Phenolic Constituents and Antioxidant Activities of Selected Onion and Potato Varieties and their Processing By-products

By

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ST.JOHN'S **NEWFOUNDLAND & LABRADOR** **CANADA**

Dedication

I dedicate this document to my husband for his support, trust, motivation and being with me at every step of this journey.

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ABSTRACT

Potato and onion processing produces a large amount of discards, mainly peels and skins. This study compared the antioxidant activity of phenolic constituents of skin and flesh of different coloured onions (pearl skin, red skin, yellow skin, white skin, red flesh, sprouted red flesh) and potatoes (purple, russet, innovator, yellow). Phenolic constituents were separated into free, esterified and bond fractions. The bound fraction was extracted into diethyl ether after consecutive alkaline hydrolysis. The contribution of free phenolics toward total phenolic content was significantly ($p<0.05$) higher than the esterified and bound for onion skin extracts tested then those in the flesh. Phenolics were present mainly in the free form in both onion skin and flesh. The content of flavonoids extracted from onion and potato skins was approximately six times higher than that of their flesh counterparts. Among onion varieties, pearl onion skin showed the highest phenolic content (26.4 mg quercetin equivalents/g freeze dried sample). Similarly, purple potato peels had the highest phenolic content (13.85 mg gallic acid equivalents/g freeze dried sample). The phenolic compounds in potatoes were predominantly present in the bound form in the peels of both Innovator and Russet varieties (45.95-51.07%) while free and esterified phenolics were predominant in purple and yellow varieties. Red onion skin was most effective in inhibiting DNA strand scission at 94.45%, and that of purple potato peel was 91.02%. Similar trends were observed for inhibition of LDL cholesterol oxidation and free radical scavenging activities of samples tested. HPLC-MS analysis showed that quercetin, quercetin 3-glucoside, and kaempferol were the predominant

phenolics in all onion extracts, while chlorogenic, caffeic, *p*-coumaric and ferulic acids were predominant in potato peels.

Acknowledgements

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CHAPTER 1

INTRODUCTION

Phenolic compounds are commonly found in both edible and nonedible plants and have been reported to possess multiple biological effects, including antioxidant activity. Crude extracts of vegetables, herbs, fruits, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufactures, and consumers as trend of the future is moving toward functional food with specific health effects (Kahkonen *et al.*, 1999). Polyphenols are recognized as the most abundant antioxidants in human diet (Manach *et al.*, 2004). As antioxidants, phenolic compounds prevent the formation of free radicals with deleterious health effects and are therefore important in disease risk reduction (Shahidi, 2000). They have been demonstrated to have positive effects on certain types of cancer (Birt, 2006), including cancer of the stomachs, colon, prostate, and breast as well as cardiovascular disease (CVD) (Hertog *et al.,* 1995), and various inflammatory disorders (Andriantsitohaina *et al.,* 1999).

Vegetables are rich sources of phytochemicals, in addition to other components that may act synergistically with phytochemicals to contribute to the nutritional and health benefits of these food commodities. Two of the most widely consumed vegetables, onion, and potato have been extensively studied. Potatoes are one of the most

commonly consumed vegetables throughout the world. Peels are the major byproduct of potato processing industries, which represents a major waste disposal problem for the industry concerned. Antioxidative compounds extracted from potato peels may therefore be of potential value for use in feed, food and healthcare industries (Habeebullah *et al.*, 2010).

Potato (*Solanum tuberosum*) is a major staple food of human diet and the fourth largest crop that is grown worldwide after rice, wheat, and maize (Singh and Saldana, 2011). Potato peels are a good source of phenolic compounds which when extracted can be used as natural antioxidants to prevent oxidation of food containing high amounts of lipid (Andrich *et al.*, 2003). Almost 50% of phenolics are located in the peel and adjoining tissues and decrease towards the centre of the tuber (Freidmen, 1997; Al-Weshahy and Rao, 2009).

Phenolic compounds in potatoes can be present in both free and bound forms. They are mostly substituted derivatives of hydroxycinnamic acid in the free form and hydroxybenzoic acid in the bound form (Shahidi and Naczk, 1995). The most common hydroxycinnamic acid derivatives in potato and potato peels were reported to be chlorogenic acid (CGA), caffeic acid (CFA), and ferulic acid (FA), while hydroxybenzoic acids present were gallic acid (GA), protocatechuic acid (PCA), and their derivatives (Kanatt *et al.*, 2005; Nara *et al.*, 2006; Al-Weshahy and Rao, 2009). Anthocyanins a sub-group within the flavonoids, are present in substantial amounts in pigmented potatoes (Brown, 2008). The purple potato peel has a higher concentration of flavonoids than white potatoes which again their peels show higher contents and activities of antioxidant

than their flesh (Rodriguez de Sotillo *et al.,* 1994; Lewis *et al.*, 1998; Velioglu *et al.*, 1998).

Though there have been extensive studies on the free phenolics and their antioxidant activities in potatoes (Mansour and Khalil, 2000; Al-Weshahy and Rao, 2009), there appears to be very few studies on the esterified and bound phenolics in these vegetable, which underestimates the total phenolic content and their contribution to the overall antioxidant activity. In the present study, the phenolic constituents of potato peel and flesh were fractionated into their respective free, esterified, and insoluble-bound forms by alkali hydrolysis and the relative proportions of the various phenolic acids were determined using different chemical assays and high-performance liquid chromatography (HPLC).

Onion (*Allium cepa*), another widely consumed vegetable, is classified based on its colour into yellow, red and white; based on taste, it is divided into sweet and non-sweet products (Shahidi and Naczk, 2004). Fresh and dehydrated onions are widely used in the human diet as a source of nutrient, spicy garnish and also non-nutritive health promoting compounds (Lee *et al.*, 2008). Onions contain scores of functional phytochemicals and their consumption has long been associated with health promotion and disease prevention; reducing the incidence of cancers in several tissues, preventing vascular and heart diseases, neurodegenerative disorders and cataract formation (Kaur *et al.*, 2008). Among phytochemicals with health benefit, the high quantities of flavonoids, fructans and organosulphur in the onions are considered to be important contributing

factors to the overall antioxidant activity of the diet (Ames *et al.*, 1993; Rice Evans *et al.*, 1997; Paganga *et al.*, 1996).

Epidemiological studies about the major sources of antioxidant intake have highlighted the importance of onions, for high levels of a specific class of flavonoids, the flavonols (Hertog *et al.*, 1993; Suh *et al.*, 1999). Red, yellow, and white onions are, in fact, known to contain a large amount of flavonols; the majority being glucosides of quercetin and keampferol (Rhodes and Price, 1996; Sellappan and Akoh, 2002). Onion skins also contain significantly higher content of flavonoids than the edible portion at 2-10 g/kg (Suh *et al.*, 1999). Regardless of high levels of flavonoids in outer scales of onion, they are peeled off and discarded before food processing such as cooking. The outer scales contain quercetin derivatives (Takahama and Hirota, 2000; Furusawa *et al.*, 2002, 2003), which constitute more than 80% to the total content of flavonoids in onion (Furusawa *et al.*, 2002, 2003; Galdon *et al.*, 2008). Takahama and Hirota (2000) have suggested that quercetin is formed by deglucosidation of its glucosides, followed by autoxidation to produce protocatechuic acid. Recently, some anti-platelet and membrane-rigidifying flavonoids have been isolated from the outer scales of onions and identified as quercetin, quercetin dimers, and querctin 4-glucoside (Furusawa *et al.*, 2002, 2003). Further, dry onion skin has different amounts of quercetin derivatives compared to fleshy scales where as much as 53% of total quercetin is present in the free form (Wiczkowski *et al.*, 2003). Although extracts from onion skins exhibit potent radical scavenging activities (Nuutila *et al.*, 2003), the specific antioxidative components are not yet fully identified for different colour onions.

Furthermore, most studies are on the free phenolics and their antioxidant activities in onions, but little literature exists on the esterified and bound phenolics which under estimates the total phenolic content and their contribution to the overall antioxidant activity of onion.

In this study, the phenolic constituents of onion skin and flesh were determined for the free, esterified, and bound forms using alkali hydrolysis before analysis. The green shoot from one of the sprouted onions was also evaluated along with the flesh to understand the changes that may take place in the phenolic constituents during germination. The study aimed to provide information about the potential of using onion skin and extracts as an effective source of antioxidants in food systems; specifically to compare four different varieties of onions which are pearl onion skin, red onion skin, yellow onion skin, white onion skin, red onion flesh, sprouted red onion flesh, and green shoots which sprouted from red onion flesh, with respect to their total phenolic content and antioxidant activity in order to investigate their potential as a source of natural antioxidants. Their antioxidant potential in food and biological model systems was also investigated to highlight their efficiency.

The study also provides data on free, esterified, bound phenolics of potato peels and flesh extracts as an effective source of antioxidants in food systems; specifically to compare the phenolic content of the peel and flesh of four different potato varieties (Russet, Innovator, Purple and Yellow potatoes) and the antioxidant capacity of their extracts in *in-vitro,* both food and biological model systems. There is an existing gap in the literature as no systematic studies exist on different onions/potatoes and their peels

for their content and nature of phenolics in different forms of free, soluble, esterified, and insoluble-bound and corresponding antioxidant efficacy.

CHAPTER 2

LITERATURE REVIEW

The importance of antioxidants contained in foods is well appreciated for both preserving the foods themselves and for supplying essential antioxidants *in vivo* (Shi and Noriko, 2001). Antioxidants are known to act at different levels in the oxidative sequence (Shahidi, 1997). There is mounting interest in natural antioxidants due to safety concerns for synthetic antioxidants. In this context, it is important to investigate the antioxidative properties of onion and potato, which are vegetables grown and consumed in large quantities all over the world. The following sections describe the importance of lipid oxidation and its implications in food and health, the role of reactive oxygen species (ROS), and synthetic and natural antioxidants.

2.1 Lipid oxidation

Lipid oxidation is a major cause of food quality deterioration and generation of off odours and off flavours, decreasing shelf-life, altering texture and colour, and decreasing nutritional value of food (Alamed *et al.*, 2009). Various methods have been used since antiquity to inhibit lipid oxidation and off-flavour development in food systems. The practices of hydrogenation of unsaturated fatty acids (FA), removal of oxygen through vacuum packaging, use of superoxide scavengers such as glucose oxidase and ascorbic acid oxidase, removal or sequestering of metal ions, irradiation, refrigeration and freezing, and use of antioxidants are among approaches that are commonplace for the control of lipid oxidation (Subhashinee *et al.*, 2006). Antioxidants

have become an indispensable group of food additives mainly because of their unique properties of extending the shelf-life of food products without any damage to their sensory or nutritional quality. Historically, gum guaiac was the first antioxidant approved for stabilization of animal fats in the 1930s (Nanditha and Prabhasankar, 2009). Halliwell *et al.* (1995) reported that antioxidants are also of interest to biologists and clinicians because they may help to protect the human body against damage by ROS. According to the United States Department of Agriculture (USDA), "antioxidants are substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation" (Shahidi and Wanasundara, 1992). Antioxidants, for use in food systems must be inexpensive, non-toxic and effective at low concentrations; high stability and capability of surviving processing; no odour, taste or colour of their own; easy to incorporate and have a good solubility in the product (Kiokias *et al.,* 2008).

