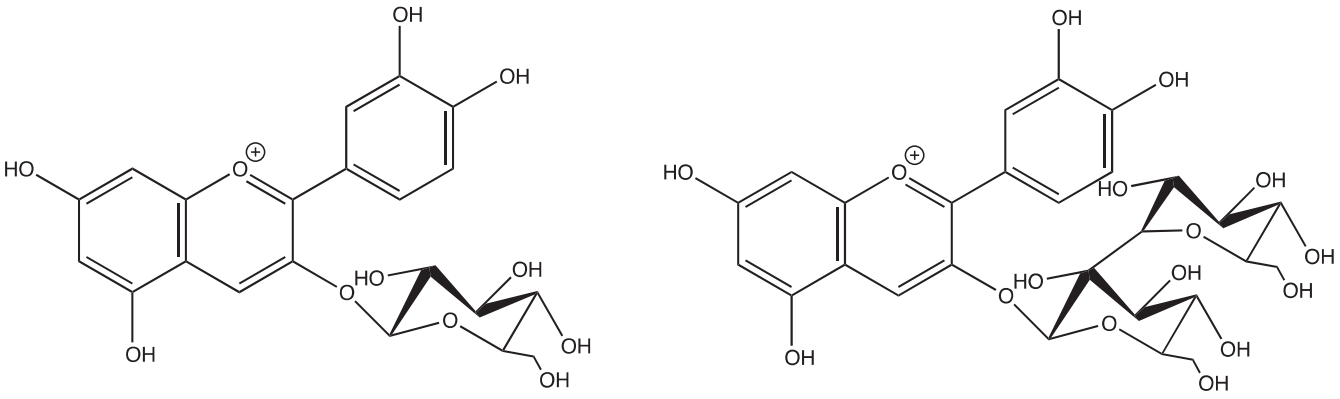


Fig. 16 – Chemical structures of raspberry anthocyanins (adapted from Sun, Cao, Bai, Liao, & Hu, 2010).

distinguishes them from other berries and fruits (Rao & Snyder, 2010). The major raspberry anthocyanin (Fig. 16) is cyanidin-3-sophoroside with smaller quantities of other anthocyanins, including cyanidin-3-(2-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, pelargonidin-3(2-glucosylrutinoside), and pelargonidin-3-glucoside (Borges et al., 2009; Bowen-Forbes, Zhang, & Nair, 2010; Mullen, Stewart, et al., 2002; Rommel & Wrolstad, 1993). The two main ellagitannins are sanguiin H-6 and lambertianin C. Raspberries also contain quercetin and kaempferol-based flavonol conjugates and trace levels of (–)-epicatechin and a procyanidin dimer (Mullen, McGinn, et al., 2002). Of these, quercetin 3-glucuronide is the predominant flavonol glycoside in red raspberry juice (Rommel & Wrolstad, 1993). Red and black raspberries are especially high in phenolic compounds compared to other lighter coloured fruits and vegetables (Liu et al., 2002). Black raspberries have much higher levels of anthocyanins (687 mg/100 g of fw) than red raspberries, attributable to their darker colouration, and the majority of these are also cyanidins (Rao & Snyder, 2010). Beekwilder, Hall, and de Vos (2005) published a review on raspberry antioxidants and reported that raspberries had the highest antioxidant capacity followed by strawberries, kiwi, broccoli, leek, apple, and, finally, tomato. Liu et al. (2002) reported that the antioxidant activity of the raspberry was directly related to their total content of phenolics and flavonoids.

8.2.1.7. Grapes. Grape phenolics, especially high in the grape skin, are classified into two groups of flavonoids and non-flavonoids. The flavonoids include flavan-3-ols (catechin), flavonols (quercetin) and anthocyanins. The non-flavonoids encompass hydroxybenzoic acids (gallic acid), hydroxycinnamic acids and stilbenes (resveratrol) (Yang, Martinson, & Liu, 2009). In the grape berry, the flavonoids are mainly localized in the skins, such as the anthocyanins and resveratrol (Palomino, Gomez-Serranillos, Slowing, Carretero, & Villar, 2000), whilst flavan-3-ols (catechins and proanthocyanidins) are present both in the skins and in the seeds (Yang, Martinson, et al., 2009). Grape berries and their skins contain phenolic acids such as caftaric acid (*trans*-caffeoyltartaric acid), coutaric acid (*p*-coumaryltartaric acid) and *trans*-fertaric acid (Cantos, Espin, & Tomas-Barberan, 2002; Souquet, Labarbe, Le Guerneve, Cheynier, & Moutounet, 2000; Vrhovsek, 1998), flavonols such as quercetin 3-glucuronide, quercetin 3-glucoside, myricetin 3-glucuronide and myricetin 3-glucuronide (Souquet et al., 2000) as well as flavanonols, such as astilbin (dihydroquercetin 3-rhamnoside) and engeletin (dihydrokaempferol 3-rhamnoside) (Lu & Foo, 1999; Souquet et al., 2000). A number of stilbenes, namely *trans*- and *cis*-resveratrols (3,5,4-trihydroxystilbene), *trans*- and *cis*-piceids (3-*O*-d-glucosides of resveratrol), *trans*- and *cis*-astringins (3-*O*-d-glucosides of 3-hydroxyresveratrol), *trans*- and *cis*-resveratrolosides (4-*O*-d-glucosides of resveratrol) and pterostilbene (a dimethylated derivative of stilbene) have been detected in both grape leaves and berries (Jaendet et al., 2002; Naczk & Shahidi, 2006; Wang, Catana, Yang, Roderick, & van Breemen, 2002). Studies have shown that grape seeds are a rich source of monomeric phenolic compounds such as (+)-catechins, (–)-epicatechin, (+)-gallocatechins, (–)-epigallocatechin, and their dimeric, trimeric, and tetrameric proanthocyanidins (Baoshan Sun & Spranger, 2005; Freitas, Glories, Bourgeois, & Vitry, 1998). Grapes are one of the major dietary sources of anthocyanins, responsible for a wide range of colours of black, red and purple grapes; however, these phenolics are absent in white grapes. In particular, anthocyanins mostly accumulate in the skins, whereas procyanidins are located in the seeds. The anthocyanins in grape skins are predominately the 3-*O*-glucosides of malvidin, cyanidin, delphinidin, peonidin and petunidin. Malvidin, the reddest of all anthocyanins, is the major one in dark red *vinifera* grapes, with higher proportions of cyanidin in red grapes. Cyanidin 3-monoglucoside and delphinidin 3-monoglucoside are the major anthocyanins in Concord grapes (Mazza, 1995; Yang, Martinson, et al., 2009). However, Orak (2007) reported that anthocyanin and phenolic compounds either alone or in combination, are responsible for the antioxidant activity of different grape cultivars. Recently, de Camargo, Regitano-d'Arce, Biasoto, and Shahidi (2014) identified that in grape by products most prominent phenolic compounds are present in the insoluble bound form.

8.2.1.8. Cherries. Sweet cherries and tart cherries are rich sources of flavonoids (Gao & Mazza, 1995). The major anthocyanin pigments in sweet and sour cherries have been

identified. Cyanidin-3-rutinoside, cyanidin-3-glucoside, peonidin-3-rutinoside, peonidin-3-glucoside, and pelargonidin-3-rutinoside have been identified in Bing and other sweet cherry cultivars (Chaovanalikit & Wrolstad, 2004; Gao & Mazza, 1995; Lynn & Luh, 1964; Mozetic, Trebse, & Hribar, 2002). Sour cherries are reported to contain cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucosylrutinoside, cyanidin-3-*O*-sophoroside, pelargonidin-3-*O*-glucoside, peonidin-3-*O*-rutinoside and cyanidin-3-*O*-arabinosylrutinoside (Chandra, Nair, & Lezzoni, 1992; Chaovanalikit & Wrolstad, 2004; Gao & Mazza, 1995). Kirakosyan et al. (2010) reported that tart cherry produces various kinds of polyphenolics in its fruits that include cyanidin derivatives (mostly cyanidin 3-glucosylrutinoside, cyanidin 3-rutinoside, cyanidin sophoroside), peonidin 3-glucoside; kaempferol, quercetin and isorhamnetin and their derivatives, as well as the alkaloid, melatonin. Several research reports have indicated that sweet cherries are rich in phenolic acids. The most abundant phenolics present are hydroxycinnamic acids, such as caffeic and coumaric acids, with antioxidant and anti-inflammatory properties (Mulabagal, Lang, DeWitt, Dalavoy, & Nair, 2009). Cherries contain higher amounts of anthocyanins and phenolics when compared to several other fruits. It is implied that regular consumption of cherries is beneficial in reducing risk factors for heart disease, diabetes and certain types of cancer (Bobe, Wang, Seeram, Nair, & Bourquin, 2006; Bolleddula, Vareed, Olson, & Nair, 2005; Mulabagal et al., 2009).

8.2.1.9. Apples. Apples are an excellent source of several phenolic compounds and also possess high total antioxidant capacity. Biedrzycka and Amarowicz (2008) reviewed apple polyphenols as antioxidants. Sun et al. (2002) found that apples had the highest soluble free phenolics when compared to 10 other commonly consumed fruits (Khanizadeh et al., 2008). There are five major groups of polyphenolic compounds in apple, including hydroxycinnamic acids, flavan-3-ols, anthocyanidins, flavonols, and dihydrochalcones (Tsao, Yang, Sockovie, & Khanizadeh, 2005). The concentration of total phenolic compounds is much greater in the peel of apples than in the flesh and the antioxidant and antiproliferative activities of unpeeled apples were greater than those of peeled apples (Burda, Oleszek, & Lee, 1990; Ju, Yuan, Liu, Zhan, & Wang, 1996; Wolfe, Wu, & Liu, 2003). The flesh of apple contains catechins, procyanidins, phloridzin, phloretin glycosides, caffeic acid and chlorogenic acid; the peel possesses all of these compounds and has additional flavonoids not found in the flesh, such as quercetin glycosides (Wolfe et al., 2003). Huber and Rupasinghe (2009) found specific antioxidant compounds such as quercetin glycosides and cyanidin-3-*O*-galactoside in apple skin extract, which are not found in the flesh of apples. McGhie, Hunt, and Barnett (2005) reported that apple skin contains approximately 46% of the total phenolics in apples. Quercetin and quercetin-3-*O*-glucoside have been shown to exhibit a better antioxidant activity than BHT in bulk fish oil (Huber, Rupasinghe, & Shahidi, 2009). Tsao et al. (2005) reported that the major polyphenolic groups present are hydroxycinnamic acids (chlorogenic acid and *p*-coumaroylquinic acid), cyanidin-3-galactoside, flavan-3-ols/procyanidins (mainly catechin, epicatechin, and procyanidins B1 and B2), flavonols (quercetin galactoside, glucoside, rhamnoside, arabinoside, and xyloside), and dihydrochalcones (mainly phloridzin and phloretin-2′-xyloglucoside). No anthocyanins were detected in the flesh (Durkee & Poapst, 1965; Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999; Tsao et al., 2005; Van der Sluis, Dekker, Skrede, & Jongen, 2002). Chlorogenic acid was the major hydrocinnamic acid family derivative identified in apple fruit accounting for up to 87% of the total amount (Guyot et al., 1998). Chinnici, Bendini, Gaiani, and Riponi (2004) found that the antioxidant activity of individual phenolic compounds of apple followed the order of quercetin glycosides > procyanidins > chlorogenic > phloridzin. However, Van der Sluis et al. (2002) reported that flavan-3-ols were the top contributor, followed by quercetin glycosides, chlorogenic acid, cyanidin-3-galactoside, and phloridzin. Tsao et al. (2005) concluded that flavan-3-ols/procyanidins are the most important contributors to the *in vitro* antioxidant activity of apple and that procyanidin B2 and epicatechin are the most important individual antioxidants in apple. Hydroxycinnamic acids may play a significant role in the flesh. Apple pomace is a valuable source of polyphenols relevant for its antioxidant activity, as flavanols and flavonols and the antioxidant activity of apple pomace can be predicted by the contents of phloridzin, procyanidin B2, rutin + isoquercitrin, protocatechuic acid and hyperin (García, Valles, & Lobo, 2009). Recently, Sekhon-Loodu, Warnakulasuriya, Rupasinghe, and Shahidi (2013) found that polyphenols from both dried and frozen apple peel have higher inhibition of lipid oxidation compared to α-tocopherol, butylated hydroxytoluene and crude apple peel extracts.

8.2.1.10. Citrus fruits. In citrus fruits, cinnamic acid derivatives, coumarins and flavonoids (flavonones, flavones and flavonols) are the major groups of phenolic compounds (Horowitz & Gentili, 1977; Manthey & Grohmann, 2001; Naczk & Shahidi, 2006). Phenolic acids are found in the flavedo of citrus fruits (Manthey & Grohmann, 2001; Peleg, Naim, Rouseff, & Zehavi, 1991) in the form of esters, amides and glycosides (Bocco, Cuvelier, Richard, & Berset, 1998; Mouly, Gaydou, Faure, & Estienne, 1997; Rapisarda, Carollo, Fallico, Tomaselli, & Maccarone, 1998). Citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of promising biological properties including anti-atherogenic, anti-inflammatory and antitumour activity, inhibition of blood clots and strong antioxidant activities (Middleton & Kandaswami, 1994; Ramful, Bahorun, Bourdon, Tarnus, & Aruoma, 2010). Three types of flavonoids occur in citrus fruits: flavanones, flavones and flavonols (Calabrò et al., 2004). Naringin, naringenin 7-neohesperidoside and narirutin, naringenin 7-rutinoside are the major flavanone glycosides found in grapefruit, whilst narirutin and hesperidin, hesperetin 7-rutinoside are present in sweet oranges and naringin, neohesperidin and hesperetin 7-neohesperidoside in sour oranges (Kanes, Tisserat, Berhow, & Vandercook, 1992). On the other hand, hesperidin, narirutin and didymin (isosakuranetin 7-rutinoside) are the predominant flavanone glycosides in navel (Gil-Izquierdo, Gil, & Ferreres, 2002) and blood oranges (Mouly et al., 1997). Polymethoxyflavones and hydroxylated polymethoxyflavones are unique phenolic compounds that exist exclusively in citrus genus, especially in the citrus peels (S. Li et al., 2009). Tangeretin and nobiletin are the most abundant polymethoxyflavones in citrus peels (S. Li et al., 2009). However, Marin and Del Rio (2001) reported

that diosmin (4′methoxy-5,7,3′-trihydroxyflavone-7-rutinoside) and neodiosmin (4′methoxy-5,7,3′-trihydroxyflavone-7-neohepseridoside) are the predominant glycosylated flavones identified in citrus fruits. Nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) and sinensetin (5,6,7,3′,4′-pentame thoxyflavone) have been identified in orange, whilst tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, 5,7,8,4′-tetramethoxyflavone, and 5,7,8,3′,4′-pentamethoxyflavone exist in grapefruits (S. Li et al., 2009; Naczk & Shahidi, 2006). Cyanidin-3-glycoside, cyanidin-3-(6″-malonyl)-glycoside and cyanidin 3-rhamnoside are the major anthocyanins in Italian blood oranges (Maccarone, Maccarrone, Perrini, & Rapisarda, 1983; Maccarone, Maccarrone, & Rapisarda, 1985; Maccarone, Rapisarda, Fanella, Arena, & Mondello, 1998) whilst cyanidin-3-glycoside and cyanidin-3-(6″-malonyl)-glycoside are present in high amounts in Budd blood oranges (Lee, 2002). Recent studies have indicated that the flavedo extracts of citrus fruits represent a significant source of phenolic antioxidants with potential prophylactic properties for the development of functional foods (Ramful et al., 2010). Limonoids are a group of highly oxygenated triterpenoids found in citrus that were also reported as an anticancer agent (Hasegawa, Lam, & Miller, 2000). An extract of multiple varieties of citrus peels containing abundant flavonoids (polymethoxyflavones) effectively suppressed azoxymethane (AOM)-induced colonic tumourigenesis and exhibited anticancer activity, especially for prostate cancer (Lai et al., 2007, 2013; Suzawa, Guo, Pan, Ho, & Li, 2014).

8.2.1.11. Pomegranate. Pomegranates are a rich source of hydrolysable tannins and anthocyanins. Studies have shown that pomegranate fruit contains anthocyanins, ellagitannins, gallotannins, non-coloured flavonoids, and lignans, amongst other (poly)phenolic compounds (Abbas, 2014; Bonzanini, Bruni, Palla, Serlataite, & Caligiani, 2009; Fischer, Carle, & Kammerer, 2011; Mena et al., 2012). Several anthocyanins such as cyanidin 3-glucoside, delphinidin 3-glucoside, cyanidin 3,5-diglucoside, delphinidin 3,5-diglucoside and pelargonidin 3-glucoside have been detected in pomegranate juice (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Noda, Kaneyuki, Mori, & Packe, 2002). The seed coat of pomegranate contains delphinidin-3-glucoside, delphinidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3,5-diglucoside, pelargonidin-3-glucoside, and pelargonidin-3,5-diglucoside (Du, Wang, & Francis, 1975). Hydrolysable tannins are the most abundant polyphenols and antioxidant compounds in pomegranates and include gallotannins, ellagitannins and gallagyl esters such as punicalagin and punicalin (Madrigal-Carballo, Rodriguez, Krueger, Dreher, & Reed, 2009). Pomegranate marc (contains about 78% peel and 22% seeds based on wet weight), a by-product after pomegranate juice processing, is a good raw material for producing natural antioxidants because of its high content of polyphenols (Qu, Pan, & Ma, 2010). Recently, Qin et al. (2013) reported that the antioxidant potential of pomegranate rind powder extract (PRP), pomegranate juice (PJ) and pomegranate seed powder extract in raw ground pork meat stored at 4 °C for 12 days follows the order of BHT > PRP > PJ > PSP > control. Based on several studies, Syed, Chamcheu, Adhami, and Mukhtar (2013) revealed that pomegranate is a potential chemopreventive agent for leukaemia, skin, lung, prostate, colon and breast cancer. Wu, Ma, and Tian (2013) found that pomegranate husk extract, punicalagin and ellagic acid inhibit fatty acid synthase and adipogenesis of 3T3-L1 adipocyte and could have potential effect in the prevention and treatment of obesity. More recently, a study showed that thinning sour–sweet cultivars of pomegranate could be considered as a good source of bioactive compounds (Nuncio-Jáuregui et al., 2015). Pomegranate peel extract has been suggested as the best source of microbial substrates at colonic level in comparison to pulp and juice (Mosele, Macià, Romero, Motilva, & Rubió, 2015).

8.2.1.12. Apricot. Apricot peel and flesh contain four main groups of phenolic compounds, procyanidins, hydroxycinnamic acid derivatives, flavonol, and anthocyanins (Ruiz, Egea, Gil, & Tomas-Barberan, 2005). Chlorogenic and neochlorogenic acids, procyanidins B1, B2, and B4, and some procyanidin trimers, quercetin 3-rutinoside, kaempferol 3-rhamnosyl-hexoside and quercetin 3-acetyl-hexoside, cyanidin 3-rutinoside, and 3-glucoside have been detected in the skin and flesh of the different cultivars of apricot (Han, Liu, Cui, & Zhang, 2013; Ruiz et al., 2005).

8.2.2. Vegetables

Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases. Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in the human body (Chu, Sun, Wu, & Liu, 2002). Phenolics in vegetables are present in both free and bound forms. Bound phenolics, mainly in the form of β-glycosides, may survive the human stomach and small intestine digestion and reach the colon intact, where they are released and exert their beneficial effects (Sosulski et al., 1982). A more complete profile of phenolic distributions, including both free and bound phenolics in some vegetables (Fig. 17) is reported using new and modified methods (Chu et al., 2002). Broccoli contained the highest total phenolic content, followed by spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery and cucumber. Red pepper had the highest total antioxidant activity, followed by broccoli, carrot, spinach, cabbage, yellow onion, celery, potato, lettuce and cucumber (Chu et al., 2002). Alvarez-Parrilla, de la Rosa, Amarowicz, and Shahidi (2010) found that fresh and processed Jalapeño and Serrano peppers are good sources of phenolics and ascorbic acid, as well as capsaicinoids, and present high antioxidant activity. The phenolics antioxidant index (PAI) was used to evaluate the quality/quantity of phenolic contents of vegetables. With this evaluation, spinach ranked first with the highest PAI (0.979), followed by broccoli, red pepper, carrot, yellow onion, cabbage, potato, celery, lettuce and cucumber (PAI = 0.004) (Table 9) (Chu et al., 2002). Antioxidant activity of vegetables is shown in Table 10. Chu, Chang, and Hsu (2000) found high amount of flavonoids in both green and purple leaves of sweet potatoes (185.01 and 426.82 mg/kg wet basis, respectively) and the outer leaves of onion (264.03 mg/kg wet basis) (Table 10), and also reported that green leaves of sweet potatoes and the outer leaves of onion showed higher reducing power and higher antioxidant activity in a linoleic acid system as compared to cabbage, spinach and potato. Sultana and Anwar (2008) reported that amongst

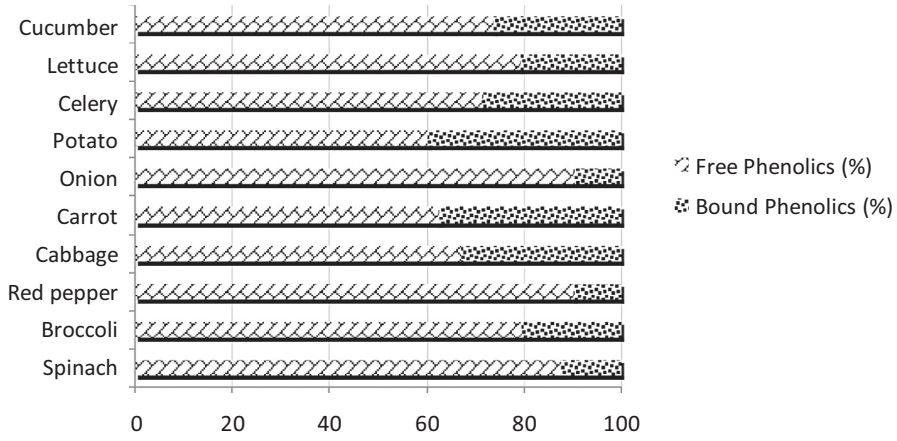


Fig. 17 – Free and bound phenolic content of vegetables (adapted from Chu et al., 2002).

Table 9 – Antioxidant activity of selected vegetables.

Vegetables	Antioxidant activity[a]	PAI[b]
Spinach	737	0.979
Broccoli	775	0.948
Red pepper	826	0.651
Cabbage	314	0.179
Carrot	750	0.448
Onion	231	0.287
Potato	83	0.030
Celery	90	0.021
Lettuce	49	0.017
Cucumber	21	0.004

Source: Adapted from Chu et al. (2002).
[a] μmol of vitamin C eq/100 g.
[b] PAI = free phenolic content/EC_{50} of corrected total antioxidant activity.
(EC_{50} of corrected total antioxidant activity = total antioxidant activity – vitamin C antioxidant activity)

vegetables, kaempferol was the dominant flavonol which was not detected in carrot and garlic. On the basis of ORAC analysis, Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002) reported that green pepper, spinach, purple onion, broccoli, beet, and cauliflower are the leading sources of antioxidants with activities against peroxyl radicals. A Recent study on the antioxidant capacities and total phenolic contents of lipophilic and hydrophilic extracts of 56 commonly consumed vegetables showed that the highest antioxidant capacities and phenolic contents were found in Chinese toon bud, loosestrife, perilla leaf, cowpea, caraway, lotus root, sweet potato leaf, soy bean (green), pepper leaf, ginseng leaf, chives, and broccoli, whilst the values were very low in marrow squash and eggplant (purple) (Deng et al., 2013).

8.2.2.1. Brassica vegetables. Brassica vegetables, which include different genus of cabbage (white, red, savoy, swamp, Chinese), broccoli, cauliflower, Brussels sprouts and kale, are consumed

Table 10 – Flavonol and flavone contents (mg/kg[a]) of methanolic extracts of several vegetables after acidic hydrolysis.

Vegetable	Flavonol			Flavone		Total
	Myricetin	Quercetin	Kaempferol	Luteolin	Apigenin	
Sponge gourd	1.29	0.29	–	0.10	0.03	1.71
Sweet potato leaves (green)	38.8	143.78	–	–	23.5	185.01
Sweet potato leaves (purple)	155.87	266.86	–	4.09	–	426.82
Leaf lettuce	0.17	0.40	–	–	0.06	0.63
Chinese kale	0.09	0.65	–	–	0.14	0.90
Cucumber	–	0.01	–	0.09	0.03	0.13
Spinach	0.42	19.60	0.56	–	–	25.02
Water spinach	0.3	1.76	–	0.41	0.05	2.11
Purple cabbage	–	0.24	–	0.16	1.07	1.47
Chinese cabbage	0.14	0.24	0.05	1.18	0.31	1.86
White cabbage	–	0.04	–	0.16	0.92	1.12
Onion (interior)	0.21	26.09	0.56	0.22	0.04	27.11
Onion (outer leaves)	–	258.85	5.18	–	–	264.03
Potato	0.01	0.05	0.50	–	0.02	0.13

Source: Adapted from Chu et al. (2000).
[a] Wet basis.

all over the world (Podsędek, 2007). Broccoli is a source of flavonol and hydroxycinnamoyl derivatives. Price, Casuscelli, Colquhoun, and Rhodes (1998) identified the main flavonol glycosides present in broccoli florets as quercetin and kaempferol 3-O-sophoroside. Three minor glucosides of these aglycones were also detected, namely isoquercitrin, kaempferol 3-O-glucoside and kaempferol diglucoside. The predominant hydroxycinnamoyl acids were identified as 1-sinapoyl-2-feruloylgentiobiose, 1,2-diferuloylgentiobiose, 1,2,2′-trisinapoylgentiobiose, and neochlorogenic acid (Borş et al., 2014; Soengas Fernández, Sotelo Pérez, Velasco Pazos, & Cartea González, 2011; Vallejo, Tomas-Barberan, & Garcia-Viguera, 2003). In addition, 1,2′-disinapoyl-2-feruloylgentiobiose and 1,2-disinapoylgentiobiose, 1-sinapoyl-2,2-diferuloyl gentiobiose, isomeric form of 1,2,2′-trisinapoylgentiobiose, and chlorogenic acids were found in broccoli (Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2012; Price, Casuscelli, Colquhoun, & Rhodes, 1997; Vallejo et al., 2003). Nielsen, Olsen, and Petersen (1993) showed that cabbage contained a mixture of more than 20 compounds of which seven were identified as 3-O-sophoroside-7-O-glucosides of kaempferol and quercetin with and without further acylation with hydroxycinnamic acids. In addition, unmodified kaempferol tetraglucosides or their derivatives acylated with either sinapic, ferulic or caffeic acid were found in cabbage leaves (Nielsen, Norbek, & Olsen, 1998). Red pigmentation of red cabbage is caused by anthocyanins. Red cabbage contains more than 15 different anthocyanins which are acylglycosides of cyanidin (Dyrby, Westergaard, & Stapelfeldt, 2001; Podsędek, 2007). More recently, Wiczkowski, Topolska, and Honke (2014) identified twenty different anthocyanins with the main structure of cyanidin-3-diglucoside-5-glucosides in red cabbages. Kaempferol and myricetin derivatives were also present in Brassica vegetables, but myricetin was not present in broccoli, white cabbage, purple cabbage, and cauliflower (Podsędek, 2007). Apigenin and luteolin as flavones were detected in the hydrolysed extracts of different Brassica vegetables, except for broccoli (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004). Chu et al. (2000) reported that the levels of flavone were higher than those of flavonol in all tested Brassica vegetables (Table 10). Apigenin was the predominant flavone aglycone in these vegetables except Chinese cabbage, where luteolin content was nearly 4-fold higher than that of apigenin. Piironen, Syvaoja, Varo, Salminen, and Koivistoinen (1986) reported that α-tocopherol was the predominant tocopherol in all Brassica vegetables, except in cauliflower, containing predominantly γ-tocopherol. In contrast, Kurilich et al. (1999) reported a lower concentration of γ-tocopherol than α-tocopherol in cauliflower (Podsędek, 2007). A recent study on raw and cooked broccoli and cauliflower showed that boiling increases the concentrations of β-carotene and vitamin A as well as steaming enhances the concentrations of lutein, zeaxanthin, cryptoxanthin, α-carotene and total carotenoids (dos Reis et al., 2015). Generally, amongst the analysed Brassica vegetables, Brussels sprouts, broccoli, and red cabbage possess the highest antioxidant capacity (Podsędek, 2007). Common cabbage had a rather low antioxidant activity. In some cases, however, cabbage and broccoli displayed similar antioxidant activities (Ou et al., 2002; Wu et al., 2004). Brassica vegetables, such as kale, broccoli, cabbage, Brussels sprouts and cauliflower, are characterized by considerable antioxidant activity, due to their high contents of polyphenol compounds and vitamin C. The greatest activity is shown by kale which, in addition to a particularly high content of these compounds, also contains a substantial amount of carotenoids (Sikora, Cieślik, Leszczyńska, Filipiak-Florkiewicz, & Pisulewski, 2009). Cao et al. (1996) also reported that kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach and broccoli flowers. Recently, Bacchetti et al. (2014) found that supplementation with brassica influenced on serum lipid profile with a significant decrease in total cholesterol, LDL-cholesterol and oxidized LDL. In addition brassica vegetables have been shown to exert a protective effect against many chronic degenerative diseases, including cancer (Herr & Buchler, 2010; Manchali, Murthy, & Patil, 2012; Melega et al., 2013).

8.2.2.2. Bitter melon. Main phenolic constituents in the bitter melon extracts are catechin, gallic acid, gentisic acid, chlorogenic acid and epicatechin (Horax, Hettiarachchy, & Chen, 2010; Kubola & Siriamornpun, 2008). Sathishsekar and Subramanian (2005) found that *in vivo* antioxidant efficacy of bitter melon extracts has highlighted the increased activity of naturally occurring hepatic antioxidant enzymes. Bitter melon possesses antioxidant, anti-tumour, anti-bacterial, anti-inflammatory, anti-viral, anti-obesity, anti-hypertension as well as anti-hyperglycaemic effects (Ahmad et al., 2012; Chen et al., 2012; Hsu, Fang, Liu, & Chen, 2013; Zhang et al., 2012). More recently, Chang et al. (2015) found that the triterpenes 3β,25-dihydroxy-7β-methoxycucurbita-5,23(E)-diene and 3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al isolated from bitter melon have insulin-sensitizing and insulin-substitution functions, which are correlated to their effects on inhibiting protein-tyrosine phosphatase-1B (PTP-1B) and activating AMP-activated protein kinase (AMPK) inhibitor, respectively.

8.2.2.3. Carrots. Carrots have been ranked 10th in nutritional value amongst 39 fruits and vegetables, and research on carrot health benefits continues (Sharma, Karki, Thakur, & Attri, 2012; Sun, Simon, & Tanumihardjo, 2009). The predominant phenolic acids in carrots are chlorogenic acid, caffeic acid, *p*-hydroxybenzoic acid, ferulic acid, and other cinnamic acid isomers. Chlorogenic acid had the highest content amongst all phenolic acids (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001). Sun, Simon, et al. (2009) suggested that purple-yellow or purple-orange carrots had high antioxidant content and capacity. Alasalvar et al. (2001) reported that all carrots studied contained mainly hydroxycinnamic acid derivatives, namely 3′-caffeoylquinic acid (neochlorogenic acid), 5′-caffeoylquinic acid (chlorogenic acid), 3′-*p*-coumaroylquinic acid, 3′-feruloyquinic acid, 3′,4′-dicaffeoylquinic acid, 5′-feruloyquinic acid, 5′-*p*-coumaroylquinic acid, 4′-feruloylquinic acid, 3′,5′-dicaffeoylquinic acid, 3′,4′-diferuloylquinic acid and 3′,5′-diferuloylquinic acid. The presence of dicaffeoylquinic acid, especially in orange carrots may exert a very strong antioxidant activity in the product (Alasalvar et al., 2001; Shahidi & Naczk, 2004). The presence of coumarins, namely 6-methoxymellein and 6-hydroxymellein, was also reported in carrot tissues (Naczk & Shahidi, 2006). These compounds were predominantly accumulated in the periderm tissue of carrot root (Talcott & Howard, 1999). Carrot peels have been reported

to be a rich source of phenolic compounds (Kähkönen et al., 1999; Zhang & Hamauzu, 2004). Zhang and Hamauzu (2004) found that phenolic contents in different tissues decreased from peels, phloem to xylem, whilst antioxidants and radical scavenging activities in different tissues decreased in the same order as the phenolic contents. Chantaro, Devahastin, and Chiewchan (2008) suggested that carrot peels could be used as a good raw material to produce a dietary fibre powder with antioxidant potential. Surjadinata and Cisneros-Zevallos (2012) have shown that phenolic profiles are influenced by wounding intensity of carrot tissue and chlorogenic acid and its related isomers are the major phenolics present and synthesized by wounding stress in carrot tissue. In addition, Leja et al. (2013) found that red carrots have higher antioxidant activity than orange, yellow and white carrots, and in the season of lower rainfall they accumulated higher amounts of phenolic compounds.

8.2.2.4. *Onions*. Onions are one of the richest sources of flavonoids in the human diet. Onions possess a high level of antioxidant activity, attributed to their flavonoid constituents, namely quercetin, kaempferol, myricetin, and catechin (Cook & Samman, 1996; Patil, Pike, & Yoo, 1995; Pérez-Gregorio, Regueiro, Simal-Gándara, Rodrigues, & Almeida, 2014). Two major components quercetin monoglucoside and quercetin diglucoside account for 80% of the total flavonoids in onions (Rhodes & Price, 1996). Levels of quercetin glucosides are much higher in onions than in other vegetables (Shahidi & Naczk, 2004). Anthocyanins are only minor components of the flavonoid spectrum in the edible portion of red onion varieties, although the edible bulb of red onions is generally higher in total flavonoids than the bulbs of white or sweet yellow onions due to the presence of anthocyanins (Rhodes & Price, 1996). Yellow onions have been found to contain higher levels of quercetin than red onions, with pink and white onions having the lowest concentrations (Patil et al., 1995). However, Gokce, Kaya, Serce, and Ozgen (2010) suggested that red onions had higher antioxidant activities than yellow and white onions although yellow onions were richest in their phenolic contents. The dominant onion flavonoids are quercetin, quercetin-3-*O*-β-glucoside, quercetin-4′-*O*-β-glucoside, and quercetin-3,4′-di-*O*-β-glucoside and the highest contribution to the antioxidant capacity of onions was provided by quercetin-4′-*O*-β-glucoside (Zielinska, Wiczkowski, & Piskula, 2008). These flavonols are mostly concentrated in the skin. The abaxial epidermis of scales contained a higher level of flavonols than did the mesophyll and approximately 50% of flavonols were detected in the top quarter part of the scales (Naczk & Shahidi, 2006). Onions also contain small quantities of phenolic acids bound to cell walls. Of these, protocatechuic acid was the most abundant phenolic component in the papery scales and was not detected in other tissues. In addition ferulic, *p*-hydroxybenzoic, vanillic and coumaric acids have been found in the papery and fleshy scales (Ng et al., 2000). Anthocyanins, namely peonidin 3-glucoside, cyanidin 3-glucoside and cyanidin 3-arabinoside and their malonylated derivatives, cyanidin 3-laminariobioside and delphinidin and petunidin derivatives (Donner, Gao, & Mazza, 1997), are located in the red onion skin and the outer fleshy layer (Gennaro et al., 2002). Fossen, Andersen, Ovstedal, Pedersen, and Ranknes (1996) reported that 3-(6″-malonyl-3″-glucosylglucoside), 3(3″,6″dimalonylglucoside), 3-(6″-malonylglucoside), 3-(3″-malonylglucoside), 3-(3″-glucosylglucoside) and 3-glucoside of cyanidin comprise over 95% of total anthocyanins in the whole red onion. Gennaro et al. (2002) have demonstrated that cyanidin and delphinidin derivatives constitute over 50% and 30% of total anthocyanins in whole red onion, respectively. Pasteurized 'Recas' paste was chosen to be the most appropriate onion by-product for developing an antioxidant food ingredient amongst all the onion by-products analysed (Roldán, Sánchez-Moreno, Ancos, & Cano, 2008). It showed several advantages such as a remarkable antioxidant activity, moderately high bioactive composition (total phenols and quercetin), and an excellent antibrowning effect from a technological point of view. Nuutila, Puupponen-Pimiä, Aarni, and Oksman-Caldentey (2003) compared the antioxidant activities of onions and garlic extract and found that onions had clearly higher radical scavenging activities than garlic; red onion being more active than yellow onion. The skin extracts of yellow and red onion possessed the highest activities. Recently, Albishi, John, Al-Khalifa, and Shahidi (2013a) also reported that pearl, red, yellow and white onion skins contain higher phenolic content (approximately six times) than that of their flesh counterparts as well as exhibited highest antioxidant activity. Furthermore, Wu and Xu (2014) showed the inhibitory effects of onion against α-Glucosidase activity and suggested their use as anti-diabetic agent.