One of the 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 and Naczk, 2004a). The classic route of autoxidation includes initiation (production of lipid free radicals), propagation and termination (Production of nonradical products) reactions (Shahidi and Wanasundara, 1992). A general schematic pathway for autoxidation of polyunsaturated lipids is shown in Figure 2.1.

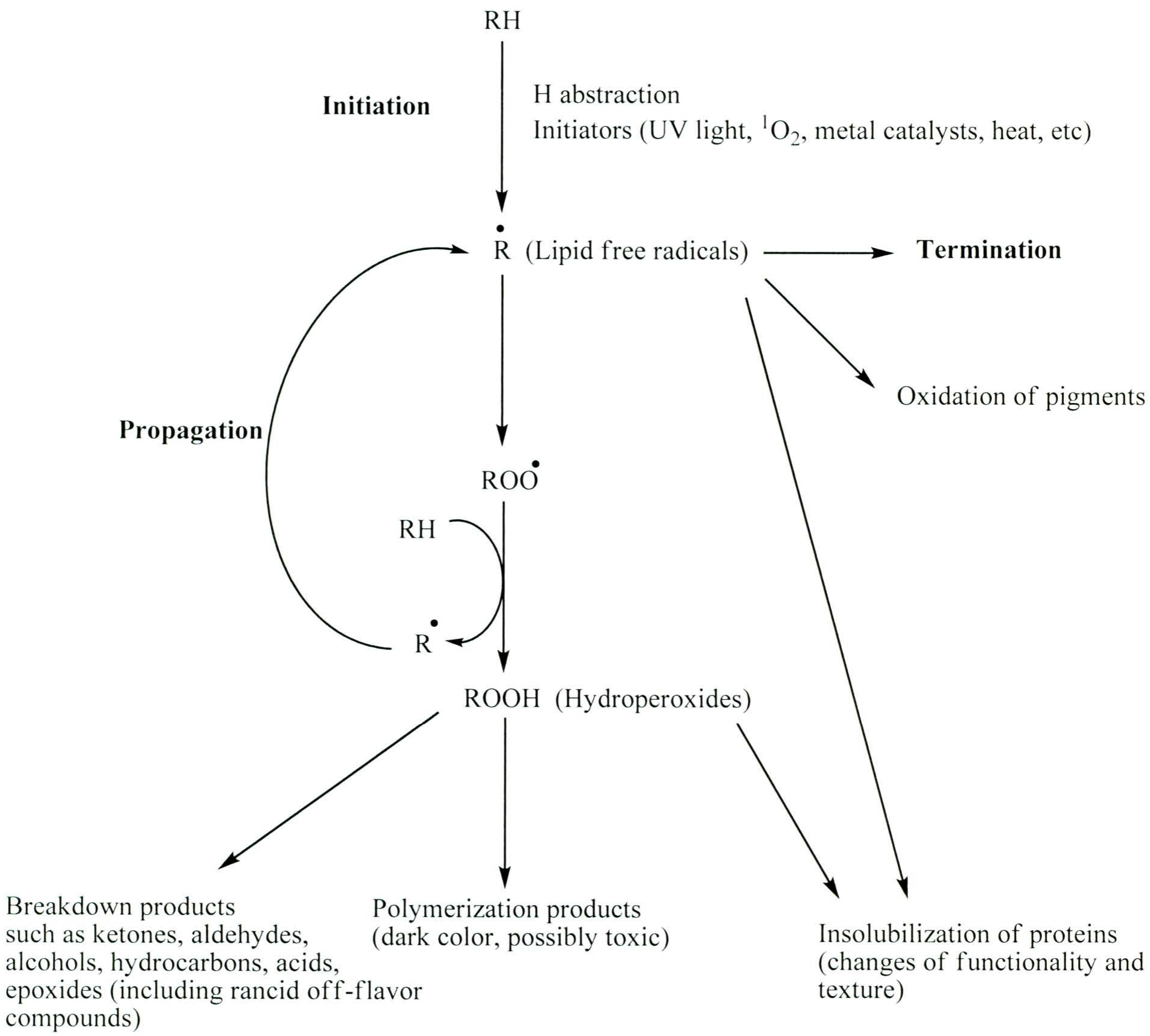


Figure 2.1. General scheme for autoxidation of lipids containing polyunsaturated fatty acids (RH) and its consequences.

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 reactions in order to prevent continued hydrogen abstraction from substrates (Shahidi, 2000, 2002; Shahidi and Naczk, 2004).

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 sulphydryl and amine groups in proteins and this may alter the functionality of proteins (McClements and Decker, 2007). Faustman *et al.* (1999) reported the ability of unsaturated aldehydes to react with histidine in myoglobin and accelerate the oxidation of oxymyoglobin.

Using antioxidants in food is to extend the shelf life of food stuffs and to reduce nutritional loss by inhibiting or delaying oxidation. In general, antioxidants are defined as organic compounds capable of neutralizing reactive oxygen or nitrogen species; these compounds can donate an electron or hydrogen atom to quench free radicals (Eskin and Bird, 2007). The bioactive phenolic compounds acting as antioxidants are substances that when present at low concentrations compared with that of an oxidizable substrate significantly delay or inhibit oxidation of that substrate (Subhashinee *et al.,* 2006). These

antioxidants are now added intentionally to foods to prevent lipid oxidation and are either synthetic or natural in their origin (Halliwell and Gutteridge, 1999). Synthetic antioxidants that are approved for use in food include phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhyroquinone (TBHQ), propyl gallate (PG) (Figure 2.3), and non-phenolics such as ascorbic acid, erythhrobic acid, and ascobyl palmitate (Shahidi *et al.,* 1987; Frankel, 1996). Natural antioxidants include tocols and their derivatives (Shahidi and Wanasundara, 1992; Hall, 2001), carotenoids, antioxidant enzymes and a large number of phenolic compounds of mainly plant origin. The importance of antioxidants contained in food is well appreciated for both preserving foods themselves and for supplying essential antioxidants *in vivo*.

2.2 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 *et al.,* 1997; Sang *et al.,* 2002). Phenolic antioxidants (AH) can donate hydrogen atoms to lipid radicals and produce lipid derivatives and antioxidant radicals (Reaction V), 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 VI and VII).

$$R^{\cdot} / RO^{\cdot} / ROO^{\cdot} + AH \longrightarrow A^{\cdot} + RH / ROH / ROOH \quad (V)$$

$$RO^{\cdot} / ROO^{\cdot} + A^{\cdot} \longrightarrow ROA / ROOA \quad (VI)$$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot} \quad (VII)$$

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 and 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 ($E^{o\prime} = 500$ mV) which have reduction potential below that of peroxyl radicals ($E^{o\prime} = 1000$ mV), are capable of donating their hydrogen to the peroxyl radical to form a hydroperoxide (McClements and Decker, 2007). The phenoxyl radical is stabilized by delocalization of its unpaired electron around the aromatic ring (Figure 2.2) which participates in the termination reaction.

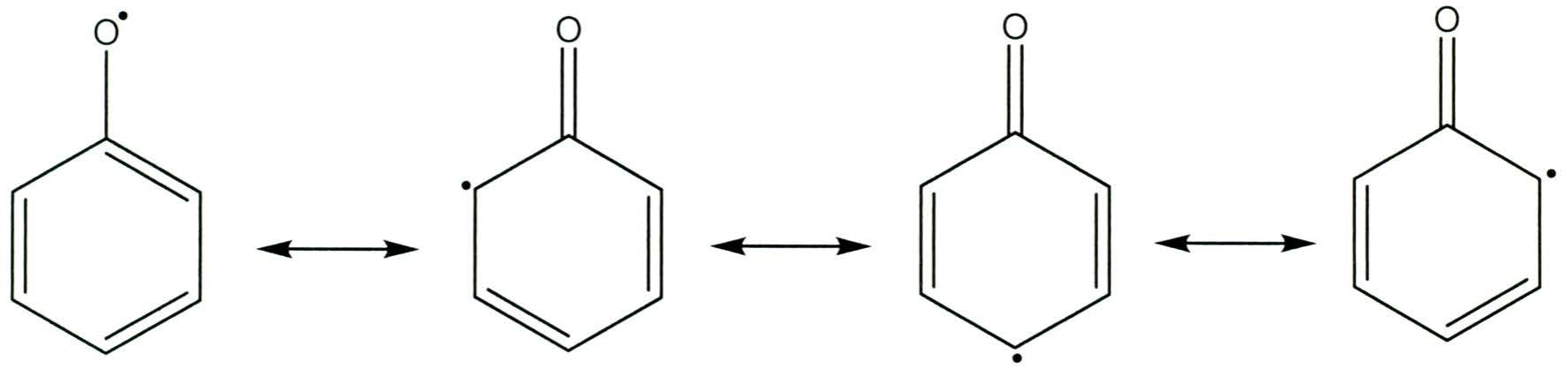


Figure 2.2. Resonance stabilization of phenoxyl radical

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 and Quakenbush, 1957), since these substituents increase the steric hinderance in the region of the radical and thereby further reduce the rate of propagation reactions involving the antioxidant radical (Reactions VIII, IX, X).

$$\dot{A} + O_2 \longrightarrow AOO^{\cdot} \qquad \text{(VIII)}$$

$$AOO^{\cdot} + RH \longrightarrow \dot{R} + AOOH \qquad \text{(IX)}$$

$$\dot{A} + RH \longrightarrow AH + \dot{R} \qquad \text{(X)}$$

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 and Naczk, 2004a). Often phenolic antioxidants lose

their activity at high concentrations and behave as prooxidants (Gorden, 1990) by involvement in initiation reactions (Reactions XI, XII).

$$AH + O_2 \longrightarrow \dot{A} + HOO^{\cdot} \qquad \text{(XI)}$$

$$AH + ROOH \longrightarrow RO^{\cdot} + H_2O + \dot{A} \qquad \text{(XII)}$$

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 and 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 and Wanasundara, 1992).

2.3 Measurement of Antioxidant Activity

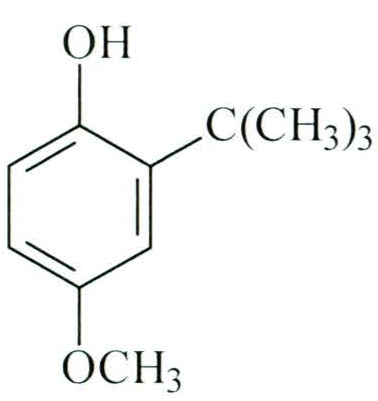
The need to measure antioxidant activity is well documented; these are carried out for meaningful comparison of foods or commercial products and for provision of quality standards for regulatory issues and health claim (Shahidi and Ho, 2007). Lipid oxidation is conventionally studied by determination of peroxide value (PV), thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), or *para*-anisidine value (*p*-AV) or by assessing volatile compounds (Kristinova *et al.*, 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 associated changes using

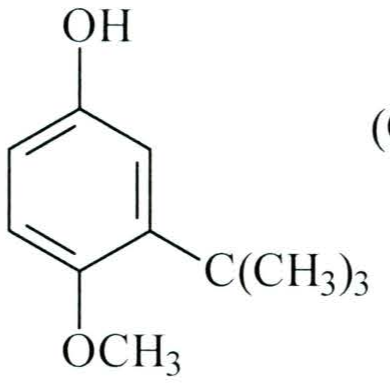
physical, chemical or instrumental means. Radical scavenging assays include methods based on hydrogen atom transfer (HAT) or electron transfer (ET) mechanisms. Oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assays are the major methods that measure HAT while trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays represent ET-based methods (Shahidi and Ho, 2007). HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation while ET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals (Shahidi and Zhong, 2005, 2007). Table 2.1 summarizes the methods commonly used to measure antioxidant activity and the units they carry.