8.2.2.5. *Potato*. Chlorogenic acid is the predominant phenolic in potato tuber, constituting up to 90% of its total phenolic content (Friedman, 1997). About 50% of the phenolic compounds were located in the peel and the adjoining tissues of potato, whilst the remainder decreased in concentration from outside towards the centre of the tubers (Friedman, 1997; Hasegawa, Johnson, & Gould, 1966). Major phenolics in potato peel are chlorogenic acid, gallic acid, protocatechuic acid, caffeic acid and quercetin (Al-Saikhan, Howard, & Miller, 1995; Al-Weshahy & Rao, 2009; Albishi, John, Al-Khalifa, & Shahidi, 2013b; Mohdaly, Hassanien, Mahmoud, Sarhan, & Smetanska, 2013; Nara, Miyoshi, Honma, & Koga, 2006; Rodriguez de Sotillo, Hadley, & Holm, 1994a, 1994b). Other phenolic compounds in potato include ferulic acid, *p*-coumaric acid as well as small amounts of rutin, quercetin, myricetin, kaempferol, naringenin and other flavonoids (Nara et al., 2006; Reyes, 2005). Purple-fleshed potato also contained petunidin- and malvidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acid whilst red-fleshed potato had pelargonidin- and peonidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acid (Reyes, 2005; Rumbaoa, Cornago, & Geronimo, 2009a). A higher anthocyanidin content and more hydroxylated anthocyanidins (malvidin) can contribute to a higher antioxidant activity of purple-fleshed potatoes (Lachman, Hamouz, Orsak, Pivec, & Dvorak, 2008). Three caffeoylquinic acid isomers, caffeic acid, 3,5-dicaffeoylquinic acid, and N-[2-hydroxy-2-(4-hydroxyphenyl) ethyl] ferulamide were identified as the main phenolic compounds in the peel and flesh of potato (López-Cobo, Gómez-Caravaca, Cerretani, Segura-Carretero, & Fernández-Gutiérrez, 2014). Polyphenolic compounds in potatoes show antioxidative activity in several food systems. Potato peel extract (with petroleum ether), exhibited strong antioxidant activity in soy bean oil during storage which was almost equal to the antioxidant activity of BHA and BHT.

However, the level of potato peel extract needed was 8–12 times higher than that of BHA and BHT to control the development of rancidity during storage of cooking oils at elevated temperatures (Rehman, Habib, & Shah, 2004). In related studies, Onyeneho and Hettiaachchy (1993) evaluated the abilities of freeze-dried extracts from six potato peel varieties to prevent soybean oil oxidation and confirmed their strong antioxidant activities.

8.2.2.6. Sweet potato. Oki et al. (2002) identified chlorogenic, isochlorogenic and cinnamic acids, and cyanidin and peonidin aglycones, as the phenolic compounds present in sweet potatoes. Philpott, Gould, Markham, Lewthwaite, and Ferguson (2003) found that hydroxycinnamic acid was the major antioxidant component of sweet potatoes. Zhan (1996) found that sweet potato extract had a high antioxidative activity resulting from the presence of phenolic acids, including chlorogenic, isochlorogenic, 4-*O*-caffeoylquinic and neochlorogenic acids. Anthocyanins from sweet potatoes were shown to possess potent antioxidative activity *in vitro* (Oki et al., 2002; Philpott, et al., 2003; Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002). Another study found that phenols, flavonoids, and anthocyanins of sweet potato flours were positively correlated with the DPPH radical scavenging effects (Huang, Chang, & Shao, 2006). Kano, Takayanagi, Harada, Makino, and Ishikawa (2005) reported that anthocyanin from purple sweet potato had a better radical scavenging activity than that of red cabbage, grape skin, elderberry and purple corn. Furuta, Suda, Nishiba, and Yamakawa (1998) reported that the purple-fleshed sweet potato cultivars had a higher radical-scavenging activity than those with white, yellow or orange flesh. The antioxidant activity of the sweet potato extract was due to a synergistic effect of phenolic components with amino acids (Hayase & Kato, 1984; Shih, Kuo, & Chiang, 2009). Sweet potato extracts showed a better radical scavenging activity, reducing power and oxidation inhibition than α-tocopherol and iron-chelating capacity than EDTA (ethylenediaminetetraacetic acid), which makes sweet potato a viable alternative source for antioxidants (Rumbaoa, Cornago, & Geronimo, 2009b). The leaves of sweet potato serve as the rich source of phenolics. Polyphenolic acids in sweet potato leaves namely 3,5-di-*O*-caffeoylquinic acid > 4,5-di-*O*-caffeoylquinic acid > chlorogenic acid (3-*O*-caffeoylquinic acid) > 3,4-di-*O*-caffeoylquinic acid > 3,4,5-tri-*O*-caffeoylquinic acid > caffeic acid. Of these 3,4,5-tri-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid are the predominant phenolics in sweet potato (Islam et al., 2002). In addition, sweet potato leaves also have been shown to possess strong antioxidant activity (Hue, Boyce, & Somasundram, 2012).

8.2.2.7. Beetroot. Ninety-two different phenol-containing plant extracts were recently screened and high antioxidant activity and high total phenolic content were found in berries, especially aronia and crowberry. Beetroot peel was shown to have the second-highest dry weight concentration of total phenols (Kähkönen et al., 1999; Vinson, Yong, Su, & Ligia, 1998). Kujala et al. (2001) isolated a highly unstable phenolic compound from red beetroot peels (*Beta vulgaris*), and proposed its structure to be 5,5′,6,6′-tetrahydroxy-3,3′-biindolyl, a dimer of 5,6-dihydroxyindole. Extra interest in 5,6-dihydroxyindoles has arisen from the recent recognition of their exceptional radical scavenging and photoprotective abilities, which makes them amongst the most effective endogenous antioxidants (Mee, Lee, Baldwin, & Cowley, 2004). Ravichandran et al. (2013) showed that different processing conditions affect betalains as well as antioxidant activity of beet. They found that betalains content and antioxidant activity increased during processing with vacuum and with certain microwave treatments. Most varieties of red beetroot contain betanin (5-*O*-*β*-d-glucopyranosylbetanidin) as the predominant colouring, and this represents 75 to 90% of the total colourant present (Kujala, Loponen, Klika, & Pihlaja, 2000). Other betacyanins found in different parts and cell cultures of red beetroot are betanidin, isobetanidin, isobetanin (5-*O*-*β*-d-glucopyranosylisobetanidin), prebetanin (betanin 6′-*O*-sulphate), neobetanin (5-*O*-*β*-d-glucopyranosylneobetanidin), amaranthin, lampranthin I (4-coumaroylbetanin), and lampranthin II (feruloylbetanin) (Alard, Wray, Grotjahn, Reznik, & Strack, 1985; Bokern et al., 1991). The main known ferulic acid ester *β*-d-fructofuranosyl-*α*-d-(6-*O*-[*E*]-feruloylglucopyranoside) was determined in the peels of beetroot (Kujala et al., 2000). In addition, *p*-hydroxybenzoic, *cis*- and *trans*-ferulic, *trans*-coumaric, and vanillic acids, as well as *p*-hydroxybenzaldehyde and vanillin have been detected in beetroot. Of these, ferulic acids are found to be the predominant phenolic acids in red beetroot (Ng, Harvey, Parker, Smith, & Walderon, 1998; Shahidi & Naczk, 2004). Sakihama, Maeda, Hashimoto, Tahara, and Hashidoko (2012) have shown that beetroot betalain could inhibit peroxynitrite-mediated tyrosine nitration and DNA strand cleavage. Beetroot juice induced the level of glutathione S-transferase pi, enzyme involved in active metabolites of 7,12-dimethylbenz[a]anthracene (DMBA) detoxification in the mammary gland (Szaefer, Krajka-Kuźniak, Ignatowicz, Adamska, & Baer-Dubowska, 2014). Recently, beetroot pomace also has been identified as a potential source of nutraceutical due to its antioxidant, antiradical and hepatoprotective activity (Vulić et al., 2012, 2014).

8.2.2.8. Asparagus. The antioxidant activity of asparagus was the highest amongst 23 vegetables analysed according to their method of inhibition of low-density lipoprotein oxidation and one of the greatest amongst 43 vegetables tested by the β-carotene bleaching method (Tsushida, Suzuki, & Kurogi, 1994; Vinson et al., 1998). Antioxidants in asparagus can protect human beings against diseases, such as cancer and cardio- and cerebrovascular diseases. The major antioxidant of asparagus is rutin (Sun, Tang, & Powers, 2005; Tsushida et al., 1994). It has been established that flavonoids are the most abundant phenolics in green asparagus (Guillen et al., 2008). Fuentes-Alventosa et al. (2008) confirmed that *triguero* asparagus flavonoids are derivatives of three different aglycones, quercetin being the major flavonol, followed by isorhamnetin and kaempferol. In addition, rutin (quercetin-3-*O*-rutinoside), nicotiflorin (kaempferol-3-*O*-rutinoside), narcissin (isorhamnetin-3-*O*-rutinoside), and isorhamnetin-3-*O*-glucoside were identified in asparagus. Rodríguez et al. (2005) suggested that asparagus byproducts should be considered as an excellent source of natural antioxidants. A number of studies have shown antioxidant, anti-inflammatory, anti-proliferative and anti-urease activity of asparagus (Maro et al., 2013; Samad et al., 2014; Shah, Khan, Sattar, Ahmad, & Mirza, 2014).

8.2.2.9. Celery and endive. Studies on celery have identified 7-*O*-apiosylglucosides and 7-*O*-glucosides of luteolin, apigenin, and chrysoeriol as well as chlorogenic acid, cinnamic acids, coumarins, and their glycosides in tested products (Galensa & Hermann, 1979; Garg, Gupt, & Sharma, 1980). Lin, Lu, and Harnly (2007) reported the presence of 10 naturally occurring glycosylated flavone malonates in celery, Chinese celery, and celery seeds. Three kaempferol conjugates [kaempferol 3-*O*-glucoside, kaempferol 3-*O*-glucuronide, and kaempferol 3-*O*-[(6-*O*-malonyl)glucoside]] were identified in endive varieties and the presence and identity of kaempferol 3-*O*-(6-*O*-malonyl)glucoside in endive was shown by DuPont, Mondin, Williamson, and Price (2000) for the first time. Of these, kaempferol 3-*O*-glucuronide comprised over 70% of the total flavonoids present (DuPont et al., 2000). Small quantities of hydroxycinnamoyl derivatives such as dicaffeoyl esters have also been detected in endive extracts (Shahidi & Naczk, 2004).

8.2.2.10. Lettuce. Lettuce is a good source of flavonoids. Five quercetin conjugates [quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-glucuronide, quercetin 3-*O*-(6-*O*-malonyl)glucoside, and quercetin 3-*O*-rhamnoside] and luteolin 7-*O*-glucuronide were detected in green-leaf lettuce. In addition, two cyanidin conjugates [cyanidin 3-*O*-glucoside and cyanidin 3-*O*-[(6-*O*-malonyl)glucoside]] were also identified in red-leaf lettuce varieties (DuPont et al., 2000; Pérez-López, Pinzino, Quartacci, Ranieri, & Sgherri, 2014). Heimler, Isolani, Vignolini, Tombelli, and Romani (2007) identified quercetin, kaempferol, luteolin, apigenin and chrysoeriol in lettuce cultivars. Recently, quercetin and luteolin rhamnosyl-hexosides were found in lettuce (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008). Red lettuce varieties contained higher levels of flavonoids than did green lettuce varieties (Crozier, Lean, McDonald, & Black, 1997; DuPont et al., 2000; Naczk & Shahidi, 2006). Red, iceberg and romaine lettuce contain caffeoyltartaric, chlorogenic, dicaffeoyltartaric and 3′,5′-dicaffeoylquinic acids (Cantos, Espin, & Tomas-Barberan, 2001; Ferreres, Gil, Castaner, & Tomas-Barberan, 1997; Llorach et al., 2008; Winter & Herrmann, 1986). Wounding induced the accumulation of phenolic compounds in iceberg and romaine lettuce leaf tissues (Kang & Saltveit, 2002). Altunkaya, Becker, Gökmen, and Skibsted (2009) showed that the degree of lipid oxidation in the liposomes in the presence of α-tocopherol, quercetin and ascorbic acid with lettuce extract was significantly lower when oxidation was initiated by free radicals formed in the water phase compared to initiation in the lipid phase. Antioxidants localized at or near the interface of the liposomes such as quercetin and α-tocopherol acted synergistically with lettuce extract as an antioxidant, whilst the hydrophilic antioxidant ascorbic acid showed no synergism.

8.2.2.11. Spinach. The flavonoids jaceidin 4′-glucuronide; 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone 4′-glucuronide and 5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone 4′-glucuronide were identified from *Spinucicr oleraceu* L. leaves (Aritomi & Kawasaki, 1984). Ferreres, Castaner, and Tomas-Barbern (1997) also isolated and identified five novel flavonoids from alcoholic extracts of spinach leaves. The new compounds (Fig. 18) were identified as spinacetin 3-*O*-*β*-D-glucopyranosyl(1→6)-[*β*-D-apiofuranosyl(1→2)]-*β*-D glucopyranoside, patuletin 3-*O*-*β*-D-(2′feruloylglucopyranosyl)(1→6)-[*β*-D-apiofuranosyl(1→2)]-*β*-D-glucopyranoside, spinacetin 3-*O*-*β*-D-(2′-p-coumaroylglucopyranosyl)(1→6)-[*β*-D-apiofuranosyl(1→2)]-*β*-D-glucopyranoside, spinacetin 3-*O*-*β*-D-(2′feruloylglucopyranosyl)(1→6)-[*β*-D-apiofuranosyl(1→2)]-*β*-D-glucopyranoside and spinacetin 3-*O*-*β*-D-(2′feruloyl glucopyranosyl)(1→6)-*β*-D-glucopyranoside. In addition, four compounds were identified as glucuronic acid derivatives of flavonoids, three components as *trans* and *cis* isomers of *p*-coumaric acid, and other components as *meso*-tartarate derivatives of *p*-coumaric acid (Bergman, Varshavsky, Gottlieb, & Grossman, 2001). The water soluble antioxidants isolated from spinach and its isolated component, glucorinated flavonoid (GF), exhibited strong antioxidant activities (Bergman, Perelman, Dubinsky, & Grossman, 2003). The occurrence of two major dietary flavonols, namely quercetin and myricetin, was also reported in spinach leaves (Chu et al., 2000). Recently, the presence of three flavonols (quercetin, kaempferol, myricetin) and two flavones (apigenin, luteolin) was reported in the extract of fresh spinach leaves (Dehkharghanian, Adenier, & Vijayalakshmi, 2010). Highest kaempferol levels in spinach amongst the 9 vegetables analysed in the present study are in good agreement with the proclamation that leafy vegetables are a good source of kaempferol (Sultana & Anwar, 2008). Lee, Lee, Lee, Park, and Choe (2002) reported that spinach powder can reduce lipid oxidation in fried products during storage in the dark. Bergman et al. (2001) showed that the antioxidant potential of the purified aromatic polyphenol fractions of spinach was similar to that of EGCG and vitamin E and even superior to that of BHT. Recently, the antioxidants from spinach leaves were combined with selected commercial polyphenolic antioxidants, such as ferulic acid, caffeic acid, and epigallocatechin-3-gallate (EGCG), and found that synergistic activity reduced reactive oxygen species generation. These findings demonstrated the importance of using antioxidant 'cocktails' which may enhance the beneficial effects in alleviating many disease conditions, including cardiovascular disease and cancer (Hait-Darshan, Grossman, Bergman, Deutsch, & Zurgil, 2009).

8.2.2.12. Tomato. Tomato is a reservoir of diverse antioxidant molecules, such as ascorbic acid, carotenoids, flavonoids and phenolic acids (George, Kaur, Khurdiya, & Kapoor, 2004). The presence of flavonoids in tomato is also important in conferring antioxidative health benefits (Takeoka et al., 2001; Vinson et al., 1998). In tomatoes, vitamin E is present mainly within the seeds, and *α*-tocopherol contribution to the antioxidant properties of tomato is minor (Raffo, Malfa, Fogliano, Maiani, & Quaglia, 2006). Quercetin conjugates are the predominant form of flavonols found in tomatoes, but lower amounts of kaempferol conjugates and traces of free aglycons have also been detected (Crozier et al., 1997). The main quercetin conjugate was identified as rutin (quercetin 3-rhamnosylglucoside) (Stewart et al., 2000). Immature green tomato fruits contain a high level of chlorogenic acid in the pericarp and pulp. The level of chlorogenic acid rapidly declines as the colour of the fruit changes from green to pink and then to red (Buta & Spaulding, 1997; Fleuriet & Macheix, 1981). George et al. (2004) suggested that cherry tomatoes, particularly variety 818 cherry, have the highest content of antioxidants (lycopene, ascorbic acid and phenols) and a higher antioxidant activity than larger size tomato cultivars. Along with a diet rich in other plant produce,

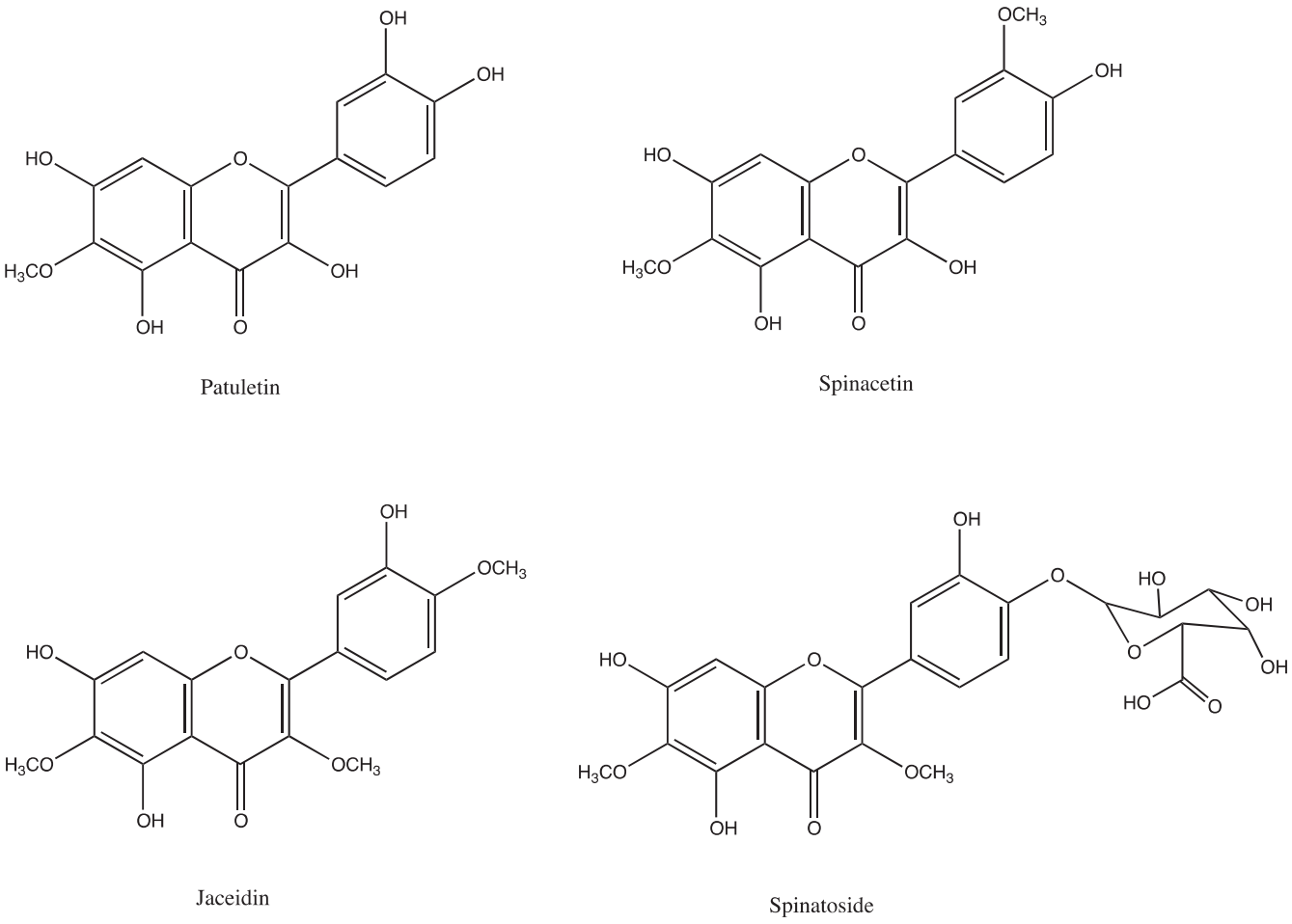


Fig. 18 – Chemical structures of some phenolic compounds found in spinach.

tomatoes, and their skins and seeds, could play an important role in improving antioxidant intake in the human diet (Toor & Savage, 2005). Recently, Valdez-Morales, Espinosa-Alonso, Espinoza-Torres, Delgado-Vargas and Medina-Godoy (2014) found that the peel and seeds of different tomato types (grape, cherry, bola and saladette type) possess antioxidant and antimutagenic activities.

8.3. *Oilseeds and plant oils*

Oilseeds constitute a major source of plant antioxidants. Amongst oilseeds of particular interest in recent years are soybean, canola, flax, borage and evening primrose because of the perceived beneficial health effects related to their oil component or their potential by-products. Canada is a major producer and exporter of several oilseeds, including canola and flaxseed. Oilseeds contain a variety of phenolic compounds with varying antioxidant activities (Shahidi, Wanasundara, & Amarowicz, 1995). The type of antioxidant present in each seed is different (Shahidi, 2000b). Total phenolic acids in some oilseeds are shown in Table 11.

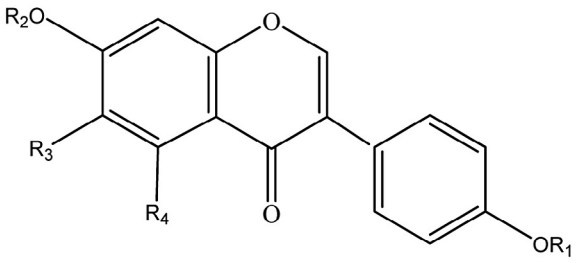
8.3.1. *Soybean*

In soybean oil, the active antioxidant is tocopherol (mainly α-tocopherol) and, to a lesser extent, γ-tocopherol (Schuler, 1990). Soybean flour and other soybean derivatives are good sources of a large variety of antioxidant compounds belonging to the family of isoflavone glycosides and their derivatives (Fig. 19), as well as phospholipids, tocopherols, amino acids and peptides (Shahidi & Naczk, 2004). It has been reported that 99% of the isoflavones are present as glycosides, of which 64% are genistein, 23% daidzein and 13% glycitein 7-*O*-β-glycoside (Naim, Gestetner, Zilkah, Birk, & Bondi, 1973). Phenolic acids, namely syringic, vanillic, caffeic, ferulic, *p*-coumaric and *p*-hydroxybenzoic acids, possessing antioxidative activity, have also been found in soybeans (Arai, Suzuki, Fujimaki, & Sakrai, 1966; Hammerschmidt & Pratt, 1978; Pratt & Birac, 1979). Amongst polyphenolic antioxidants extracted with methanol or water from dried and fresh soybean, chlorogenic, isochlorogenic, caffeic and ferulic acids were identified, in addition to isoflavones (Pratt & Birac, 1979). Juan and Chou (2009) reported that fermentation enhanced the total phenolic and flavonoid contents as well as the antioxidant activity of black soybean extract due to the liberation of isoflavonoids from their corresponding glycosides by acids formed during the fermentation (Shahidi & Naczk, 2004). The antioxidant activity of soy isoflavones in a β-carotene/linoleate model system in the increasing order was glycetin, diadzein, genistein, quercetin and 6,7,4′-trihydroxyisoflavones, respectively. Meanwhile, for the phenolic acids, the increasing order of antioxidant activity was *p*-coumaric, ferulic, chlorogenic and

Table 11 – Total phenolic acids in some oilseed flours.[a]

Phenolic acid	Soybean	Flax	Sesame	Cottonseed[b]	Sunflower	Peanut
p-Hydroxybenzoic	13.9	2.6	Trace	1.1	7.4	2.0
Vanillic	–	trace	–	–	0.8	–
Syringic	28.9	0.4	–	–	3.6	–
trans-*p*-Coumaric	9.4	6.1	7.2	4.3	5.6	146.4
trans-Ferulic	15.7	37.6	5.7	4.5	7.2	16.2
trans-Caffeic	6.0	5.3	9.8	0.5	979.1	2.8
trans-Sinapic	–	29.1		–	–	8.1
Total	73.6	81.1	22.7	10.4	1003.7	175.5

Source: Adapted from Shahidi and Naczk (2004).
[a] Milligram per 100 g of flour.
[b] Glanded.



Compound	R_1	R_2	R_3	R_4
Daidzein	H	H	H	H
Formononetin	CH_3	H	H	H
Genestein	H	H	H	OH
Prunetin	H	CH_3	H	OH
4',6',7'-trihydroxyisoflavone	H	H	OH	H
Glycitein (7-*O*-glucoside)	H	Gluc	OCH_3	H

Fig. 19 – Chemical structures of isoflavone and isoflavone glycosides of soybean.

caffeic acids, respectively (Pratt & Birac, 1979; Shahidi & Naczk, 2004).

8.3.2. *Rapeseed and canola*

Canola seeds, canola meal and their by-products are rich in phenolics, including caffeic, cinnamic, *p*-coumaric, ferulic, gentisic, *p*-hydroxybenzoic, salicylic, sinapic and syringic acids (Shahidi & Naczk, 2004) with high radical-scavenging activities. Total phenolic content in rapeseed flour is much higher than other oleaginous seeds, exceeding about 28 times the level of phenolics in soybean flour (Table 11) (Shahidi & Naczk, 1992, 2004). Phenolic acids and their derivatives, as well as soluble and insoluble tannins, are the predominant phenolic compounds found in canola and rapeseed (Naczk, Amarowicz, Pink, & Shahidi, 2000; Naczk, Amarowicz, Sullivan, & Shahidi, 1998; Naczk, Nichols, Pink, & Sosulski, 1994; Wanasundara & Shahidi, 1994). According to Nowak, Kujawa, Zadernowski, Roczniak, and Kozlowska (1992), amongst oilseeds, rapeseed contains the highest amount of phenolic compounds. The most significant phenolic compounds in rapeseed are sinapic acid derivatives such as sinapine, the choline ester of sinapic acid (constitutes 80% of the total phenolic compounds). Sinapic acid in rapeseed can also exist as a glucosidic ester, glucopyranosyl sinapate (Amarowicz & Shahidi, 1994). Shahidi and Naczk (2004) reported the presence of two flavonoid glucosides in rapeseed meal, namely, 3-(*O*-sinapoyl sophoroside)-7-*O*-glucoside of kaempferol and 3-(*O*-sinapoyl glucoside)-7-*O*-sophoroside of kaempferol. Canolol (4-vinylsyringol) is one of the main phenolic compounds present in processed canola/rapeseed oil from the precursor sinapic acid (Huidrom & Thiyam-Holländer, 2012). Wanasundara and Shahidi (1994) reported that the antioxidant activity of ethanolic extracts of canola meal in canola oil was equivalent to that of TBHQ and stronger than that of BHA, BHT, and BHA/BHT/monoacylglycerol citrate (MGC). The most active component of these extracts was 1-*O*-*β*-D-glucopyranosyl sinapate (Wanasundara, Amarowicz, & Shahidi, 1994). Shahidi, Wanasundara, Amarowicz, and Naczk (1995) demonstrated that addition of canola flour to meat resulted in 73–97% inhibition of lipid oxidation, as determined by the 2-thiobarbituric acid (TBA) test. Use of canola hulls as a source of natural antioxidants was explored by Naczk, Pegg, Zadernowski, and Shahidi (2005). According to Wanasundara et al. (1996), the antioxidant activity of different rapeseed phenolic fractions was lower than the activity of crude ethanolic extract due to synergism between different phenolics in a β-carotene–linoleate model system. The most active rapeseed meal phenolic fraction contained several classes of phenolic compounds including phenolic acids, flavones, and flavonols. Canola and rapeseed hulls have been reported to contain up to 6% tannins (Naczk et al., 2000). Therefore, use of hulls, after dehulling, as a source of natural antioxidants may provide a means for their utilization (Amarowicz, Naczk, & Shahidi, 2000).

8.3.3. *Flaxseed*

Flaxseed is a rich source of the lignan secoisolariciresinol diglucoside (Eliasson, Kamal-Eldin, Andersson, & Aman, 2003). Other phenolic compounds have also been found in flaxseed, e.g. the hydroxycinnamic acid derivatives, *p*-coumaric acid-4-*O*-glucoside and ferulic acid-4-*O*-glucoside (Johnsson et al., 2002), and the flavonoid herbacetin diglucoside (Struijs et al., 2007). The ratio of the tocopherol homologues α-, γ-, and δ- in flaxseed hull at 17:61:22 was different from that in the seed (Oomah, Kenaschuk, & Mazza, 1997). Compared to other oilseeds, flaxseed contains low amount of phenolic acids (Amarowicz,

Karamac, Wanasundara, & Shahidi, 1997). Besides lignans, flaxseed was reported to contain a number of phenylpropanoids, namely *p*-coumaric, *o*-coumaric, ferulic, *p*-hydroxybenzoic, gentisic, vanillic, and sinapic acids in the free and/or bound forms (Babrowski & Sosulski, 1984; Kozlowska, Zadernowski, & Sosulski, 1983). *Trans*-ferulic and *trans*-sinapic acids are the major phenolic acids and *trans*-caffeic, *p*-coumaric and *p*-hydroxybenzoic are the minor phenolic acids found in dehulled, defatted meal (Dabrowski & Sosulski, 1984). However, Harris and Haggerty (1993) reported that the content of ferulic and chlorogenic acids accounted for 84% of total phenolic acids in methanolic extracts of defatted meal (Shahidi & Naczk, 2004).

8.3.4. *Sesame seeds*

The oil from the sesame seed has a superior oxidative stability when compared with other vegetable oils largely due to the presence of sesamol in sesame seeds (Shahidi & Wanasundara, 1992). The compounds present in sesame cake extract included sesamol, sesamin, sesamolin, sesaminol diglucoside, and sesaminol triglucoside (Suja, Jayalekshmy, & Arumughan, 2004). Both sesamin and sesamolin have the same 2,7-dioxabicyclo-(3,3,0)-octane backbone with two 3,4-methylene dioxyphenyl substituents. These components along with γ-tocopherol confer superior oxidative stability to sesame oil as compared to other sources of vegetable oils (Shahidi, Amarowicz, Abou-Gharbia, & Shehata, 1997). Chemical structures of some phenolic compounds found in sesame seeds are shown in Fig. 20. Abou-Gharbia, Shehata, and Shahidi (2000) reported that oxidative stability of sesame oil may be due to the presence of unique unsaponifiable constituents namely lignans and tocopherols. These compounds possess strong antioxidant activity and may have the potential of inhibiting the process of ageing in man and in biological systems. Chang, Yen, Huang, and Duh (2002) have shown that sesame hulls possess considerable antioxidant activity, partly due to their high level of phenolic compounds. Shahidi, Liyana-Pathirana, and Wall (2006) suggested that defatted sesame extracts and their hulls possess good antioxidant activity. This activity was higher for black sesame than white sesame and concentrated mainly in the hull fraction. In the linoleic acid system, the brown pigment of black sesame seed showed an equal antioxidant activity to BHA and higher than trolox and α-tocopherol indicated that the brown pigment of sesame seed possessed excellent antioxidant activity (Xu, Chen, & Hu, 2005).

8.3.5. *Cottonseed*

Gossypol is the major phenolic component present in cottonseed kernel in the free and bound forms, a polyphenolic binaphthyl dialdehyde, which is toxic to man and monogastric animals and also act as a male contraceptive (Shahidi & Naczk, 2004). Zhang, Yang, Zhao, Luan, and Ke (2001) identified that glandless cottonseed contains five, kaempferol 3-0-β-D-apiosyl(1→2)-[α-L-rhamnosyl (1→6)]-β-D-glucoside, quercetin-3-0-β-D-apiosyl (1→2)-[α-L-rhamnosyl (1→6)]-β-D-glucoside, quercetin-3-0-β-D-apiosyl (1→2)-α-D-glucoside, rutin and quercetin-3-0-β-D-glucoside. However, Piccinelli et al. (2007) identified seven flavonol glycosides, namely quercetin 3-*O*-{β-d-apiofuranosyl-(l → 2)-[α-l-rhamnopyranosyl-(l → 6)]-β-d-glucopyranoside}, kaempferol 3-*O*-{β-d-apiofuranosyl- (1 → 2)-

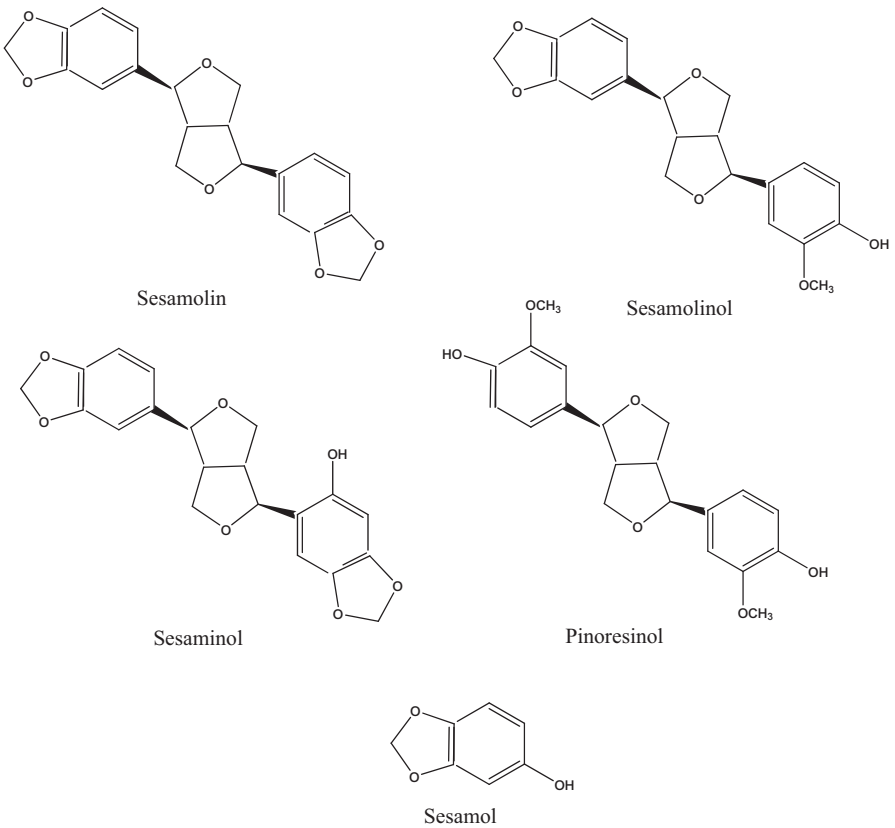


Fig. 20 – Chemical structures of some phenolic compounds found in sesame seeds.