Table 2.1. Antioxidant activity measurement methods and units

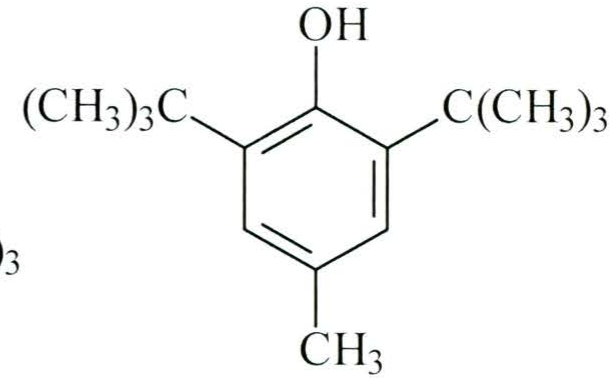
Methods	*Measurement units*
Peroxide value (PV)	milliequivalents of oxygen per kilogram of sample (meq/kg)
Conjugated dienes and trienes	conjugable oxidation products (COPs)
Thiobarbituric acid reactive substances (TBARS)	Milligrams of malondialdehyde (MDA) equivalents per kilogram sample or micromoles 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	Oil stability index (OSI) value, which is defined as the point of maximal change of the rate of oxidation
Oxygen radical absorbance capacity (ORAC)	µmol of trolox equivalents
Total radical-trapping antioxidant parameter (TRAP)	µmol per litre peroxyl radical deactivated
Trolox equivalent antioxidant capacity (TEAC)	mM Trolox equivalent to 1 mM test substance
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	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)	Absorbance of Fe^{2+} complex at 593 nm produced by antioxidant reduction of corresponding tripyridyltriazine Fe^{2+}complex



2-BHA



3-BHA

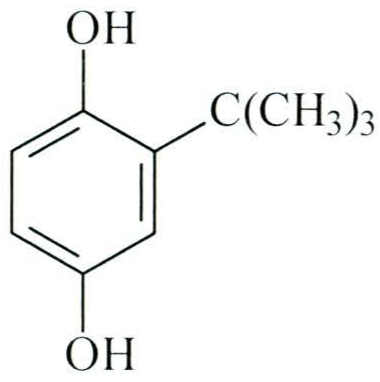


Butylated hydroxyanisole

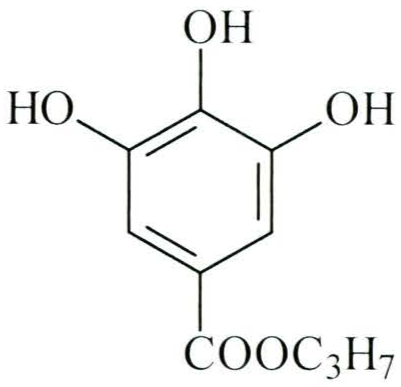
(BHA)

Butylated hydroxytoluene

(BHT)



Tertiary-butylhydroquinone
(TBHQ)



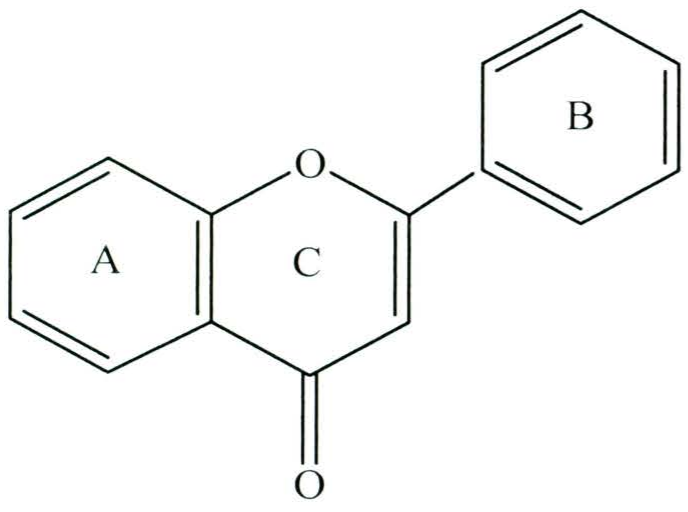
Propyl gallate

(PG)

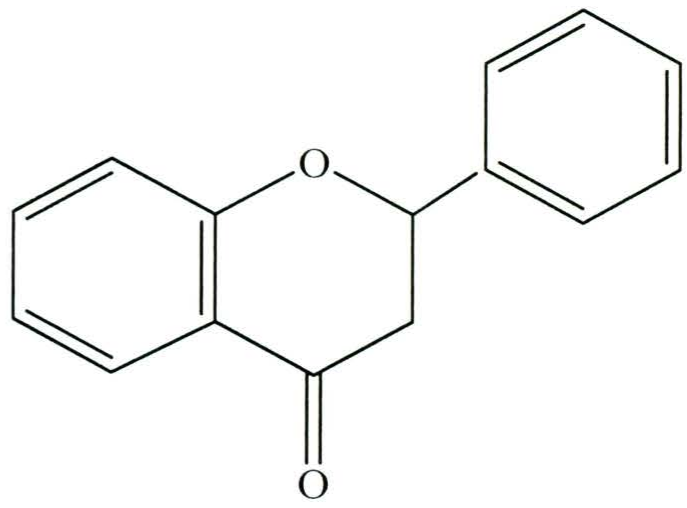
Figure 2.3. Structures of some synthetic antioxidants

Natural sources of antioxidants are mainly found in a variety of foods. These include fruits, vegetables, and whole grains. According to Thiel and Nutropath (2010) natural antioxidants found in food have distinctive benefits, which cannot be duplicated in synthetic antioxidants. This section will explore some sources of natural antioxidants and will specifically discuss fruits and vegetables, onions, and potatoes as sources of natural antioxidants.

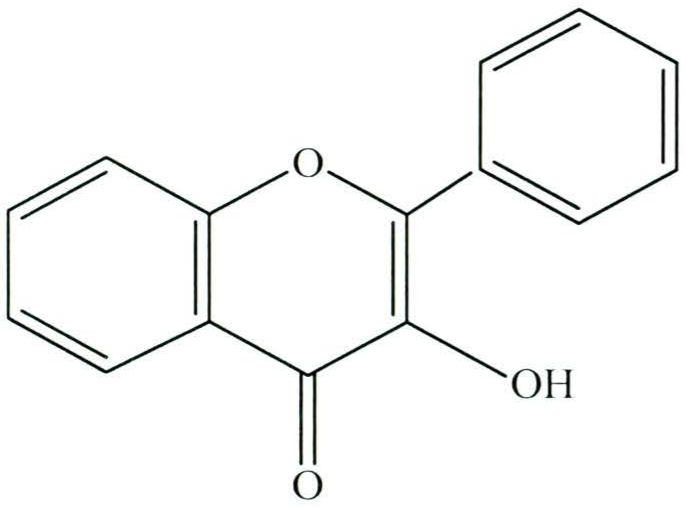
Naturally-occurring antioxidative compounds in food include flavonoids, phenolic acids, lignans, terpenes, tocopherols, phospholipids and polyfunctional organic acids, among others. As already mentioned, sources of natural antioxidants are primarily plant phenolics that occur in all parts of the plants. They can be found in fruits, vegetables, nuts, seeds, leaves, flours, roots and barks (Wanasundara *et al.,* 1996). There have been numerous studies on the biological activities of phenolics, which are potent antioxidants and free radical scavengers (Naczk and Shahidi, 2004; 2006; Tung *et al.,* 2007). Figure 2.4 provides the chemical structures of certain natural antioxidant compounds.