[α-l-rhamnopyranosyl-(1 → 6)]-β- d-glucopyranoside}, quercetin 3-*O*-[β-d-apiofuranosyl- (1 → 2)-β-d-glucopyranoside], quercetin 3-*O*-β-d-glucopyranoside, kaempferol 3-*O*-[α-l-rhamnopyranosyl-(1 → 6)-β-d- glucopyranoside], quercetin 3-*O*-[α-l-rhamnopyranosyl- (l → 6)-β-d-glucopyranoside], kaempferol 3-*O*-α-l-rhamnopyranoside. In addition, gallic acid and 3,4-dihydroxybenzoic acid were identified in whole cottonseed by-products. According to Rhee, Ziprin, and Calhoun (2001), cottonseed meal may be a potential source of natural antioxidants for cooked meat products as well as other oxidation-susceptible food products. However, they suggested that further research is needed to clearly establish the relationship amongst processing technology, gossypol profile and antioxidant activity of cottonseed meal in food products with different types/amounts of prooxidative constituents.

8.3.6. *Evening primrose*

Evening primrose has earned an important place in the pharmaceutical industry due to the presence of a high amount (9–10%, w/w) of γ-linolenic acid in its seed oil (Gibson, Lines, & Neumann, 1992; Shahidi, Amarowicz, He, & Wettasinghe, 1997; Wettasinghe & Shahidi, 1999b). Evening primrose meal contain three major low-molecular-weight phenolic compounds, namely (+)-catechin, (–)-epicatechin, and gallic acid (Wettasinghe, Shahidi, & Amarowicz, 2002). Shahidi (2000b) reported that the stronger antioxidant activity of evening primrose meal may be attributed to its tannin components. More recent studies have suggested that the antioxidant properties of evening primrose may arise from phenolic acids such as gallic, caffeic, *p*-hydroxybenzoic, vanillic, ferulic and salicylic acids, as well as proanthocyanidins and flavanols (Zadernowski, Nowak-Polakowska, & Konopka, 1996), catechin and epicatechin derivatives (Wettasinghe & Shahidi, 2002) or protocatechuic and gallic acids and esters (Peschel, Dieckmann, Sonnenschein, & Plescher, 2007; Schmidt, Niklová, Pokorný, Farkaš, & Sekretár, 2003). Salicylic, *p*-hydroxybenzoic, 2-hydroxy-4-methoxybenzoic, vanillic, *m*- and *p*-coumaric, gallic, ferulic and caffeic acids are found in smaller quantities in evening primrose seeds (Zadernowski, Naczk, & Nowak-Polakowska, 2002).

8.3.7. *Borage*

Borage seeds and leaves have been employed as a source of phenolics/polyphenolics, showing good antioxidant activity and retarding lipid oxidation in various food model systems (Wettasinghe & Shahidi, 1999a, 2000, 2002). Rosmarinic acid, syringic acid and sinapic acid are the major phenolic compounds present in the ethanolic extract of borage meal (Wettasinghe, Shahidi, Amarowicz, & Abou-Zaid, 2001). Although borage oil is rich in polyunsaturated fatty acids, it is highly resistant to oxidation in intact seed tissues due to the presence of tocopherols and several other phenolic compounds in oil-bearing tissues (Wettasinghe & Shahidi, 1999a). Wettasinghe and Shahidi (1999a) suggested that borage extract may be added to bulk oils and meat products in place of synthetic antioxidants in order to retard lipid oxidation. However, the low content of hydrophobic phenolics in the extract might make it less antioxidative in oil-in-water emulsion systems (Wettasinghe & Shahidi, 2000, 2002). Recently, Gómez-Estaca, Giménez, Montero, and Gómez-Guillén (2009) reported that the incorporation of a borage antioxidant extract improved the antioxidant power of the commercial fish gelatin films with only minor modifications of the physico-chemical properties (lower breaking force). The use of polyphenolic extracts from natural sources as the borage extract seems to be a more promising way to improve the antioxidant power of edible films than the use of BHT or α-tocopherol (Gómez-Estaca et al., 2009).

8.3.8. *Olive and olive oil*

Olive oil contains a high amount of natural antioxidants such as tocopherols, carotenoids, sterols and phenolic compounds (Minioti & Georgiou, 2008). Secoiridoids, oleuropein, demethyloleuropein, ligstroside and verbascoside (caffeoylrhamnosylglucoside of hydroxytyrosol) (Fig. 21) are the main phenolic compounds of olive fruits (Amiot, Fleuriet, & Macheix, 1986; Angerosa, d'Alessandro, Konstantinou, & Di Giacinto, 1995, 1996; Borzillo, Iannota, & Uccella, 2000; Czerwińska, Kiss, & Naruszewicz, 2012; Ucella, 2001). Oleuropein is the major phenolic compound in olive fruit, which can be

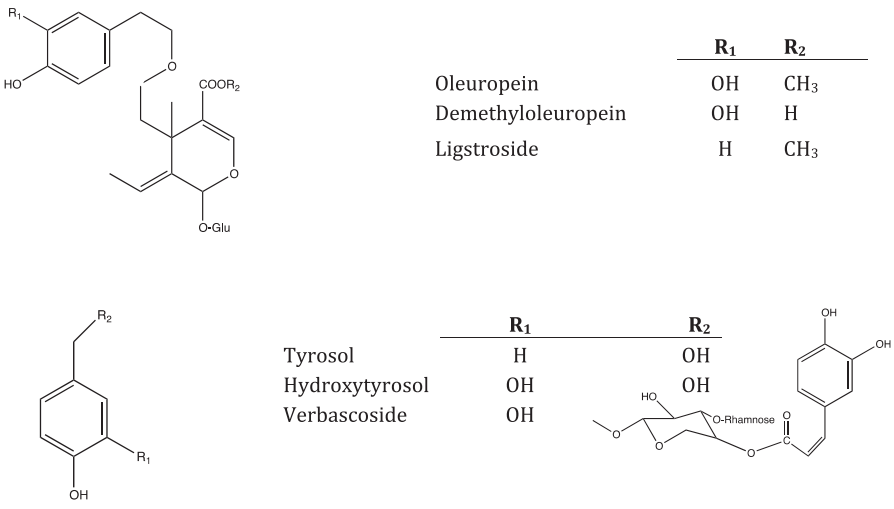


Fig. 21 – Chemical structures of some phenolics found in olive oil.

as much as 14% in dried fruit, whilst hydroxytyrosol is the major phenolic component in olive oil (Amiot et al., 1986). According to Cabrini et al., (2001), amongst olive phenolics, tyrosol is present at the highest concentration; however, this compound appears to contribute very little, if any, to the stability of olive oil. Other phenolic compounds include hydroxytyrosol, 3,4-dihydroxyphenylacetic, 4-hydroxyphenylacetic, and 4-hydroxybenzoic acids as well as protocatechuic, vanillic, syringic, and *p*-coumaric acids (Cabrini et al., 2001). However, Owen et al. (2000) reported that hydroxytyrosol is one of the main components of virgin olive oil and olive mill waste with a strong antioxidant potential (González-Santiago, Fonollá, & Lopez-Huertas, 2010). Amongst the phenolic compounds found in extra-virgin olive oils, *o*-dihydroxy-phenolics are very potent antioxidants (Boskou, Blekas, & Tsimidou, 2005). A new class of phenolic compounds, hydroxy-isochromans, l-phenyl-6,7-dihydroxy-isochroman and 1-(3′-methoxy-4′-hydroxy) phenyl-6,7-dihydroxy-isochroman were found in different samples of extra-virgin olive oil (Bianco, Coccioli, Guiso, & Marra, 2002). Rutin and luteolin 7-glucoside are the two main flavonoids in olive fruits. In addition, cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside are the most abundant anthocyanins in olive fruits (Romani, Mulinacci, Pinelli, Vincieri, & Cimato, 1999). Moreover, Brenes et al. (2000) have identified two new phenolic compounds in olive oil, namely pinoresinol and 1-acetoxypinoresinol. Cioffi et al. (2010) have reported that phenolic compounds in virgin olive oil are a complex mixture of components that include α- and γ-tocopherols, hydroxytyrosol, tyrosol, phenolic acids (caffeic acid, vanillic acid, syringic acid), lignans (pinoresinol, 1-acetoxypinoresinol) (Montedoro, Baldioli, & Miniati, 1992), and secoiridoids (oleuropein aglycone, oleuropein, demethyloleuropein, ligstroside) (Lavelli & Bondesan, 2005). It has also been reported that one of the well-known phenolic compounds present in newly-pressed extra-virgin olive oil, the dialdehydic form of deacetoxy-ligstroside aglycone, called oleocanthal, is one of the main substances responsible for the bitter taste of olive oil and possesses ibuprofen-like cyclooxygenases (COX-1 and 2) inhibitory activity and responsible for its anti-inflammatory effect (Beuchamp, Keast, Morel, & Lin, 2005). Czerwińska et al. (2012) showed that oleacein is a more potent scavenger of hypochlorous acid than oleuropein as well as a stronger inhibitor of reactive oxygen species production and myeloperoxidase release in neutrophils.

8.4. *Beverages*

8.4.1. *Tea*

Phenolics in tea are responsible for its antioxidant activity. Polyphenolic compounds and caffeine are major constituents of tea. The pure catechins and phenolic acids found in tea are more powerful than the antioxidant vitamins C and E as well as β-carotene in an *in vitro* lipoprotein oxidation model (Vinson & Dabbagh, 1998). Vinson and Dabbagh (1998) indicated that both catechins and theaflavins contribute to the antioxidant characteristics of tea. Green tea is a gently processed tea where the catechin profile closely resembles that originally present in the leaves at harvest (Astill, Birch, Dacombe, Humphrey, & Martin, 2001). Black tea, unlike green tea, is a more processed product. Fresh tea leaves are fermented in a process by which oxidative enzymes naturally occurring in tea leaves including polyphenol oxidase and peroxidase, oxidize catechin monomers and generate a complex mixture of polyphenol derived products including theaflavins, theasinensins, and other poorly characterized complex oxidation polymers known as thearubigins (Ferruzzi, 2010). Thearubigins have been reported as the most abundant pigment in black tea, which comprises polyphenolic oxidation products (Yassin, Koek, & Kuhnert, 2015), Recently, Yassin et al. (2015) reported that oxidation is mainly taking place on the B-ring and the galloyl group, where the oxidized components subsequently undergo oxidative coupling for the formation of theaflavins, theasinensins and polyhydroxylated flavan-3-ols, all precursors for thearubigin formation. The total flavanol level is reduced from 50–53% in green tea to 10% in black tea (Sun, Ho, & Shahidi, 2009). The antioxidant activity of various teas have been shown to descend in the following order: green > oolong > black > pu'erh tea (Yashin, Yashin, & Nemzer, 2011). Catechins are found in particularly high concentration in teas, especially the unfermented green and white varieties (Freeman & Niemeyer, 2008). Green tea leaves contain relatively large amounts (approximately 30%) of (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin gallate (EGCG) (Fig. 22) (Amarowicz & Shahidi, 1996; He & Shahidi, 1997). Theaflavins, which include theaflavin, theaflavin-3-gallate, theaflavin-3′ gallate and theaflavin-3,3′-digallate (Fig. 23), are key to the characteristic colour and taste of black tea, and account for 2–6% of the solids in brewed black tea (Khan & Mukhtar, 2010). According to Chen,

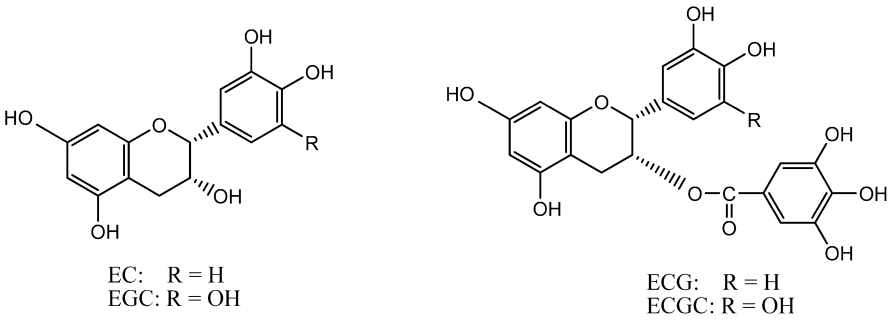
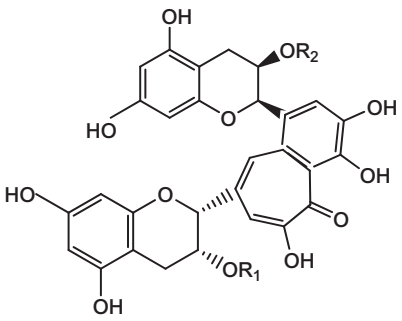


Fig. 22 – Structures of tea catechins.



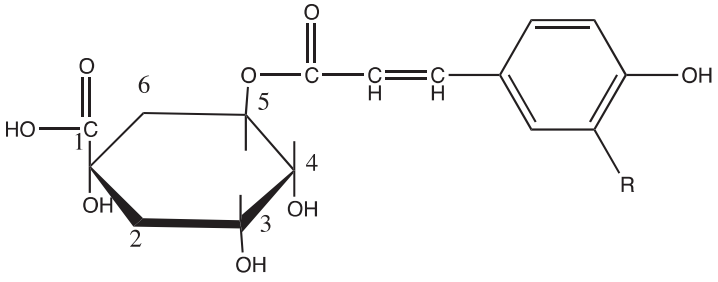
	R1	R2
Theaflavin	H	H
Theaflavin-3-gallate	Galloyl	H
Theaflavin-3'-gallate	H	Galloyl
Theaflavin-3,3'-digallate	Galloyl	Galloyl

Fig. 23 – Structures of major theaflavin in black tea (adapted from Wan et al., 2009).

Zhu, Tsang, and Huang (2001), the total content of epicatechin derivatives in Chinese black teas is between 2.4 and 5.1 g/kg; in Chinese oolong tea it ranges from 41.4 to 46.3 g/kg and in Chinese green tea between 80 and 144.4 g/kg. EGCG is the most abundant epicatechin derivative in teas. Besides catechins, flavonol, and their glycosides, anthocyanidin and leucoanthocyanidin, phenolic acids and depsides are also present in fresh tea leaves (Wan, Li, & Zhang, 2009). Hara (1994) has evaluated the antioxidative potency of crude extracts of green tea and individual catechins in lard by the active oxygen method and found that crude tea catechins reduce the formation of peroxides more effectively than α-tocopherol or BHA. Wanasundara and Shahidi (1996) found that tea catechins exhibit an antioxidative activity similar to or better than that of BHA, BHT, and TBHQ. The order of their potency, at 200 ppm, in marine oils was ECG > EGCG > EGC > EC (Shahidi, Wanasundara, He, & Shukla, 1997). Several studies have shown that green tea extract were effective in stabilizing meat and fish (Chan, Soh, Tie, & Law, 2011; He & Shahidi, 1997; Mustafa, 2013; Senanayake, 2013; Shahidi & Alexander, 1998; Shahidi & Wanasundara, 1992). Zhu et al. (2009) reported that EGCG's direct trapping of reactive carbonyl species may also contribute to the significant reduction of acrolein and other aldehydes in the peroxidation of seal blubber oil. Black and green teas were not significantly different in their phenolic contents, and in antioxidant strength as measured by IC_{50}, or in antioxidant potential as measured by the phenolic antioxidant index (PAOXI) (Vinson & Dabbagh, 1998). Vinson and Dabbagh (1998) found that the PAOXI of teas was higher than those of grape juices and wine. Tea has been shown to possess several health benefits, such as inhibition of mutagenesis, protection against metabolic syndrome, antioxidative, anticarcinogenic, anti-inflammatory, antiobesity, anti-hypertensive and hypocholesterolaemic properties (Basu et al., 2013; Ramji, Huang, Shahidi, & Ho, 2009; Suliburska et al., 2012; Yarmolinsky, Gon, & Edwards, 2015). In addition, lyophilized derivatives of green tea have also been shown to exert antioxidant and anticancer activities (Zhong et al., 2012; Zhong & Shahidi, 2011). In recent years, scientists throughout the world have investigated the potential benefits of green tea and its most abundant catechin, EGCG (Karaosmanoglu & Kilmartin, 2015). The potential for use of EGCG in human cancer prevention and treatment seems very promising because EGCG acts against cancer through a number of complementary mechanisms (Khan & Mukhtar, 2010). Recently, Kadasi et al. (2014) found that green tea extracts could affect basic ovarian cell functions such as inducing the suppression of proliferation, stimulation of apoptosis, promotion of progestagen and changes in testosterone release.

8.4.2. *Coffee*

Phenolics play an important role in the formation of coffee flavour. From over 800 volatile compounds found in roasted coffee aroma, only 42 have been identified as phenolics (Flament, 1989, 1995; Nijssen, Visscher, Maarse, Willemsens, & Boelens, 1996). Catechol is the predominant volatile phenolic compound found in coffee aroma, followed by 4-ethylguaiacol, 4-ethylcatechol, pyrogallol, quinol and 4-vinylcatechol (Smith, 1985). Green coffee beans may contain at least five major groups of chlorogenic acid isomers (Fig. 24), namely caffeoylquinic acids, dicaffeoylquinic acids, feruoylquinic acids, *p*-coumaroylquinic acids and caffeoylferuloylquinic acids (Clifford & Ramirez-Martinez, 1991). Chu, Lin, Yu, and Ye (2008) determined catechin, rutin, ferulic acid, o-dihydroxybenzene, chlorogenic acid, caffeic acid, gallic acid and protocatechuic acid in coffee using capillary electrophoresis with amperometric detection (CE-AD). Brezová,



	R
5-*p*-Coumaroylquinic acid	H
5-Caffeoylquinic acid	OH
5-Feruloylquinic acid	OCH_3

Fig. 24 – Structure of chlorogenic acid and its derivatives.

Šlebodová, and Staško (2009) reported that antioxidant capacity of instant coffee was approximately 3–4 times higher than that of ground coffee. However, considering the traditional preparation procedures of coffee brews (approximately four times higher amount of ground than instant coffee in the same water volume) the antioxidant capacities of both ground and instant brews were quite comparable. More detailed investigations of two coffee components, namely caffeic acid and caffeine, using moderate oxidants, such as $ABTS^{\bullet+}$, DPPH and TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine *N*-oxyl) radicals, indicated a high antioxidant activity of caffeic acid but no antioxidant action for caffeine (Brezová et al., 2009). However, employing hydroxyl radical, a more reactive oxidant source (generated in the photochemical decomposition of H_2O_2), both compounds showed a remarkable scavenging activity (Brezová et al., 2009). Recently, Mnatsakanyan et al. (2010) reported that the overall antioxidant profile of the "Decaffeinato" coffee sample was similar to "Gold" and "Ristretto" coffee samples which are rich in phenolic compounds. Furthermore, decaffeinated coffee showed similar positive effects on human health.

8.4.3. *Cocoa*

Cocoa bean and its products (cocoa liquor, cocoa powder, and dark chocolate) are rich in phenolic compounds (Othman, Ismail, Ghani, & Adenan, 2007). Indeed cocoa products contain greater antioxidant capacity and higher amounts of flavonoids per serving than either tea or red wine (Lee, Kim, Lee, & Lee, 2003; Steinberg, Bearden, & Keen, 2003). Cocoa beans have a high phenolic content of about 12–18% (dry weight) in unfermented beans (Kim & Keeney, 1984). Dreosti (2000) reported that 60% of the total phenolics in raw cocoa beans are flavan-3-ol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer). Theobromine and caffeine are two major ingredients in chocolate (Vinson, Proch, & Zubik, 1999). The polyphenolic composition of cocoa has been characterized and quantified (Sanchez-Rabaneda et al., 2003; Wollgast & Anklam, 2000; Zumbe, 1998). The compounds identified include epicatechin, gallocatechin and epigallocatechin and other phenolics such as procyanins, anthocyanins, and flavone and flavonol glycosides such as luteolin-7-*O*-glucoside and quercetin-3-*O*-arabinoside. Reports on the polyphenolic content of cocoa products vary greatly in the literature, with values ranging from 3.3 to 65 mg/g in cocoa powder or 1.7 to 36.5 mg/g total polyphenols in dark chocolate (Adamson et al., 1999; Vinson et al., 1999). There are mainly flavan-3-ols (monomeric epicatechin and catechin, as well as their oligomers from dimers to decamers, the procyanidins), with small amounts of anthocyanins (mainly cyanidin glycosides) and flavonols (quercetin glycosides) (Adamson et al., 1999; Hammerstone, Lazarus, Mitchell, Rucker, & Schmitz, 1999). Counet, Callemien, and Collin (2006) identified *trans*-resveratrol and *trans*-piceid in dark chocolate and cocoa liquor extracts and found that chocolate products have higher antioxidant activity than concentrated commercial stilbene extracts. Epicatechin, the major monomeric polyphenol in chocolate (Sanbongi et al., 1998) and an extract of chocolate liquor were both found to stimulate cellular immune response *in vitro* (Sanbongi, Suzuki, & Sakane, 1997). Chocolate has also been reported to be a good source of dietary catechins, second only to green tea (Arts, Hollman, & Kromhout, 1999). According to Saura-Calixto and Goñi (2006), the total polyphenolic content of cocoa was higher than that reported in other foods like cereals, legumes, vegetables, nuts and fruits. Studies have demonstrated that the consumption of cocoa or chocolate reduces the risk of cardiovascular disease (Keen, 2001; Osakabe et al., 1998). Moreover, extracts prepared from cocoa powder and cocoa beans were shown to exhibit antihyperglycaemic effects on streptozotocin-induced diabetic rats (Amin, Faizul, & Azli, 2004; Ruzaidi, Amin, Nawalyah, Hamid, & Faizul, 2005). Recently, Pimentel, Nitzke, Klipel, and de Jong (2010) found that 49 g of dark chocolate (71% cocoa) had the same quantity of flavonoids as that of 196 ml of Tannat wine, which is the daily wine intake recommended to produce health benefits in an adult of 70 kg body weight.

8.4.4. *Beer*

Phenolic compounds identified in beer include phenolic acids, flavonoids, proanthocyanidins, tannins and amino phenolic compounds, all of which have been reported to possess antioxidant and antiradical properties as well as other biological effects (Gorinstein, Caspi, Zemser, & Trakhtenberg, 2000; Montanari, Perretti, Natella, Guidi, & Fantozzi, 1999). Zhao, Chen, Lu, and Zhao (2009) identified nine phenolic compounds, including gallic acid, protocatechuic acid, (+)-catechin, vanillic acid, caffeic acid, syringic acid, (–)-epicatechin, *p*-coumaric acid and ferulic acid in different beer samples. The major free phenolic acids in beers were *m*-, *p*- and *o*-coumaric and ferulic acids (Montanari et al., 1999), whilst vanillic, ferulic and *p*-coumaric acids were the dominant free phenolic acids in Spanish, German and Danish brands (Bartolome, Pen-Neira, & Gomez-Cordoves, 2000). Sinapic, vanillic, chlorogenic, homovanillic, *p*-hydroxybenzoic, 2,6- and 3,5-dihydroxybenzoic, syringic, gallic, protocatechuic and caffeic acids are also identified in beer (Bartolome et al., 2000; Montanari et al., 1999). Moreover, anthocyanins and anthocyanidins (cyanin, cyanidin, pelargonin, pelargonidin and delphinidin), flavones and flavonols such as vitexin, isoquercetin, quercetin, rutin, kaempferol, myricetin and myricitrin have been found in beer at less than 1 mg/l (Drawert, Leupold, & Lessing, 1977). Gorinstein, Zemser, Weisz, Haruenkit, and Trakhtenberg (1998) reported a significant beneficial effect in rats supplemented for 4 weeks with both alcohol-containing and alcohol-free beer. Ghiselli et al. (2000) indicated that beer, which has a moderate antioxidant capacity coupled with a low ethanol content, is an alcoholic beverage that is able to improve plasma antioxidant capacity without the negative effects produced by high doses of ethanol. Later, Gasowski et al. (2004) found that beer has a positive effect on plasma lipid profile and plasma antioxidant capacity, and increases bile volume and bile acid concentrations mainly in rats fed cholesterol-containing diets. The degree of this positive influence of beer is directly connected to the bioactive compounds (flavonoids) in beer. Beer also contains an array of health-promoting isoflavonoids (Lapcik, Hill, Hampl, Wahala, & Adlercreutz, 1998). However, despite all the health benefits of alcoholic beverages, it should be noted that excessive alcohol consumption has clear detrimental effects, which cause mortality (Shahidi, 2009).

8.4.5. *Wine*

Wine is a particularly rich source of phenolics, and its moderate intake has been associated with decreased levels of lipid peroxidation and a lower incidence of certain types of cancer (Bianchini & Vainio, 2003; Waffo-Teguo et al., 2001). The moderate consumption of red wine has a relatively higher benefit in the prevention of atherosclerosis and coronary heart disease (CHD) (Faustino, Sobrattee, Edel, & Pierce, 2003; Gambelli & Santaroni, 2004). According to Vinson, Teufel, et al. (2001), consumption of 196 ml (~1 glass) of red wine per day is enough to significantly reduce the atherosclerosis risk in a 70 kg adult. *Trans*-resveratrol and some phenolic antioxidants (catechin, epicatechin, quercetin and rutin) were identified in red wine (Anli, Vural, Demiray, & Özkan, 2006). *Trans*- and *cis*-resveratrol have been shown to exist in wine as both the aglycone and the bound glucoside piceid (Vian, Tomao, Gallet, Coulomb, & Lacombe, 2005). The most common flavonoids in wine are flavonols (quercetin, kaempferol, and myricetin), flavan-3-ols (catechin and epicatechin), tannins, and anthocyanins (cyanin). Nonflavonoids comprise stilbenes, hydroxycinnamic acids and benzoic acids (S. Li et al., 2009). Phenolic acids and stilbenes are important non-flavonoid compounds present in grapes and wine. Phenolic acids are present in their free form or as glycosylated derivatives and esters of tartaric, quinic and shikimic acid in both red and white wines (Monagas, Bartolome, & Gomez-Cordoves, 2005). Phenolic compounds in red wine are derived from the grape's skin, as well as from grape seeds, grape stems, or grape pulp, all of which are important sources of flavanols that are transferred to the wine during fermentation. On the contrary, white wines are usually made from the free running juice, without the grape mash, having any contact with the grape skins. This is thought to be the main reason for the relatively low polyphenol content and for the lower antioxidant activity of white wine in comparison to red wine (Fuhrman, Volkova, Suraski, & Aviram, 2001; S. Li et al., 2009). Epidemiological studies have shown that moderate wine intake may be beneficial for human health (Mudnic et al., 2010). Amongst many beneficial effects, wine inhibits low density lipoprotein (LDL) oxidation (Frankel, Kanner, German, Parks, & Kinsella, 1993), increases antioxidative capacity in humans (Maxwell, Cruickshank, & Thorpe, 1994) and modulates vascular function by inducing vasodilation through increased production of nitric oxide (NO) (Flesch, Schwarz, & Bohm, 1998). The "French paradox", that is, the low incidence of cardiovascular events despite a diet high in saturated fat, was attributed to the regular drinking of red wine in southern France (Renaud & de Lorgeril, 1992). Recently, Fukui, Choi, and Zhu (2010) have shown the protective effect of resveratrol against oxidative neuronal death based on an *in vitro* model.

8.5. *Tree nuts*

Frequent consumption of nuts has been linked to a lowered risk of cardiovascular disease (Kris-Etherton et al., 1999; Shahidi & John, 2010). Phytochemicals, especially phenolics, in nuts may be considered as the major bioactive compounds for health benefits. In particular, nuts include plant protein, unsaturated fatty acids, dietary fibre, plant sterols, phytochemicals and micronutrients like tocopherols (Kris-Etherton, Zhao, Binkoski, Coval, & Etherton, 2001). Maguire, O'Sullivan, Galvin, O'Connor, and O'Brien (2004) reported that α-tocopherol was the most dominant tocopherol in almonds, peanuts, hazelnuts and macadamias. However, Kornsteiner, Wagner, and Elmadfa (2006) reported that β- and γ-tocopherols, were the most predominant tocopherols in 7 nut types and the mean amounts in descending order were pistachios > walnuts > pecans > Brazil nuts > pines ≥ peanuts > cashews. Fig. 25 shows the total phenolic and flavonoid content of tree nuts. Walnuts, pistachios, peanuts and pecans contain the highest total phenolic and flavonoid content amongst all types of nuts (Yang, Liu, & Halim, 2009). Kornsteiner et al. (2006) reported that the antioxidants were found to be lower in cashews and pines. Macadamias also contained the lowest level of antioxidative constituents (polyphenols, tocopherols). Nuts are a good source of phytochemicals, including phenolics (tannins, ellagic acid, and curcumin), flavonoids (luteolin, quercetin, myricetin, kaempferol, and resveratrol), isoflavones (genistein and daidzein), terpenes, organosulphuric compounds, and vitamin E (Bravo, 1998; Yang, Martinson, et al., 2009). Miraliakbari and Shahidi (2008) found that minor component extracts of

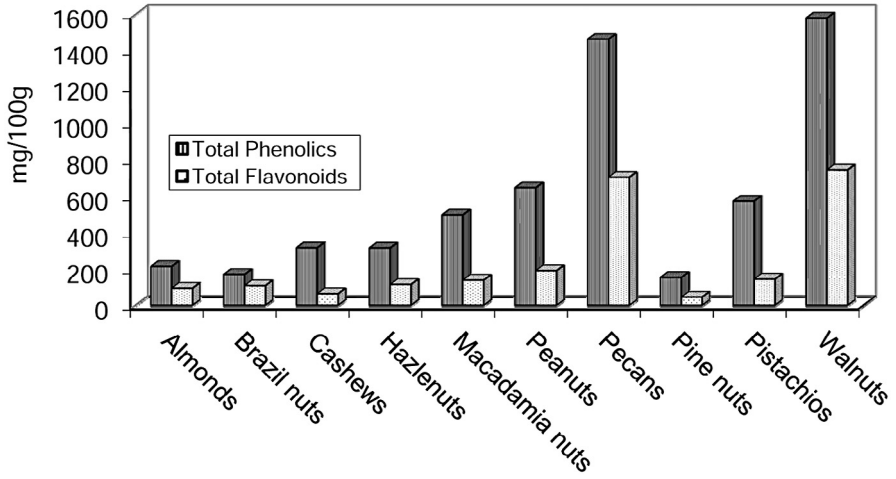


Fig. 25 – Content of total phenolics and flavonoids in tree nuts (mg/100 g) (adapted from Yang, Liu, et al., 2009).

chloroform/methanol extracted pecan and walnut oils possessed the highest antioxidant activities, which is likely due to their high amounts of tocopherols and other antioxidative minor components such as phospholipids and possibly other unidentified phenolic and/or non-phenolic components present in the extracts.

8.5.1. *Almonds*

Extracts of whole almond seed, brown skin, and green shell cover possess potent free radical scavenging capacities (Siriwardhana & Shahidi, 2002). These activities may be related to the presence of flavonoids and other phenolic compounds in nuts (Wijeratne, Abou-Zaid, & Shahidi, 2006). The antiradical activity of almond extracts against free radicals examined follow the order: skin > shell > seed (Siriwardhana & Shahidi, 2002). Almond hulls have been shown to serve as a rich source of triterpenoids, betulinic, urosolic, and oleanolic acids (Takeoka et al., 2000) as well as flavonol glycosides and phenolic acids (Sang, Lapsley, Rosen, & Ho, 2002). In addition, Sang, Lapsley, Rosen, et al. (2002) isolated catechin, protocatechuic acid, vanillic acid, *p*-hydroxybenzoic acid, and naringenin glucoside, as well as galactoside, glucoside, and rhamnoglucoside of 3′-*O*-methylquercetin and rhamnoglucoside of kaempferol (Esfahlan, Jamei, & Esfahlan, 2010; Wijeratne, Abou-Zaid, et al., 2006). Amarowicz, Troszynska, and Shahidi (2005) reported the presence of phenolic compounds such as vanillic, caffeic, *p*-coumaric, ferulic acids, quercetin, kaempferol, isorhamnetin, delphinidin and cyanidin as well as procyanidins B2 and B3 in almond extract using an HPLC method and found that procyanidins B2 and B3 and vanillic acid were the dominant phenolic compounds in almond extract. Wijeratne, Amarowicz, and Shahidi (2006) suggested that almond green shell cover extract, which contained a high phenolic acid content, could serve as a valuable source for phenolic acids. Recently, hydroxybenzoic acids (as protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic and *trans*-*p*-coumaric acids), flavan-3-ols (as (+)-catechin and (–)-epicatechin), flavonols (as aglycones; isorhamnetin, quercetin, kaempferol and glycosides; quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside) and flavanones (as aglycones; naringenin and eriodictyol and glycosides; eryodictiol-7-*O*-glucoside and naringenin-7-*O*-glucoside) were identified in the almond skin samples (Mandalari et al., 2010). Recently, Esfahlan, Jamei, and Esfahlan (2010) reported quercetin, isorhamnetin, quercitrin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, and morin as the major flavonoids in almond extracts. Sfahlan, Mahmoodzadeh, Hasanzadeh, Heidari, and Jamei (2009) found a strong antioxidant activity for methanolic extracts of almond hull and shell. Wijeratne, Abou-Zaid, et al. (2006) evaluated antioxidant efficacy of defatted almond whole seed, brown skin, and green shell cover extracts by monitoring inhibition of human low-density lipoprotein (LDL) oxidation, inhibition of DNA scission and metal ion chelation activities and also found that the brown skin of almond exerted the highest preventive effect against LDL oxidation at 10, 50, and 100 ppm levels, compared to those of whole almond and its green shell (leafy) cover. Almonds, when incorporated in the diet, have been reported to reduce colon cancer risk in rats (Davis & Iwahashi, 2001) and increase HDL cholesterol and reduce LDL cholesterol levels in humans (Hyson, Schneeman, & Davis, 2002). Almond appears to be effective in reducing the risk of heart disease and cancer prevention, and consumption of almond is recommended by FDA for better health conditions (Shahidi, Zhong, Wijeratne, & Ho, 2009).