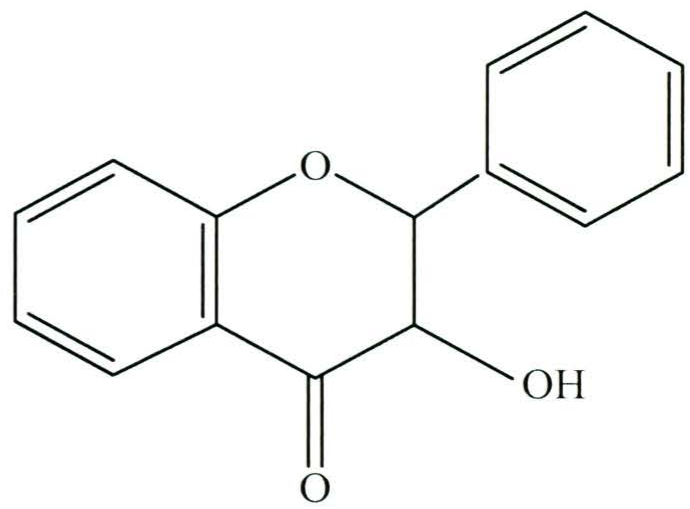
Flavone



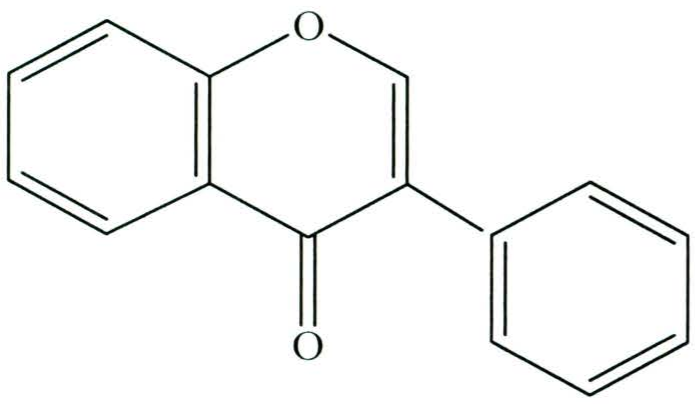
Flavanone



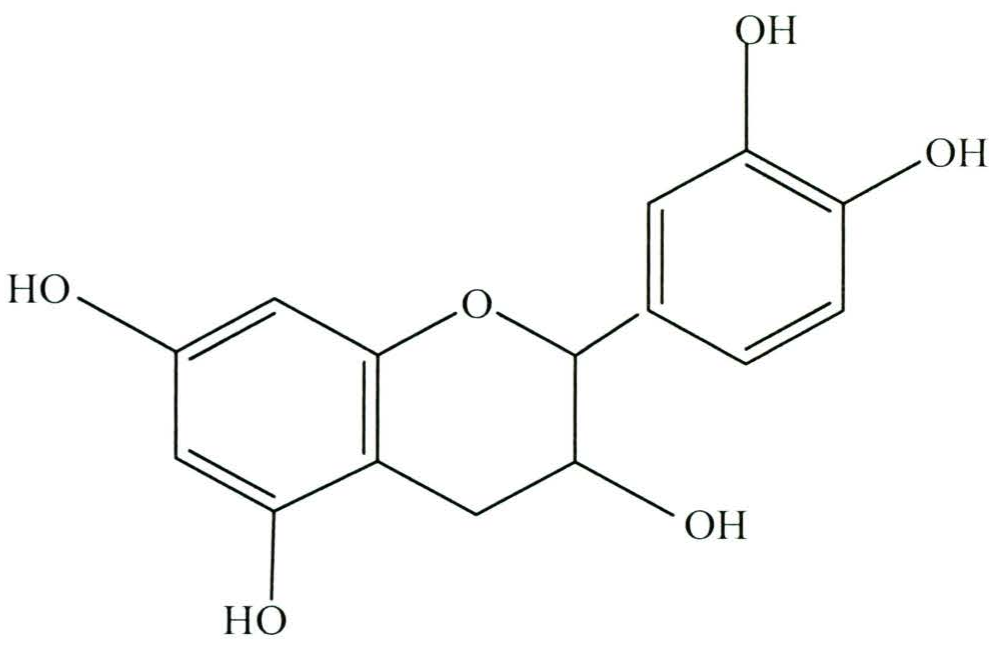
Flavonol



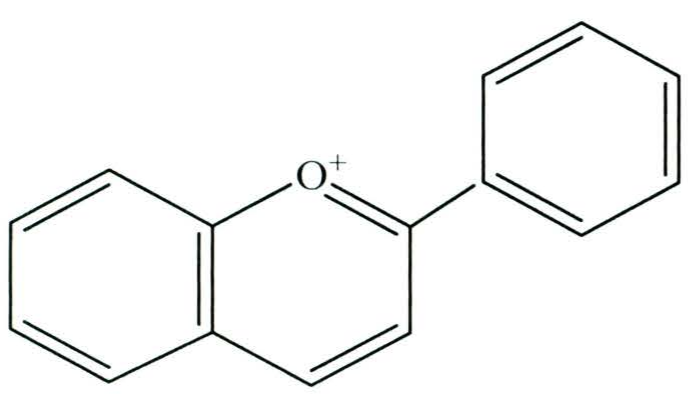
Flavanonol



Isoflavone



Catechin



Anthocyanidin
(Flavylium cation)

Figure 2.4. Chemical strucures of selected natural antioxidant compounds

2.4 Benefits of bioactive of polyphenolics

Polyphenols are one of the antioxidant groups of compounds that are found in fruits and vegetables (Chodak and Tarko, 2011). There are over 4000 different species of plant phenols which have unique biological, chemical and physical properties that make them powerful antioxidants (Figure 2.5). Phenolic acids are structurally related to flavonoids and serve as precursors in their biosynthesis. Phenolic acids such as hydroxycinammic acids (caffeic, *p*-coumaric, ferulic, and sinapic acids, hydroxycoumarine (scopoletin), and hydroxylbenzoic acids (4-hydroxybenzoic, gallic, protocatechuic, vanillic, salicylic, and gentisic acids) are phenolic compounds that can form complexes with metal ions. Antioxidant activity of these compounds varies greatly and is also dependent on the food system.

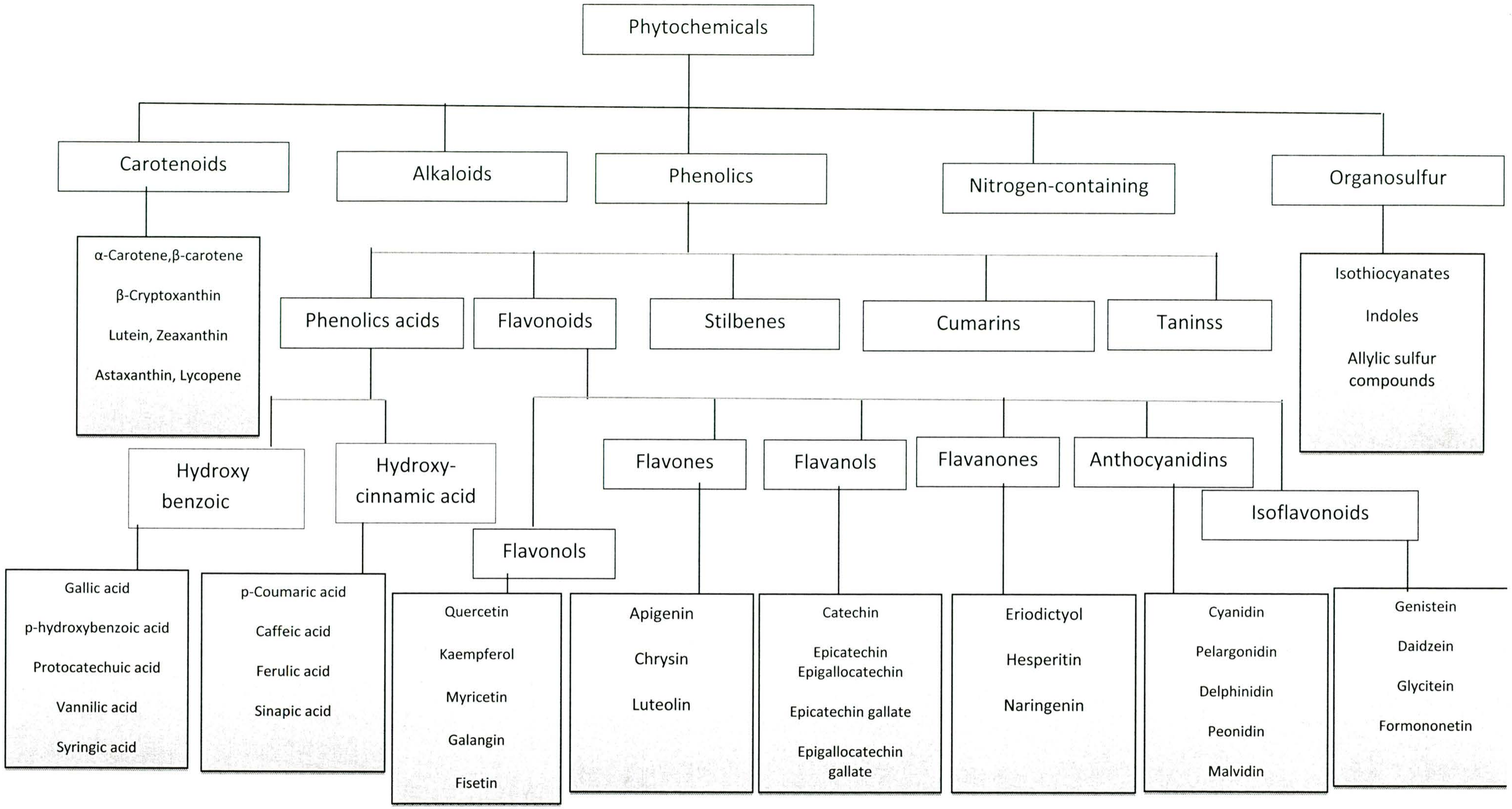


Figure 2.5. Classification of dietary phytochemicals (Adapted from Shahidi and Ho, 2007).

2.4.1 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, anti-inflammatory, antimicrobial, and other biological properties (Xu *et al.,* 2008). Substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids in plants, with hydroxycinnamic acids being the more common. These derivatives differ in the pattern of the hydroxylation and methoxylation in their aromatic rings (Shahidi and Naczk, 2004; Mattila and Hellström, 2007). Technically speaking, only benzoic acid derivatives are phenolic acids and cinnamic acid derivatives are phenylpropanoids (Figure 2.6 and 2.7).

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 catalyzed by phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL), respectively (Amarowicz *et al.,* 2009) (Figure 2.6).

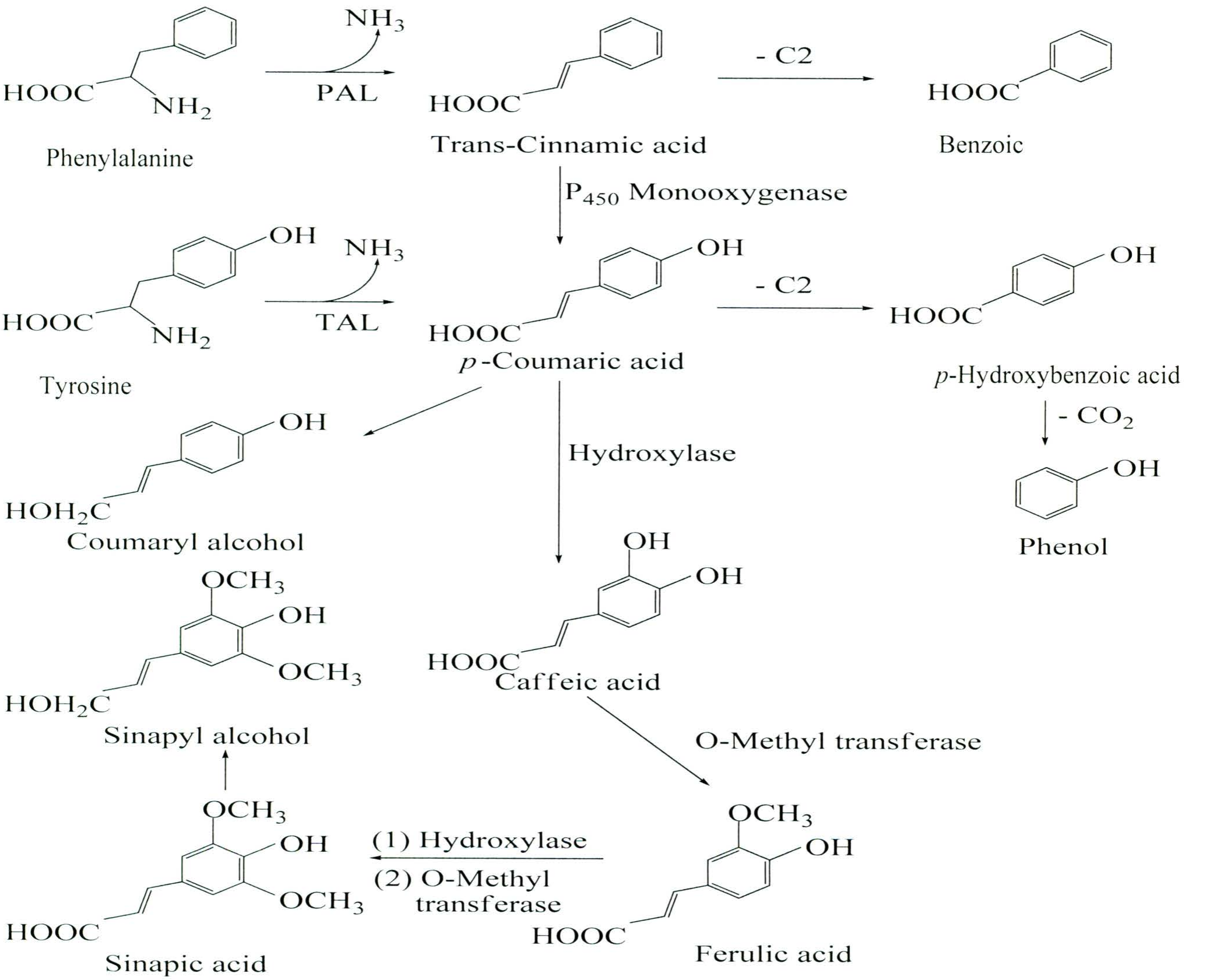
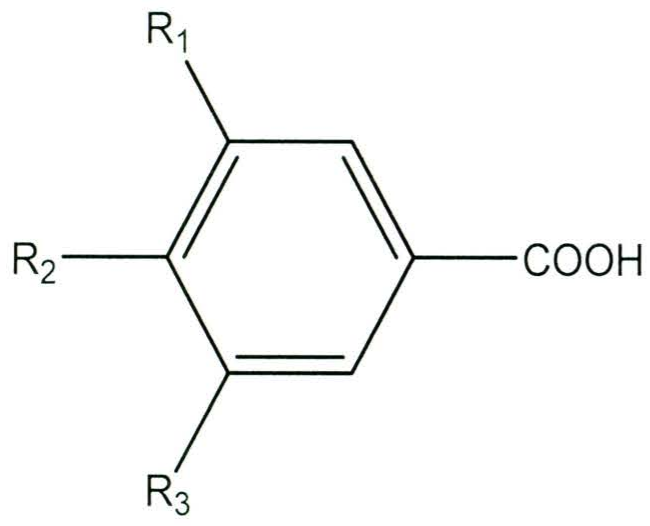
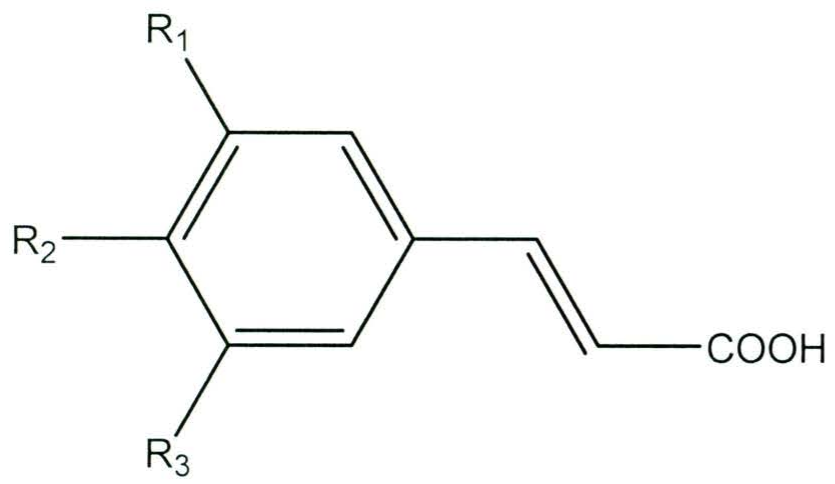


Figure 2.6. Formation of phenylpropanoids of cinnamic acid family as well as benzoic acid derivatives and corresponding alcohols from phenylalanine and tyrosine; PAL: phenylalanine ammonia lyase; and TAL: tyrosine ammonia lyase



Hydroxybenzoic acid

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	OH



Hydroxycinnamic 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

Figure 2.7. Chemical structures of naturally occurring phenolic acids and related compounds

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 are mainly present in foods in the form of glucosides; *p*-hydroxybenzoic, vanillic and protocatechuic acids are the most common forms (Herrmann, 1989; Shahidi and Naczk, 2004; Mattila and Hellström, 2007; Shahidi and Chandrasekara, 2010).

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 and Wanasundara, 1992). Substituents on the aromatic ring affect the stabilization and therefore the radical-quenching ability of these phenolic acids. Different acids therefore have different antioxidant activities (Rice-Evans *et al.,* 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 have received the most attention in the 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

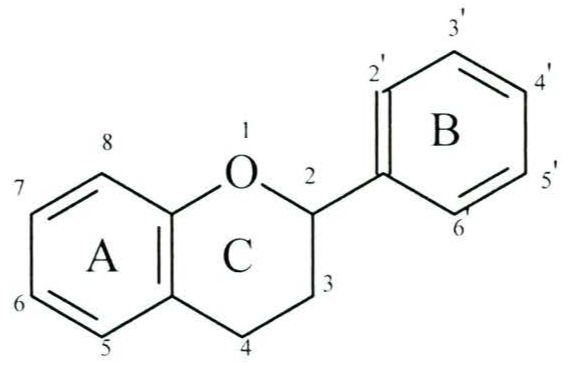
25 mg - 1 g per day depending on the diet consumed (fruit, vegetables, grains, teas, coffees, and spices) (Clifford, 1999).

2.4.2 Flavonoids

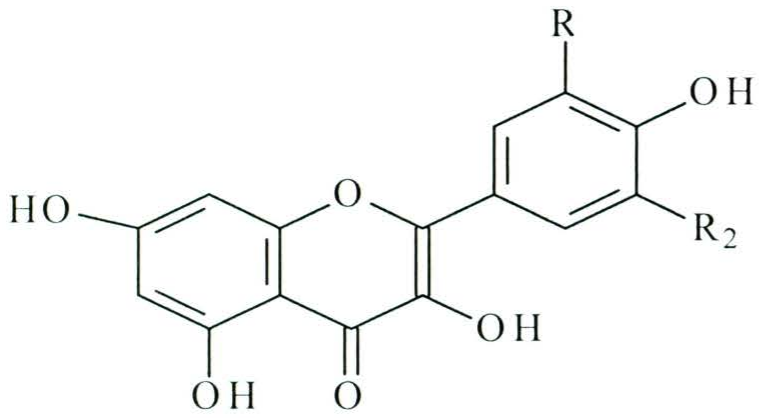
Flavonoids contain a phenolic benzopyran structure (C6-C3-C6) where two aromatic rings A and B are attached to a heterocyclic ring C. As a function of hydroxylation pattern of ring C, flavonoids are further classified into anthocyanin, flavan-3-ols, flavones, flavanones and flavonols (Tsao, 2010) (Figures 2.8 and 2.9). The sub group chalcones is also considered in the flavonoid family, even though it lacks ring C. About 8000 flavonoids have been discovered and there are many more to be identified as of yet (Harborne and Williams, 2000). In plants, flavonoids exist as either glycones or aglycones depending upon the glycosylation patterns. Like other phenolics, flavonoids are crucial for normal growth and development and defence system in plants. Some flavonoids are responsible for importing colour, flavour, odour to the flowers, fruits and leaves (Harborne, 1989; Gharras, 2009). Flavonoids constitute the largest subgroup of phenolics due to their presence as glycosides, methoxides and various acylation pattern on the three rings (Figure2.8). The examples are quercetin and kaempferol which have 279 and 347 different glycosidic compounds, respectively (Tsao and McCallum, 2009; Williams, 2006).

Human intake of all flavonoids is estimated at hundred milligrams, varying by nearly 50-fold, from 20 to 1,000 mg/day (Mullie *et al.,* 2007). The total average intake of

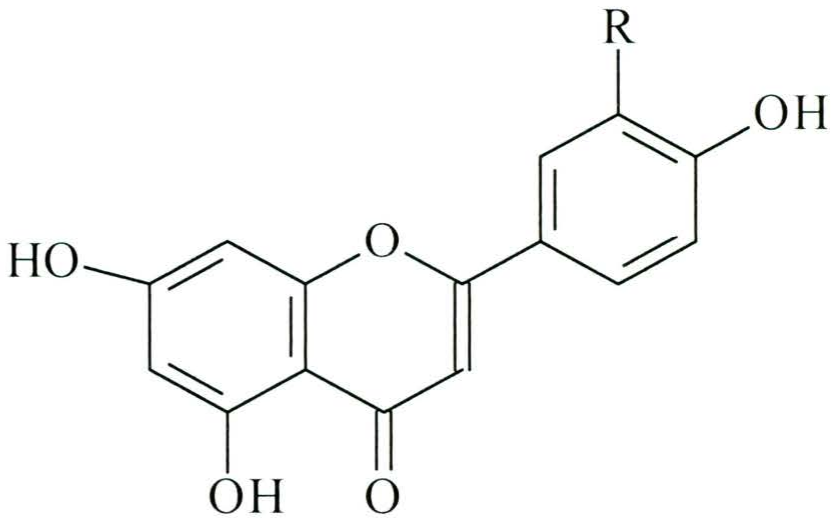
flavonols (quercetin, myricetin, and kaempferol) was estimated at 23 mg/d, of which quercetin contributed ~70%, kaempferol 17%, and myricetin 6% (Hertog *et al.,* 1993



General structure of flavonoids

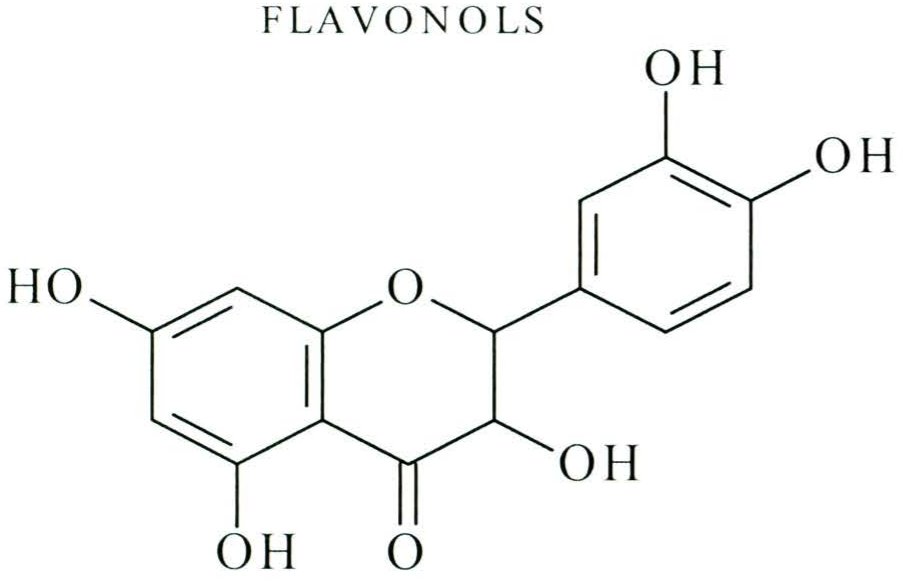


Kaempferol $R_1 = H, R_2 = H$
Quercetin $R_1 = H, R_2 = OH$
Myricetin $R_1 = OH, R_2 = OH$
Isorhamnetin $R_1 = OCH_3, R_2 = H$