8.5.2. *Cashew nuts*

The major phenolics found in cashew nuts are anacardic acids, cardanols, cardols, tocopherols and other minor phenolic constituents (Shahidi & Tan, 2009). The kernel of cashew nut valued in trade is covered with a thin reddish-brown skin or testa. The testa has been reported to be a good source of hydrolysable tannins (Pillai, Kedlaya, & Selvarangan, 1963) with catechin and epicatechin as the major polyphenols (Mathew & Parpia, 1970). The polymeric proanthocyanidins account for 40% of total testa phenolics; other components of testa phenolics include leucocyanidins and leucopelargonidins (Mathew & Parpia, 1970). The presence of gallic, caffeic and quinic acids apart from catechin and leucocyanidin was reported by Kantamoni (1965). γ-Tocopherol is prevalent in cashew, whilst α- and δ-tocopherols are present in lower amounts (Trevisan et al., 2006). Kamath and Rajini (2007) suggested that cashew nut skin, a byproduct of the cashew processing industry, can be used as an economical source of natural antioxidants. Cashew nut shell liquid, a byproduct obtained during the processing of cashew nuts is reported to possess antioxidant activity (Singh, Kale, & Rao, 2004). Recently, Chandrasekara and Shahidi (2011) reported that whole cashew and nuts testa were better sources of antioxidants compared to the kernel as assessed in different food and biological model systems.

8.5.3. *Hazelnut*

The presence of gallic, vanillic, caffeic and ferulic acids in extracts of hazelnut was reported by Alasalvar, Karamac, Amarowicz, and Shahidi (2006). Amongst the identified phenolic acids, *p*-coumaric acid was most abundant in hazelnut kernel, hazelnut green leafy cover, and hazelnut tree leaf, whereas gallic acid was most abundant in hazelnut skin and hazelnut hard shell (Alasalvar et al., 2006, 2009). However, in a previous study, protocatechuic acid was reported to be the predominant phenolic acid in testa (skin) of hazelnut (Senter, Horvat, & Forbus, 1983). Amaral et al. (2005) identified and quantified four phenolic acids, namely, 3-caffeoylquinic acid, 5-caffeoylquinic acid, caffeoyltartaric acid, and *p*-coumaroyltartaric acid, in hazelnut leaves from 10 different cultivars grown in Portugal. Hazelnut contained one anthocyanidin (cyanidin) and four flavan-3-ols [(–)-epicatechin, (–)-epigallocatechin, (–)-epigallocatechin 3-gallate and (+)-catechin]. Cyanidin was the most abundant flavonoid and no flavonones and flavonols were found in hazelnut (Alasalvar, Hoffman, & Shahidi, 2009). Hoffman and Shahidi (2009) found small amounts (8.61 to 68.22 μg/g) of paclitaxel (Taxol®) in tombul hazelnut (*Corylus arellana* L.) hard shells, green shell covers and leaves. In addition to paclitaxel, 10-deacetyl baccatin III, baccatin III and cephalomannine were also identified in tombul hazelnut extracts. In addition, hazelnut was found to contain the highest amount of condensed tannins amongst seven tree nuts (hazelnut, almond, cashew, chestnut, pecan, pistachio and walnut) (Alasalvar, Shahidi, Amaral, & Oliveira,

2009). A research carried out on crude extracts obtained from hazelnut by-products supports the hypothesis that hazelnut wastes, especially skin and hard shell, could serve as reliable source of efficient natural antioxidants (Shahidi, Alasalvar, & Liyana-Pathirana, 2007). Recently, Alasalvar, Shahidi, et al. (2009) suggested that hazelnut skin can be considered as a value-added byproduct for use as dietary antioxidants.

8.5.4. *Walnuts and heartnut*

Most commonly identified phenolic compounds in walnut are condensed tannins and phenolic acids. Walnut phenolics are found in the highest concentration in the hull fraction (the pellicle surrounding the kernel) (Labuckas, Maestri, Perelló, Martínez, & Lamarque, 2008). A walnut extract containing ellagic acid, gallic acid, and flavonoids was reported to inhibit the oxidation of human plasma and low density lipoproteins (LDL) *in vitro* (Anderson et al., 2001). Luczak, Swiatek, and Zadernowski (1989) identified 10 phenolic acids in the pericarp of walnut: *p*-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, *p*-coumaric, gallic, ferulic, caffeic and sinapic acids. Syringic acid was identified as a principle phenolic acid. Subsequently, Anderson et al. (2001) reported that ellagitannins were the most abundant phenolics in walnuts. Of these, ellagic acid, valoneic acid dilactone and pedunculagin have been identified in phenolic extracts from shelled walnuts. Walnut leaves are considered as a good source of healthcare compounds, and have been widely used in traditional medicine for the treatment of skin inflammations, hyperhidrosis and ulcers as well as for antidiarrhoeic, anti-helminthic, antiseptic and astringent properties (Carvalho et al., 2010). The compounds 3- and 5-caffeoylquinic acids, 3- and 4-*p*-coumaroylquinic acids, *p*-coumaric acid, quercetin 3-galactoside, quercetin 3-pentoside derivative, quercetin 3-arabinoside, quercetin 3-xyloside and quercetin 3-rhamnoside were identified in different cultivars of walnut leaves grown in Portugal (Pereira et al., 2007). Recently Zhang, Liao, Moore, Wu, and Wang (2009) identified seven phenolic compounds, namely, pyrogallol, *p*-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid and 3,4,8,9,10-pentahydroxydibenzo[*b*,*d*]pyran-6-one in English walnut seed by spectroscopic methods. Walnuts contained a number of structurally related naphthoquinones, including 1,4-naphthoquinone, juglone (5-hydroxy-1,4-naphthoquinone), 2-methyl-1,4-naphthoquinone and plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) (Muller & Leistner, 1978a, 1978b). Phenolics extracted from different fractions of English walnut showed marked antioxidant activities in different *in vitro* model systems (Samaranayake, John, & Shahidi, 2008). A recent study represents the first evidence that walnut seed, green husk and leaf methanolic extracts possess effective antihaemolytic and human renal cancer cell antiproliferative activities. It is therefore suggested that English walnut may be used as an inexpensive and easily accessible source of effective natural antioxidants and chemopreventive agents (Carvalho et al., 2010).

Heartnut (*Juglans ailanthifolia* var. *cordiformis*), a naturally occurring genetic oddity of the more common Japanese walnut, has recently become very popular amongst consumers and nut tree growers in southern Ontario, Canada (Li et al., 2006). Ellagic acid and valoneic acid dilactones are the major polyphenolics in heartnut (Li et al., 2006). γ-Tocopherol was the main tocopherol homologue present in heartnut, followed by δ- and α-tocopherol (Li, Tsao, Yang, Kramer, & Hernandez, 2007). Colaric, Veberic, Solar, Hudina, and Stampar (2005) identified phenolic acids (chlorogenic, caffeic, *p*-coumaric, ferulic, sinapic, ellagic, and syringic acid) as well as syringaldehyde and juglone in ripe fruits of 10 walnut cultivars. Syringic acid, juglone and ellagic acid were found as predominant phenolics. Phenolic compounds from walnut fruits have a positive influence on human health such as a decrease of coronary heart diseases, prevention of several kinds of cancer, and anti-inflammatory and antimutagenic activities (Anderson et al., 2001).

8.5.5. *Peanuts*

Numerous phytochemical compounds, including polyphenolics and tocopherols are present in peanuts with potential antioxidant activity (Hashim, Koehler, & Eitenmiller, 1993; Talcott, Passeretti, Duncan, & Gorbet, 2005). Peanuts are good sources of folic acid and resveratrol (Yang, Martinson, et al., 2009). The predominant phenolic acid present in peanuts with antioxidant potential is *p*-coumaric acid (Talcott, Passeretti, et al., 2005). Fajardo, Waniska, Cuero, and Pettit (1995) demonstrated a stress-elicited synthesis of free and bound phenolics in peanuts, with *p*-coumaric and ferulic acids as the major compounds identified. Talcott, Duncan, Pozo-Insfran, and Gorbet (2005) reported that free *p*-coumaric acid, three esterified derivatives of *p*-coumaric, and two esterified derivatives of hydroxybenzoic acid were the predominant polyphenolics present in peanuts. Yu, Ahmedna, and Goktepe (2005) reported that 3 classes of polyphenols were present in peanut skins, including phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, and coumaric acid), flavonoids (catechins and procyanidins), and stilbene (resveratrol). Lou, Yamazaki, Sasaki, Uchida, and Tanaka (1999) identified 6 A-type procyanidins in peanut skin. Identification of phenolic compounds from dry-blanched peanut skins by liquid chromatography-electrospray ionization mass spectrometry was also reported by Ma et al. (2014). Recently, de Camargo et al. (2015) identified proanthocyanidins as the major phenolic compounds present in peanut skin. Three compounds possessing potential antioxidant activities in the methanolic extract of peanut shell were identified as 5,7-dihydroxychromone, eriodictyol and luteolin using the HPLC–DAD–TOF/MS technique (Qiu et al., 2012). Craft, Kosinska, Amarowicz, and Pegg (2010) reported the antioxidant properties of extracts obtained from raw, dry-roasted, and oil-roasted peanuts. Potential contributors to antioxidant capacity in peanuts were identified as an ethyl ester of protocatechuic acid isolated from peanut testa (Huang, Yen, Chang, Yen, & Duh, 2003), luteolin in peanut hulls (Duh, Yeh, & Yen, 1992), Maillard-derived compounds and protein hydrolysates (Hwang, Shue, & Chang, 2001), tocopherols (Hashim et al., 1993) and resveratrol (Ibern-Gonzalez, Roig-Perez, Lameula-Raventos, & Torre-Boronat, 2000; Sobolev & Cole, 1999) in peanuts and peanut-based products. Research has shown that the consumption of high-oleic acid peanuts has potential health benefits, such as lowering of blood cholesterol levels in hypercholesterolaemic women (O'Byrne, Knauft, & Shireman, 1997). The content of oleic acid in high-oleic acid peanuts is similar to that of olive oil (Cabrini et al., 2001), which is known for its heart-healthy characteristics. However, literature shows that peanut contains multiple allergens (De Jong et al., 1998; Isanga, & Zhang, 2007). A total of 6 protein subunits were recognized by more than 50% of the

specific IgE-containing plasma samples of peanut allergic patients and were, therefore, defined as major allergens (De Jong et al., 1998).

8.5.6. *Pistachio*

Phytochemicals identified from pistachios include fatty acids, phytosterols, lutein, resveratrol, and anthocyanins (Phillips, Ruggio, & Ashraf-Khorassani, 2005). Galloyl quinic derivatives were identified in aqueous extracts from leaves and gum of *Pistacia lentiscus* (Romani, Pinelli, Galardi, Mulinacci, & Tattini, 2002), and α-tocopherol was evaluated in lipophilic extracts from the leaves of *P. lentiscus* and *Pistacia therebintus* (Kivcak & Akay, 2005). The polyphenol phytoalexin *trans*-resveratrol was detected in the aqueous extracts from the edible nut of five Turkish cultivars of *Pistacia vera* (Tokusoglu, Unal, & Yemis, 2005). Gentile et al. (2007) found *trans*-resveratrol, proanthocyanidins, and a remarkable amount of the isoflavones daidzein and genistein (3.68 and 3.40 mg per 100 g of edible nut, respectively) in hydrophilic extract of *P. vera*. Seeram et al. (2006) identified quercetin (14.9 μg/g), luteolin (10.0 μg/g), eriodictyol (10.2 μg/g), rutin (1.6 μg/g), naringenin (1.2 μg/g), apigenin (0.2 μg/g), and the anthocyanins, cyanidin-3-galactoside (696 μg/g) and cyanidin-3-glucoside (209 μg/g) in pistachio skin. Recently, Saitta, Giuffrida, Torre, Potortì, and Dugo (2009) identified sixteen different 3-alkylphenols (cardanols) with a saturated, monounsaturated and diunsaturated chain in pistachio kernel. The most abundant cardanols were 3-(8-pentadecenyl)-phenol, 3-(10-pentadecenyl)-phenol, 3-pentadecyl-phenol and 3-(10-eptadecenyl)-phenol. *Trans*-resveratrol and *trans*-resveratrol-3-*O*-β-glucoside (*trans*-piceid) were identified in Sicilian pistachio variety (Grippi et al., 2008). Halvorsen et al. (2006) ranked the edible pistachio nut amongst the first 50 food products having the highest antioxidant potential. Recent studies show that essential oils and lipophilic extracts of leaves, fruits, gum, and galls of Pistachio may exert various characteristics such as anti-microbial, anti-inflammatory, insecticidal, and anti-nociceptive activities, with various terpenes playing a major role in the observed activities (Alma et al., 2004).

8.5.7. *Pecan*

Pecans are a rich source of γ- and a poor source of α-tocopherol, containing 24.4 and 1.4 mg per 100 g of nut, respectively (Haddad, Jambazian, Karunia, Tanzman, & Sabaté, 2006). Phenolic acids (gallic acid) (Senter, Horvat, & Forbus, 1980), proanthocyanidins (Polles, Hanny, & Harvey, 1981), prodelphinidins (3-*O*-gallates) including epigallocatechin, epicatechin-3-*O*-gallate, and the more common flavan-3-ols, catechin and epicatechin were identified in pecan kernel (Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos, 2007). Miraliakbari and Shahidi (2008) found that chloroform/methanol extracted pecan oil had the highest antioxidant activity and total phenolic content (711 mg/kg gallic acid equivalents) amongst the seven tree nut oils tested. Recent studies have shown that pecan kernels may improve human serum lipid profile and lower low density lipoprotein levels, due to their high monounsaturated fatty acid content (Rajaram, Burke, Connell, Myint, & Sabate, 2001). de la Rosa, Alvarez-Parrilla, and Shahidi (2010) identified five phenolic compounds in Mexican pecan kernels (ellagic, gallic, protocatechuic, and *p*-hydroxybenzoic acids and catechin), and two (ellagic and gallic acids) in shells. In addition they also reported the presence of seven phenolic compounds in kernels, namely, protocatechuic aldehyde, (epi)gallocatechin, one gallic acid–glucose conjugate, three ellagic acid derivatives, and valoneic acid dilactone by means of MS and UV spectral comparison.

8.5.8. *Chestnut*

The principal extract and medicinal constituent of *Aesculus hippocastanum* seeds is aescin, a mixture of triterpenoid saponin glycosides (Sirtori, 2001). Its components include glycosides of protoaescigenin and barringtogenol. A number of other products have been isolated from chestnut seeds, i.e., coumarin derivatives (aesculin, fraxin, scopolin), essential oils (oleic acid, linoleic acid), and tannins (leucocyanidine, proanthocyanidin A_2) (Bombardelli, Morazzoni, & Griffini, 1996). The skins of chestnut are rich in tannin. Tannins of chestnut fruit consist mainly of 3,6-digalloylglucose, pyrogallol, and resorcinol (Hwang, Hwang, & Park, 2001). Ogawa et al. (2008) reported procyanidin trimers, polymeric proanthocyanidins having a series of heteropolyflavan-3-ols, (+)-catechin, (–)-epicatechin in seed shells of the Japanese horse chestnut. Nine flavonol oligosides of quercetin and kaempferol with glucose, xylose, and rhamnose as sugars were isolated from the seeds of *A. hippocastanum* L (Hubner, Wray, & Nahrstedt, 1999). Kapusta et al. (2007) identified a new glycoside, tamarixetin 3-*O*- [β-d-glucopyranosyl(1→3)]-*O*-β-d-xylopyranosyl-(1→2)-*O*-β-d-glucopyranoside in horse chestnut. Horse chestnut is most often used as a treatment for venous insufficiency. This is a condition associated with varicose veins, when the blood pools in the veins of the legs and causes aching, swelling, and a sense of heaviness (Kapusta et al., 2007). Kimura et al. (2006) found that saponins from edible seeds were appreciably effective in inhibiting pancreatic lipase *in vitro* and exhibiting antiobesity effects in mice that had been fed high-fat diets. Kapusta et al. (2007) suggested that industrial horse chestnut waste water can be used to obtain quercetin and kaempferol glycosides for cosmetic, nutraceutical, and food supplement industries.

8.5.9. *Pine nut*

Traditionally known as cedar nuts, the seeds of *Pinus sibirica* are considered to be a valuable medicinal raw plant material, being ascribed a wide spectrum of traditional properties. Lantto et al. (2009) identified benzoic acid (protocatechuic, syringic and vanillic acids) and cinnamic acid derivatives (*m*-coumaric and (*E*)-cinnamic acids), flavanones (taxifolin, eriodictyol and naringenin) and flavan-3-ols (catechin, epicatechin and epigallocatechin gallate) in Siberian pine. The most abundant component is eriodictyol (383 ± 1.0 mg/100 g) followed by taxifolin (172 ± 3.1 mg/100 g), whilst the least abundant components are *trans*-cinnamic (12.2 ± 1.2 mg/100 g) and *m*-coumaric (trace) acids. Some other components were identified as proanthocyanidins, hydroxylated benzoic and cinnamic acid derivatives, flavan-3-ol and flavonol derivatives. The beneficial properties of *Pinus* species in general primarily related to their anti-inflammatory, antioxidant, antineoplastic and immuno-modulatory properties (Guri, Kefalas, & Roussis, 2006; S. Li et al., 2009; Rohdewald, 2002).

8.5.10. *Macadamia nut*

Tocopherols, tocotrienols, and squalene are present in macadamia kernels (Maguire et al., 2004). Macadamia kernels

contained low concentrations of α-tocopherol and δ-tocopherol compared to almonds and pecans (Fourie & Basson, 1989), but Kaijser, Dutta, and Savage (2000) found high concentrations of α-tocotrienol in *Macadamia tetraphylla*, a species grown in Australia (Wall, 2010). Macadamia nuts have generated considerable interest because they are believed to have blood cholesterol-lowering properties (Colquhoun, Humphries, Moores, & Somerset, 1996). Quinn and Tang (1996) found catechol, pyrogallol, 3,4,5-trihydroxy phenolic compounds, 2,6-dihydroxybenzoic acid, 2′-hydroxy-4′-methoxyacetophenone, 3′,5′-dimethoxy-4′-hydroxyacetophenone, and 3,5-dimethoxy-4-hydroxycinnamic acid in oil extract of macadamia nut kernels and shells using thin-layer chromatography.