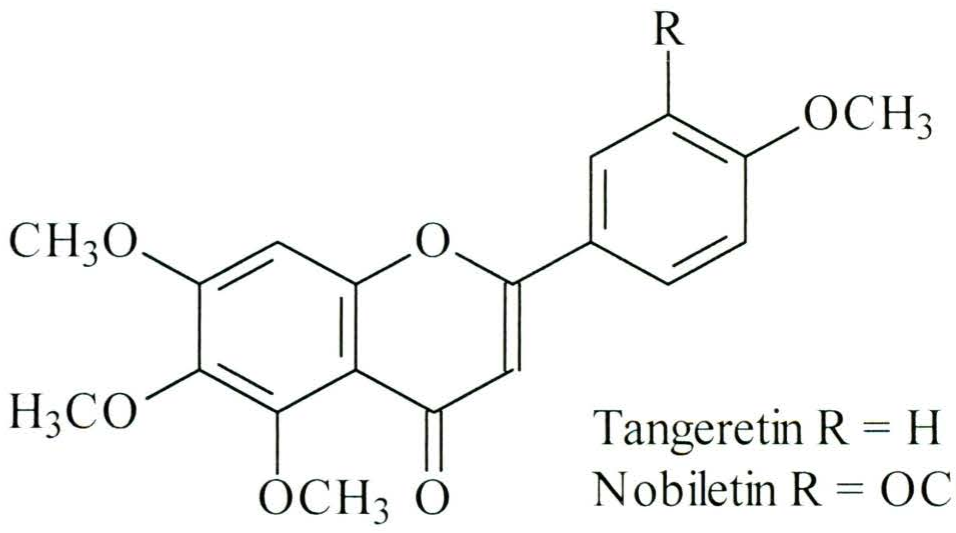
Apigenin R = H
Luteolin R = OH

FLAVONOLS



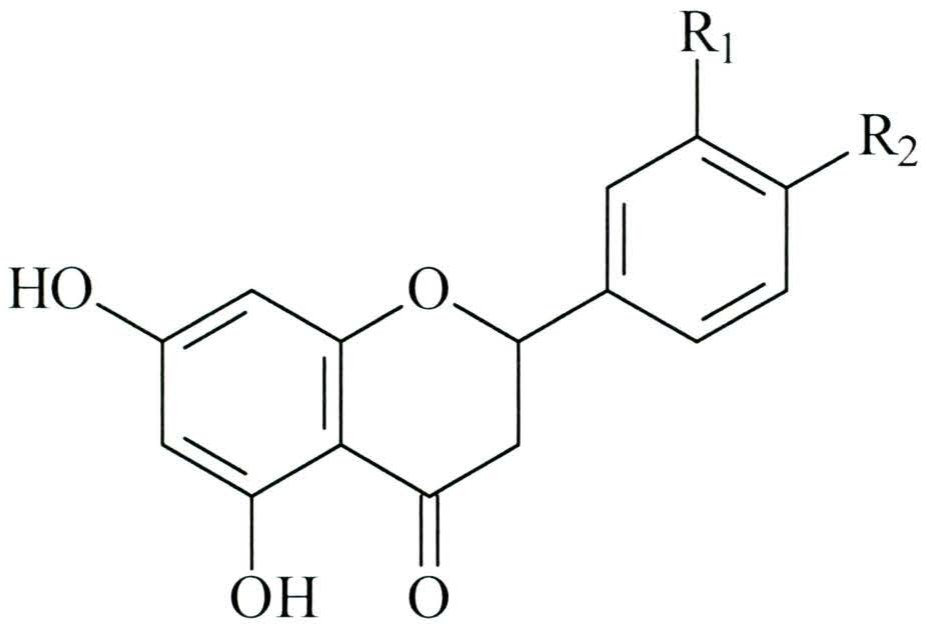
Taxifolin

FLAVANONOLS



Tangeretin R = H
Nobiletin R = OCH_3

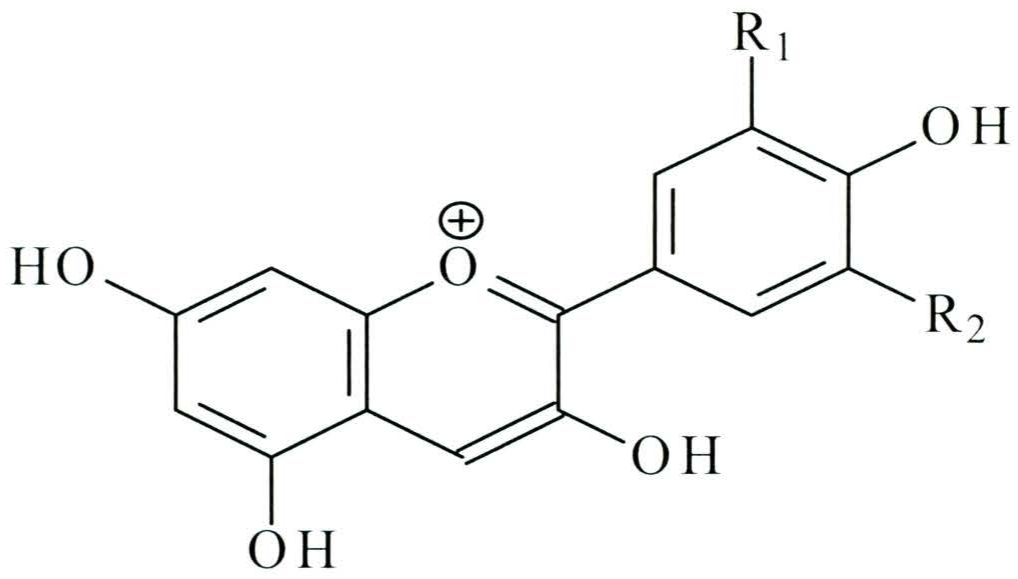
FlAVONES



Naringenin $R_1 = H, R_2 = OH$

Hesperetin $R_1 = OH, R_2 = OCH_3$

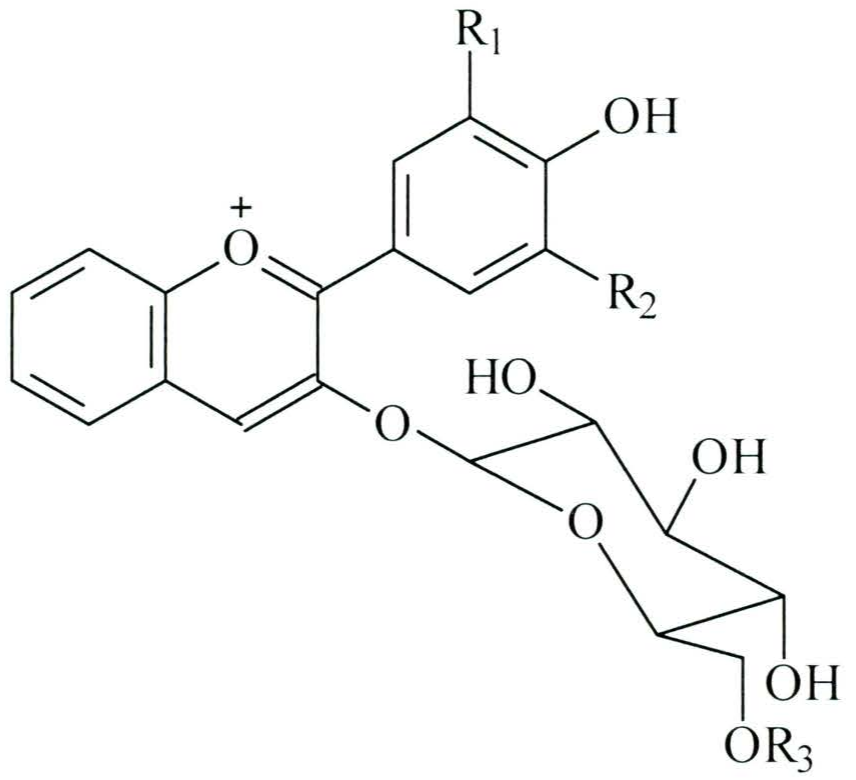
FLAVANONES



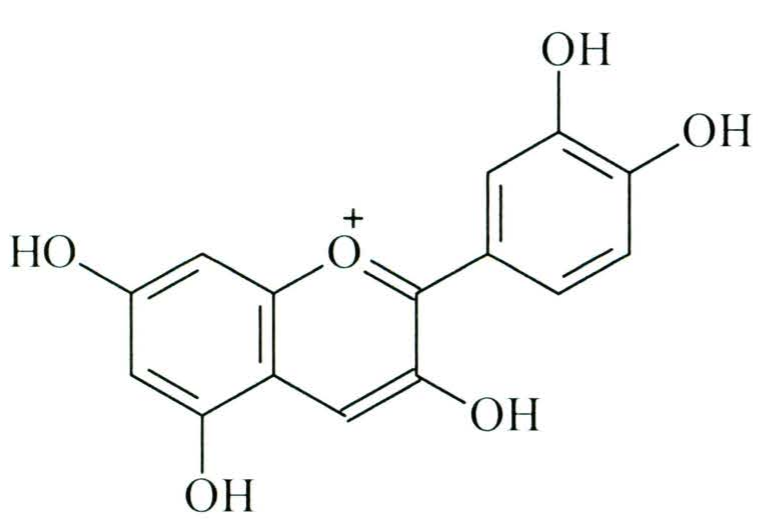
Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Pelargonidin	H	H
Malvidin	OCH_3	OCH_3
Peonidin	OCH_3	H
Petunidin	OH	OCH_3

ANTHOCYANIDINS

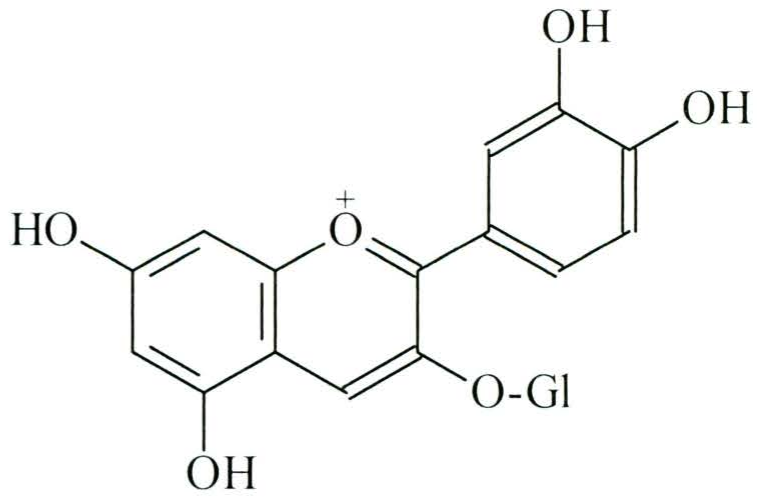
Figure 2.8. Chemical structures of selected flavonoids



ANTHOCYANINS



Cyanidin



Cyanidin-3-glucoside

Figure 2.9. Chemical structures of anthocyanins and cyanadin

2.5 Fruits and Vegetables as Sources of Antioxidants

Numerous naturally occurring phenolic antioxidants have been identified in plant sources and vegetable extracts. A study conducted by Asgard *et al.* (2007) revealed that fruits and vegetables have optimal antioxidative status that boosts defense among patients with type 2 diabetes. In this study, it was noted that patients with type 2 diabetes suffer from oxidative stress due to diminished antioxidative defense. These result in further complications among these patients, including kidney problem, eye problem and problems associated with the circulatory system. This study attributed this diminished defense to the formation of hyperglycaemia which leads to the development of ROS. Asgard *et al.* (2007) also noted that patient suffering from type 2 diabetes have higher levels of peroxidation products. The study revealed that high vegetable and fruit intake can lead to a significant reduction in the level of inflammation.

A similar study was conducted by Ziebland and Roe (2002) who investigated the antioxidant potential of fruits and vegetables. The specific aim of this study was to explore the impact of consumption of fruits and vegetables on the concentration of plasma antioxidants and effects on blood pressure. Ziebland and Roe (2002) conducted an experiment that entailed a six-month controlled and randomized trial on 690 participants. These healthy participants were dividing into two groups; the control and the experimental group. Individuals in the experimental groups were contacted regularly over the period of six months and given message to reinforce and encourage fruits and vegetables consumption. The control group was not contacted during this period. After the six months period the concentration of α-tocopherol and β-cryptoxanthin was

analyzed in both the control and the experimental group. The results revealed that there was higher plasma concentration of α-tocopherol and ß-cryptoxanthin in the experimental group than in the control group. There was also a notable fall in blood pressure among individuals in the experimental group. This result supports the views that fruits and vegetables are essential sources of antioxidants.

According to Ziebland and Roe (2002) the increase in plasma antioxidant level has a clinical significance as it can reduce the risk of coronary heart disease; carotid arteries; epithelial cancer and stroke. They also noted that though the small drop in blood pressure would not produce significant clinical effects, it would lead to substantial reduction of cardiovascular ailments at the population level. According to research a reduction of diastolic blood pressure leads to a reduction of hypertension by 17%; reduction in cardiovascular incidence by 6% and reduction of risk of stroke by 15%. Fruits and vegetables contain hundreds of different antioxidant substances and other phytochemicals (Thiel , 2010). These deoxidizing/ antioxidant substances are useful in managing oxidative stress which is caused by an imbalance between free radicals within the body and the antioxidant defense mechanism (Asgard *et al.,* 2007).

2.5.1 Onion as a Rich Source of Flavonoid

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 (Patil *et al.,* 1995; Cook and Samman, 1996). Two major components quercetin monoglucoside and quercetin

diglucoside account for 80% of the total flavonoids in onions (Rhodes and Price, 1996). Levels of quercetin glucosides are much higher in onions than in other vegetables (Shahidi and Naczk, 2004). Anthocyanins are only minor components of the flavonoid spectrum in the edible portion of red onion varieties. However, 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 and Price, 1996). Yellow onions have been found to contain higher levels of quercetin than red onions, with pink and white onions having the lowest concentration (Patil *et al.,* 1995). However, Gokce *et al.* (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 *et al.,* 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 and 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 *et al.,* 1997), are located in the red onion skin and the outer fleshy layer (Gennaro *et al*., 2002). Fossen *et al.* (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 whole red onion. Gennaro *et al.* (2002) have demonstrated that cyanidin and delphinidin derivatives constitue over 50 and 30% of total anthocyanins in whole red onion, repectively.

Recently pasteurized 'Recas' paste was chosen to be the most appropriate onion by-product for developing an antioxidant food ingredient among all the onion by-products analyzed (Roldán *et al.,* 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 *et al.* (2003) compared the antioxidant activities of onion and garlic extracts and found that onion had clearly higher radical scavenging activities than garlic; red onion being more active than yellow onion. The skin extracts of red and yellow onion possessed the highest activities. Table 2.2 summarizes the flavonoid content of some selected onions as mg/100g of edible portion.

The onion bulb consists of several layers with the outmost layers being a crispy and scaly skin that is often thrown discarded. However, studies have revealed that this outer layer of onion consists of very high content of flavonoids, mainly quercetin (Park *et*

al., 2007). Park *et al.* (2007) examined the impact of onion peel or flesh on lipid peroxide and DNA damage in old male rats. They reported that the total quercetin and antioxidant levels where highest in the diet containing onion peel extract, while onion peel powder came second, and ethanol extract of onion flesh had the third highest content.

Table 2.2. Flavonoid content of some selected onions (Adapted from USDA, 2007).

Onion Description	Class	Flavonoid	Minimum (mg/100g)	Maximum (mg/100g)
Onion, spring, red, leaves (*Allium cepa*)	Flavanols	Kaempferol	4.10	4.10
		Quercetin	12.60	12.60
Onions, cooked, boiled, drained, without salt	Flavanols	Kaempferol	0.29	0.41
		Quercetin	19.87	31.00
Onions, raw (*Allium cepa*)	Flavanols	Isorhamnetin	1.26	7.16
		Kaempferol	0.00	1.00
		Myricetin	0.00	0.03
		Quercetin	1.50	118.70
Onions, red, raw (*Allium cepa*)	Anthocyanidins	Cyanidin	1.3	23.99
		Delphinidin	0.10	3.15
		Pelargonidin	0.02	0.02
		Peonidin	1.22	1.22
	Flavonols	Isorhamnetin	0.00	22.60
		Kaempferol	0.00	4.50
		Myricetin	0.00	3.80
		Quercetin	0.00	191.70
Onions, scallions (includes tops and bulb), raw (*Allium cepa*)	Flavonols	Kaempferol	0.00	3.45
Onions, sweet , raw (*Allium cepa*)		Myricetin	0.00	0.00
		Quercetin	6.7	30.60
	Flavonols	Kaempferol	0.00	1.98
		Myricetin	0.00	4.13
		Quercetin	0.97	46.32
Onions, welsh, raw (*Allium fistulosum*)	Flavonols	Kaempferol	22.62	27.28
Onions, white, raw (*Allium cepa*)	Flavonols	Kaempferol	0.00	0.00
		Myricetin	0.00	0.00
		Quercetin	0.00	63.40
Onions, young green, tops only (*Allium cepa*)	Flavonols	Kaempferol	2.40	2.40
		Myricetin	0.03	0.03

2.5.2 Potato as a Rich Source of Phenolic acids

Potato is the third most important food crop in the world after rice and wheat in terms of human consumption. More than a billion people worldwide eat potato, and global total crop production exceeds 300 million metric tones based on FAO (2007) statistics. As the first modern "convenience food," potato is energy-rich, nutritious, and easy to grow on small plots, cheap to purchase, and ready to cook without expensive processing (Visser *et al*., 2009).

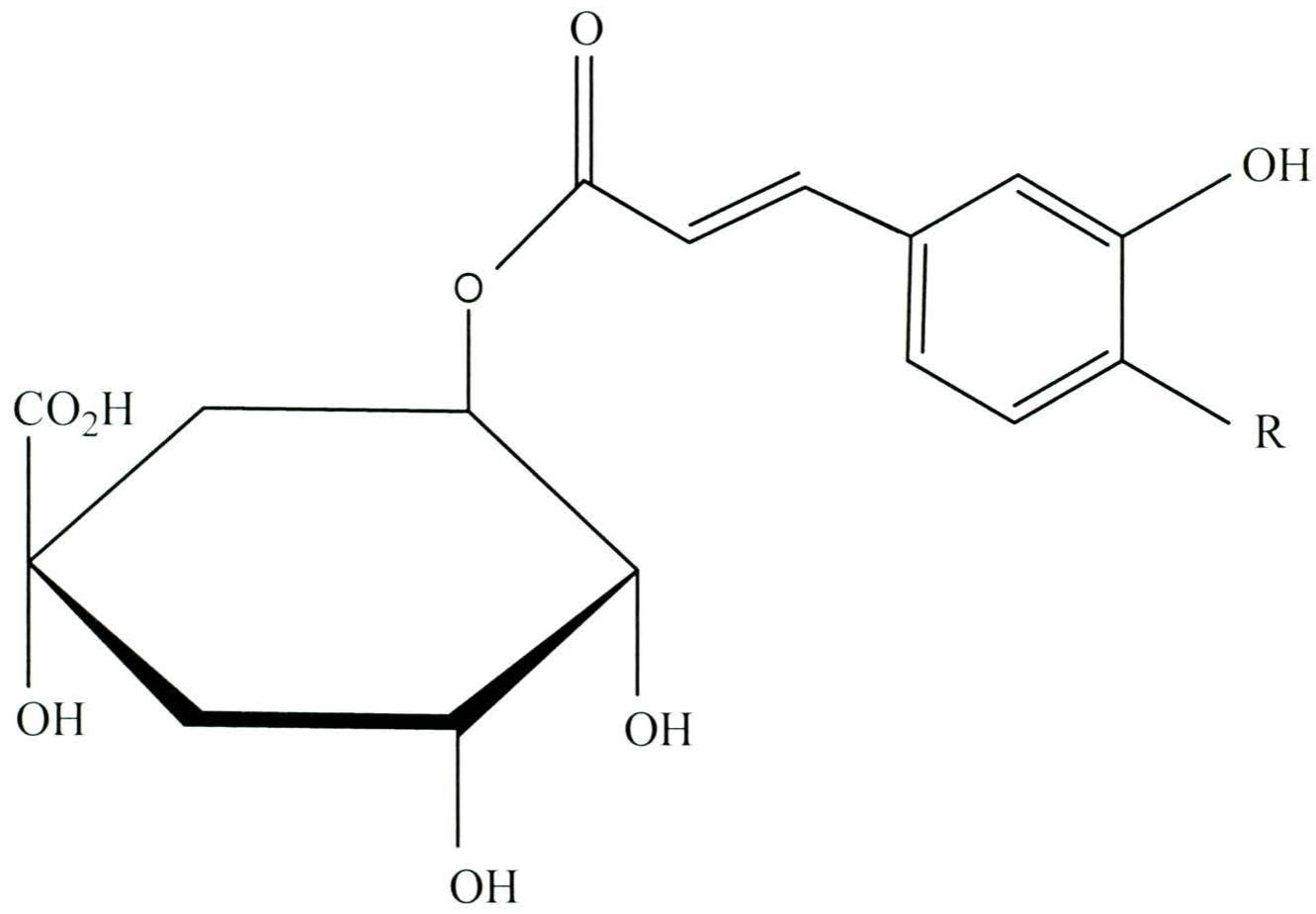
Potatoes have been included in the list of foods as natural sources of antioxidants. Recent studies have proven that potatoes contain a variety of substances that makes them powerful sources of antioxidants. The chemistry, biochemistry, and dietary role of potato polyphenols have been reviewed by Friedman (1997). Phenolic compounds in potatoes can be present in both free and bound forms. They are mostly substituted derivatives of hydroxycinnamic acid as free form and hydroxybenzoic acid as bound form (Shahidi and Naczk, 2004). The most common hydroxycinnamic acid derivatives in potato and potato peels were reported to be chlorogenic acid (CGA), caffeic acid (CFA), and ferulic acid (FA), while the hydroxybenzoic acids were gallic acid (GA), protocatechuic acid (PCA), and their derivatives (Kanatt *et al.*, 2005; Nara *et al.*, 2006; Al-Weshahy and Rao, 2009). Anthocyanins a sub-group within the flavonoids was present in substantial amounts in pigmented potato flesh (Brown, 2008).