8.6. *Herbs and spices*

There is increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants (Suhaj, 2006). These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens and minerals, amongst others, that render spices and some herbs their antioxidant components as preservative agents in food (Calucci et al., 2003). Important fractions of spices are their volatile oils and oleoresins (Shahidi, Pegg, & Saleemi, 1995). Many herbs and spices, usually used to flavour dishes, are an excellent source of phenolic compounds which have been reported to show good antioxidant activity (Carlsen et al., 2010; Embuscado, 2015; Rice-Evans et al., 1996; Shahidi, Pegg, et al., 1995; Zheng & Wang, 2001). Viegas, Amaro, Ferreira, and Pinho (2012) studied the inhibitory effect of antioxidant-rich marinades containing beer and white wine (with/without alcohol) alone or mixed with herbs commonly used as meat flavouring (garlic, ginger, thyme, rosemary, and red chili pepper) on the formation of heterocyclic aromatic amines (HAs) in pan-fried beef. The authors found that all selected marinades exhibit a reduction in total HAs formation in pan-fried meat. Shahidi, Pegg, et al. (1995) investigated the antioxidant efficacy of ground clove, ginger, oregano, rosemary, sage and thyme in comminuted pork systems. Spices at 200–2000 ppm levels of addition inhibited the formulation of the 2-thiobarbituric acid reactive substances (TBARS) by 12–96% over 21-days of storage at 4 °C. The antioxidant efficiency of spices was reported as clove ≥ sage ≥ rosemary = oregano ≥ thyme = ginger. In addition, some herbs such as rosemary and sage are used to produce drugs classified as phytopharmaceuticals, representing a significant part of the world pharmaceutical market (Kähkönen et al., 1999; Madsen & Bertelsen, 1995). A problem which is often encountered when using spices and herbs is that they generally have a distinct odour, taste, and colour, a fact that makes their systematic use in foods (for example, in spreads) rather difficult (Kiokias et al., 2008). Besides, the extraction procedures are quite complicated and cannot easily be made into an economically attractive process. Purification of the extracts and further processing to convert them to additives suitable for foods (Kiokias et al., 2008) are usually needed. Furthermore, plants show seasonal and topographical variations, giving rise to differences in their antioxidant activities, whereas the safety of the extracts is a topic of current discussion (Kiokias, 2006). However, deflavoured spice extracts and oleoresins, such as those of rosemary and sage are commercially available.

8.6.1. *Rosemary and sage*

The greatest level of attention amongst herbs and spices as sources of antioxidants has been focused on rosemary (Berdahl & McKeague, 2015; Erkan, Ayranci, & Ayranci, 2008; Rodríguez-Rojo, Visentin, Maestri, & Cocero, 2012). In earlier studies, sage (*Salvia officinalis* L.) and rosemary were shown to have similar patterns of phenolic compounds and their antioxidant activity was attributed mainly to carnosic acid, carnosol and rosmarinic acid present (Fig. 26) (Frankel, Huang, Aeschbach, & Prior, 1996; Okamura, Fujimoto, Kuwabara, & Yagi, 1994; Senanayake, 2013; Thorsen & Hildebrandt, 2003; Y. Zhang et al., 2012). These polyphenols also possess important biological activities *in vitro* as anti-tumour, chemopreventive and anti-inflammatory agents (Al-Sereiti, Abu-Amer, & Sen, 1999; Cheung & Tai, 2007; Danilenko et al., 2003; Shuang-sheng & Rong-liang, 2006). It has been proposed that polyphenols of rosemary may greatly increase functionality of food for health and wellness (Shahidi & Naczk, 2004). Antioxidative effect of rosemary ethanolic extract on butter was reported by Zegarska et al. (1996, 1998). The main compounds responsible for rosemary's antioxidant properties have been identified as phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epi- and iso-rosmanol, rosmadial and methyl carnosate (Bandonienė, Murkovic, Pfanhauser, Venskutonis, & Gruzdiéne, 2002; Bandonienė, Venskutonis, Gruzdiene, & Murkovic, 2002; Ibañez et al., 2003; Pizzale, Bortolomeazzi, Vichi, & Conte, 2002). Other compounds, such as rosmarinic acid, caffeic acid and flavonoids, have also been associated with the antioxidant activity of rosemary (del Baño et al., 2003; Suhaj, 2006). Recently, Escriche, Kadar, Juan-Borrás, and Domenech (2014) found kaempferol, chrysin, pinocembrin, caffeic acid and naringenin in rosemary honey. Wang et al. (1998) extracted sage with ethanol and identified seven flavonoids, 15 diterpenoids, 17 triterpenes and steroids including rosmarinic acid, 4-*O*-β-d-glucopyranosylacetophenone (picein), 6-*O*-(*E*)-feruloyl-(α and β)-glucopyranoside, (+)-1-hydroxypinoresinol-1-β-d-glucoside, homoplantaginin, (–)-isolariciresinol 3α-*O*-β-d-glucopyranoside, luteolin-7-*O*-β-glucopyranoside, 4-hydroxyacetophenone-4-*O*-β-d-apiofuranosyl-(1→6)-*O*-β-d-glucopyranoside, icariside F2, and 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol 4′-*O*-β-glucopyranoside. Recently, Kontogianni et al. (2013) reported that the rosemary extract exhibits more pronounced antioxidant, cytotoxic and immunomodifying activities, due to the presence of betulinic acid and a higher concentration of carnosic acid. Romano, Abadi, Repetto, Vojnov, and Moreno (2009) suggested that the methanolic extract of rosemary synergistically enhances the antiradical efficiency of BHT and the antibacterial activity of BHA. It has also been observed that ascorbic acid (500 mg/kg of ascorbic acid + 200 mg/kg of rosemary extract) enhanced the antioxidant activity of rosemary extract in lard (Chang, Ostric-Matijasevic, Hsieh, & Huang, 1977). Whilst rosemary extracts have been found to be effective antioxidants, they are generally considered to be less effective than BHA and BHT (Berdahl et al., 2010; Sebranek, Sewalt, Robbins, & Houser, 2005). For example, Ahn, Gruen, and Fernando (2002) reported that rosemary was significantly less effective than BHA/BHT for

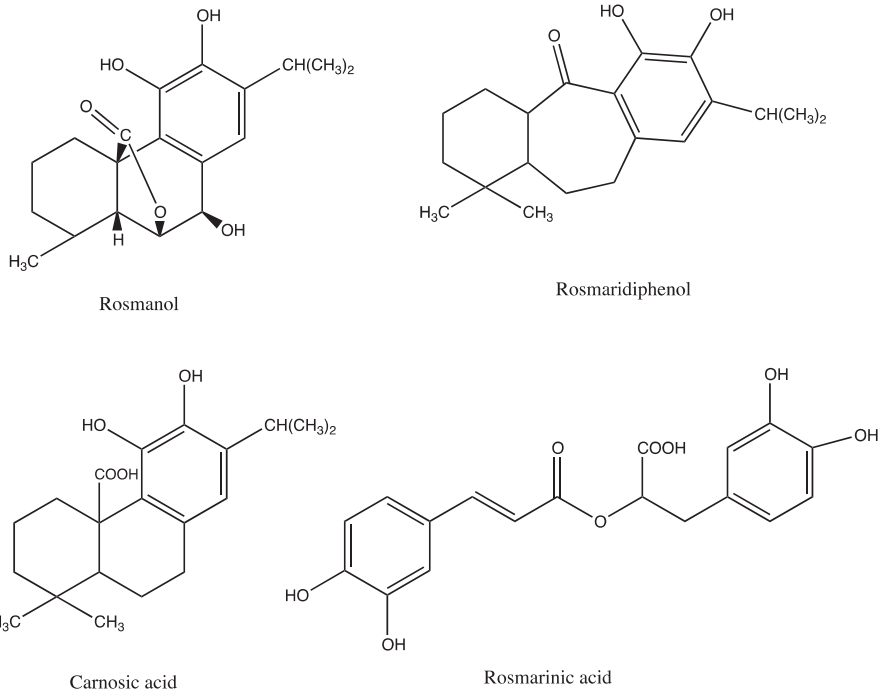


Fig. 26 – Chemical structures of selected antioxidant compounds in rosemary (Source: Adapted from Shahidi & Naczk, 2004).

suppression of oxidative changes in cooked ground beef. Several studies have shown that rosemary extracts are effective oxidative stabilizers of various meat and poultry products as well as vegetable and fish oils (Senanayake, 2013; St.Angelo, Crippen, Dupuy, & James, 1990; Ucak, Ozogul, & Durmus, 2011; Wanasundara & Shahidi, 2005). In addition rosemary extracts are effective antioxidants in snack foods, nuts, baked goods and pet foods (Gruiner-Richter, Otto, & Weidner, 2012; Lalas & Dourtoglou, 2003). Rosemary and sage extracts are commercially available as StabilEnhance®, OxyBlock®, Xtrablend®, Fortium®, Herbalox®, Duralox®, Aquarox®, Inolens®, Vivox®, and are manufactured by Naturex, Kemin, Kalsec, Flavex and Vitiva (Embuscado, 2015).

8.6.2. Clove

Cloves are the dried flower buds of *Syzygium aromaticum* and serve as sources of anti-microbial agents against oral bacteria that are commonly associated with dental caries and periodontal disease (Cai & Wu, 1996). The major aroma constituents of *S. aromaticum* buds are eugenol (2-methoxy-4-allylphenol) and eugenyl acetate (Lee & Shibamoto, 2002). Eugenol is reported to have antifungal activity (Martini, Weidenborner, Adams, & Kunz, 1996) and inhibits malonaldehyde formation from cod liver oil and the formation of hexanal (Lee & Shibamoto, 2001). Sesquiterpenes, in clove may serve as potential anticarcinogenic agents (Zheng, Kenney, & Lam, 1992). The main components of clove leaf essential oil are eugenol (76.8%), followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%) (Jirovetz et al., 2006).

8.6.3. Ginger

Kikuzaki and Nakatani (1993) reported that chemical constituents like gingerols and shogaols present in ginger exhibit strong antioxidative activity. This has clearly been demonstrated by inhibition of phospholipid peroxidation induced by the $FeCl_3$–ascorbate system (Aeschbach et al., 1994) and its inhibitory effect on xanthine oxidase system (Chang, Chang, Lu, & Chiang, 1994) which is responsible for the generation of reactive oxygen species, such as superoxide anion. Sekiwa, Kubota, and Kobayashi (2000) isolated two glucosides of 6-gingerdiol, namely 1-(4-*O*-*β*-d-glucopyranosyl-3-methoxyphenyl)-3,5-dihydroxydecane and 5-*O*-*β*-d-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxy phenyl)decane and reported that 5-*O*-*β*-d-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxy phenyl)decane had a strong antioxidant activity in a linoleic acid model system. Other gingerol-related compounds are formed from phenylalanine via ferulic acid, and diarylheptanoids and terpenoids (Kikuzaki, Tsai, & Nakatani, 1992). Ginger and the components in it are also known to possess such physiological features as antimicrobial, antioxidative, antitumour, and antiplatelet aggregation activities (Conner, 1991; Sekiwa et al., 2000; Yin & Cheng, 1998). The anticancer properties of ginger are attributed to the presence of certain pungent vallinoids, gingerol and paradol, as well as some other constituents like shogaols, zingerone, amongst others (Shukla & Singh, 2007). Nirmala, Prasanna Krishna, and Polasa (2007) reported the *in vivo* antimutagenic effect of ginger in rats and suggested that the antimutagenic and chemopreventive potential of ginger could be due to its antioxidant activity.

8.6.4. *Black pepper*

Agbor, Vinson, Oben, and Ngogang (2006) reported that black pepper is more effective than white pepper in scavenging free radicals and ROS. This property of black pepper is related to its higher polyphenol content. Chemically, peppercorn contains lignans, alkaloids, flavonoids, aromatic compounds, and amides (Jirovetz, Buchbauer, Ngassoum, & Geissler, 2002). Essential oil and oleoresins of black pepper exhibit high antioxidant and radical scavenging activities against various antioxidant assays *in vitro* (Kapoor et al., 2009). The antioxidative action of black pepper, for instance, has been attributed to its content of piperine and piperine isomers, such as chavicine, isopiperine, and isochavicine, and monoterpene (Chipault, Mizuno, & Lundberg, 1956; Milbourne, 1987; Nakatani, Inatani, Ohta, & Nishioka, 1986; Yanishlieva, Marinova, & Porkorny, 2006). However, Kapoor et al. (2009) showed that the antioxidant activity of black pepper was due to the presence of β-caryophyllene, limonene, β-pinene, piperine and piperolein in essential oil and oleoresins. Suhaj (2006) reported that according to USDA (2002), ascorbic acid, beta-carotene, camphene, carvacrol, eugenol, gamma-terpinene, lauric acid, linalyl-acetate, methyleugenol, myrcene, myristic acid, myristicin, palmitic acid, piperine, terpinen-4-ol and ubiquinone were the antioxidants present in black pepper.

8.6.5. *Turmeric*

Curcuminoids [i.e., curcumin (1,7-bis(4-hydroxy-3-methoxyfenil)-1,6-heptadiene-3,5-dione), demethoxy-curcumin, and bis-demethoxy-curcumin] are the major antioxidative compounds of turmeric (Schieffer, 2002). In addition to curcuminoids, other compounds possessing antioxidant capabilities that are present in turmeric include γ-terpinene, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, campestrol, camphene, dehydrocurdione, eugenol, *p*-coumaric acid, protocatechuic acid, stigmasterol, syringic acid, turmerin, turmeronol and vanillic acid (Cousins, Adelberg, Chen, & Rieck, 2007; Relajakshmi & Narasimhan, 1996). Curcumin, the principal polyphenolic curcuminoid is reported to have antioxidant, anticarcinogenic, anti-HIV, antibacterial and anti-inflammatory activities (Smitha, Dhananjaya, Dinesha, & Srinivas, 2009). Further studies have revealed that curcumin may suppress cancer development by helping in inhibiting enzymes that lead to tumour production (Surh, 2002) and prevents cancer along with inflammation by inducing production of enzymes used to detoxify electrophyllic species produced in lipid peroxidation (Piper et al., 1998). Verma, Salamone, and Goldin (1997) demonstrated that curcumin inhibited the growth of human breast cancer cells.

8.6.6. *Oregano*

Oregano (*Origanum vulgare* L.), a widely used herb, also commonly used in Western dishes possesses antithrombin, anti-*Helicobacter pylori*, antibiotic, antihyperglycaemia and antioxidation effects (Goun, Gunningham, Solodnikov, Krasnykch, & Miles, 2002; Lin et al., 2008; Stamatis et al., 2003). The compound 4-(3,4-dihydroxybenzoyloxymethyl)phenyl-*O*-β-d-glucopyranoside is a major constituent of oregano and might contribute to its antioxidant activity (Nakatani & Kikuzaki, 1987). Kikuzaki and Nakatani (1993) also isolated five different phenolic compounds from the methanol extract of leaves of oregano, namely, caffeic, protocatechuic, and rosmarinic acids, a phenyl glucoside and a new compound 2-caffeoyloxy-3-[2-(4-hydroxybenzyl)-4,5-dihydroxy]phenylpropionic acid; amongst these rosmarinic acid was present in highest concentration. Rosmarinic acid is a caffeoyl ester and has now been shown to be an important antioxidant and anti-inflammatory compound (Chun, Vattem, Lin, & Shetty, 2005). Antioxidative effect of oregano ethanolic extract on butter was reported by Amarowicz, Carle et al. (2009) and Amarowicz, Zegarska et al. (2009). Phenolic compounds and flavonoids such as luteolin, hispidulin, apigenin, acacetin, diosmetin, herbacetin, quercetin and naringin had also been described in oregano extracts (Cavero et al., 2006; El-Ansari, El-Negoumy, & El-Desoky, 1996; Justesen & Knuthsen, 2001; Pizzale et al., 2002; Zheng & Wang, 2001).

8.6.7. *Fenugreek seed*

Fenugreek seeds are a rich source of polyphenols (Kenny, Smyth, Hewage, & Brunton, 2013). Several compounds were identified by HPLC, including apigenin and a number of kaempferol and quercetin glycosides (Chatterjee, Variyar, & Sharma, 2009) as well as the flavonoids; vitexin, tricin, naringenin, quercetin and tricin 7-*O*-*β*-D-glucopyranoside (Shang et al., 1998). Studies have shown that fenugreek extracts are effective in reducing the total cholesterol and triglyceride levels in patients with coronary artery disease (CAD) (Bordia, Verma, & Srivastava, 1997), alleviating physiological oxidative stress and inflammation in arthritic mice (Sindhu, Ratheesh, Shyni, Nambisan, & Helen, 2012) and protecting against hepatotoxicity caused by the anti-cancer drug adriamycin (ADR) in albino rats (Sakr & Abo-El-Yazid, 2012).

8.7. *Future considerations*

There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of their antioxidant activity, amongst other mechanisms. Mode of action of different phenolic compound in specific body organs needs to be found. Due to safety and other limitation surrounding the use of synthetic antioxidants, natural antioxidants obtained from edible sources, by-products and co-products are alternative sources of interest. Further studies on the isolation of phenolic compounds using complementary methods and their effects on antioxidant status in animal models and human subjects are needed to evaluate their potential benefits. In addition it is necessary to further confirm lack of toxicity and bioavailability of such natural phenolic extract. Delivery of isolated phenolics as dietary supplements or functional food ingredients for health promotion and disease risk reduction may also be helpful in improving the efficacy of such materials.

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