Chlorognic acid (Figure 2.10) the predominant phenol in potato constituted 90% of total phenolics, 50% found in potato peel which decreased gradually from outside toward the centre of the potato tuber (Friedman, 1997). Protocatechuic acid, vanillic acid,

p-coumaric acid and caffeic acid were the second most abundant after chlorogenic acid. In the other studies major phenolics in potato were peel reported to be chlorogenic acid, gallic acid, protocatechuic acid, caffeic acid and quercetin (Rodriguez de Sotillo *et al.,* 1994a; Rodriguez de Sotillo *et al*., 1994b; Al-Saikhan *et al*., 1995; Nara *et al.,* 2006). Other phenolic compounds in potato included ferulic acid, *p*-coumaric acid as well as small amounts of rutin, quercetin, myricetin, kaempferol, naringenin and other flavonoids (Reyes, 2005; Nara *et al.,* 2006). Purple-fleshed potato also contained petunidin- and malvidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acids while red-fleshed potato had pelargonidin- and peonidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acids (Reyes, 2005; Rumbaoa and Geronimo, 2009a, 2009b). A higher anthocyanidin content and more hydroxylated anthocyanidins (malvidin) can contribute to a higher antioxidant activity of purple-fleshed potatoes (Lachman *et al.,* 2008). Warner (2012) found that Russet potato (cooked) had 4,649 mg/kg total antioxidant capacity which was higher than most fruits and vegetables.

Polyphenolic compounds in potatoes exhibited 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 *et al.,* 2004). In related studies, Onyeneho and Hettiarachchy (1993) evaluated the ability of freeze-dried extracts from

six potato peel varieties to prevent soybean oil oxidation and confirmed their strong antioxidant activities.



Chlorogenic acid

Figure 2.10. Chemical structure of chlorogenic acid

2.6 Extraction of polyphenolics

The chemistry of polyphenols varies in different classes of phenolics and this is one of the important factors influencing their extraction. Extraction of phenolics is dependent on the other factors such as sample particle size, solvent system, extraction method, storage time and presence of other substances (Naczk and Shahidi, 2004). The different extraction solvents used for phenolics are methanol, ethanol, acetone, water, propanol, ethyl acetate and their various combinations. The extraction of phenolics can

be improved by adjusting sample- to-solvent ratio (Naczk and Shahidi, 1991). Sample particle size significantly influenced tannin recovery from dry beans (Deshpande, 1985). Optimization of polyphenolic extraction is essential due to large variation in their polarity and biochemical modifications such as glycosylation and esterification that affect the extraction output (Pellegrini *et al.,* 2007). Michiels *et al.* (2012) evaluated various solvent to sample ratios and found that higher extraction of polyphenolics can be achieved at higher solvent-to-sample ratios. The proposed extraction conditions included extraction solvent, acetone-water-acetic acid mixture (70:28:2, v/v/v) with solvent-to-solid ratio of 20:1 (v/w) and extraction for 1 h at 4°C. Komes *et al.* (2011) demonstrated that hydrolysed extracts of medicinal plants (using 60% ethanol and 5 mL of 2 M hydrochloric acid) had higher total phenolic content than non-hydrolysed extracts.

Krygier *et al.* (1982) extracted free, and esterified phenolic acids from oilseeds using a mixture of methanol-acetone-water (7:7:6, v/v/v) at room temperature. First, the free phenolics were extracted with diethyl ether, and then the extract was treated with 4M NaOH under nitrogen. The hydrolyzate was acidified and the liberated phenolic acids were extracted with diethyl ether. After exhaustive extraction with a mixture of methanol-acetone- water, the left-over sample was treated with 4M NaOH under nitrogen to liberate insoluble bound phenolic acids. Similarly, phenolic acids from onions and potatoes were extracted under the same conditions.

2.7 Separation and Identification of Flavonols

The extract of polyphenolics always contains a mixture of different classes of phenolic and non-phenolic substances. Further purification may be needed to isolate the desired phenolic compounds from the crude extract. Several difficulties arise because no universal method can be used to isolate all phenolics. Flavonoids, the most prominent class of phenolics, include flavones, flavonols, flavonones, flavanols, isoflavonoids, and anthocyanins and all have same basic structure. Numerous gas-solid and liquid-solid phase adsorption techniques have been employed to adsorb target phenolics (Zagorodni, 2007; LeVav and Carta, 2007). Krammerer *et al.* (2010) evaluated non-polar adsorbent and ion-exchange resins to optimize the recovery of different phenolics. In their study, phloridzin and rutin were successfully recovered using acidic resin cation-exchange chromatography.

Conventional methods used for purification of polyphenolic include ion-exchange resins and reversed-phase liquid chromatography. Counter-current chromatography (CCC) has recently been explored to be an excellent alternative for isolating various phenolic classes (Pauli *et al.,* 2008). In CCC, separation of compounds is achieved on the basis of partition ratio between stationary liquid phase and mobile liquid phase. Modern commercial CCC includes high-speed counter-current chromatography, multilayer coil counter-current chromatography and centrifugal partition chromatography. Anthocyanins from wine (Salas *et al.,* 2005; Schwarz *et al.,* 2003), flavanols and proanthocyanidins from green tea (Cao *et al.,* 2000) have been fractionated and isolated using CCC.

High performance liquid chromatography (HPLC) is the most widely used analytical technique for the separation and characterization of polyphenolics (Carrasco-Pancorbo *et al.,* 2007; Naczk and Shahidi, 2006; Valls *et al.,* 2009). Reversed-phase columns with C18 stationary phase have enhanced the separation of different compounds (Gruz *et al.,* 2008). Mostly, electrochemical, UV-visible, fluorescent, photodiode array detectors are used with HPLC methods for analysing food phenolics. The different classes of phenolic compounds have been successfully identified and quantified using mass spectrometry detectors coupled to HPLC. Mass spectrometry is selective and hence improves the characterization and identification of phenolic compounds (Nicoletti *et al.,* 2007; Buiarelli *et al.,* 2007). Ion-trap mass spectrometry is an advanced and highly sensitive technique for identification of isomeric flavonoid glycosides which are not identified by mass spectrometry. It is based on the principle of sequential fragmentation of molecular ions (Prasain *et al.,* 2004). The other techniques for polyphenolic analysis are electrospray ionisation (ESI), atmospheric-pressure chemical ionization(APCI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (Valls et al., 2009). Liu *et al.* (2008) separated and quantified different polyphenolics from water samples using HPLC coupled with photodiode array detector. Owen *et al.* (2003) elucidated the structures of polyphenolics present in carob fibre using nano ESI-MS and LC-ESI.

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials

Onion samples, namely pearl, red, yellow, and white varieties were purchased from local markets in St. John's, NL, Canada. Some of the red onions were allowed to sprout to test the phenolic composition of the sprouted red onion flesh and the green shoots. Peels from Russet and Innovator variety of potatoes were procured from McCain Foods Limited, Florenceville, NB, Canada; while the yellow and purple potatoes were purchased from local markets in St. John's, NL, Canada.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Acros Organics (Fair Lawn, NJ). Organic solvents and reagents such as methanol, acetone and sodium carbonate were purchased from Fisher Scientific Co. (Nepean, ON). 2,2'-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), Folin and Ciocalteau's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and all phenolic compound standards with a purity of ≥96% were obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON). Hydrogen peroxide, sodium hydroxide, butylated hydroxyanisole (BHA), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), ferrous sulphate, ferric chloride as well as mono- and dibasic sodium and potassium phosphates, ethylenediaminetetraacetic acid (EDTA), deoxyribonucleic acid (DNA) of pBR 322 (*E.coli* strain RRI) and human LDL

cholesterol were also purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

3.2 Methods

3.2.1 Preparation of crude extracts

Onions were peeled manually, and the skin was freeze dried for 3 days at -48°C and 30 x 10^{-3} mbar (Freezone 6, model 77530, Labconco Co., Kansas City, MO). The flesh of red onion and sprouted red onion were also separated, cut and freeze dried. The dried samples were then ground and sieved using a 0.5mm sieve, vacuum packed and stored in a freezer at -20°C until used for analysis within 5 days. All experiments were carried out in triplicates and the results were reported as mean ± standard deviation. The methodologies followed are described below. Yellow and purple potatoes were peeled manually, and their skins freeze dried for 3 days at -48°C and 30 x 10^{-3} mbar (Freezone 6, model 77530, Labanco Co., Kansas City, MO, USA). The flesh of Russet, Purple and Yellow potatoes was also separated, cut and freeze dried. Peels from Russet and Innovator variety of potatoes, procured from McCain, were also immediately freeze dried; these were received as fresh-frozen samples). The dried samples were then ground and to pass through a 0.5mm sieve, vacuum packed and stored in a freezer at -20°C until analyzed.

Extraction of phenolics

Free, esterified, and insoluble-bound phenolic compounds were extracted and fractionated as described by Krygier *et al.* (1982) as modified by Naczk and Shahidi (2006). Freeze dried onion skins and flesh samples (5g) were ultrasonicated for 20 min at 30°C with 150 mL of a mixture of methanol–acetone–water (7:7:6, v/v/v). The resulting slurries were centrifuged at 4000 x *g* (ICE Centra MS, International Equipment Co., Needham Heights, MA) for 5 min and the supernatants collected. The residue was re-extracted under the same conditions. After centrifugation, the combined extracts were analyzed for free phenolic acids and soluble phenolic esters, and the residue was reserved for determination of insoluble-bound phenolics. The combined supernatants were evaporated under vacuum at 40°C to remove the organic solvents, and the aqueous phase was adjusted to pH 2 before extraction with hexane to remove interfering lipids (Krygier, 1982).

The free phenolic acids were then extracted 4 times with diethyl ether-ethyl acetate (1:1, v/v), dried under vacuum using a rotary evaporator and the extract was dissolved in 5 mL of 80% methanol (HPLC grade). The esters remaining in the aqueous phase were hydrolyzed with 4M NaOH and the liberated phenolic acids were extracted with diethyl ether-ethyl acetate (1:1, v/v), dried and dissolved in 5 mL methanol as in the case of free phenolics. The residues were initially dispersed in 50 mL of 4 M NaOH and stirred for 4 hours under nitrogen. The solution was then acidified to pH 2, centrifuged and the bound phenolics were extracted with diethyl ether- ethyl acetate (1:1, v/v).

3.2.2 Chlorophyll removal

Green shoots which sprouted from red onion flesh were separated as described in Section 3.2.1. However, most experiments for green shoots were carried out after dechlorophyllization of green shoot extracts. Chlorophyll removal was done using liquid-liquid extraction according to Alvarez-Parrilla *et al.* (2011). The crude phenolic extracts (1.5 g) were dissolved in 50mL of 80% methanol and poured into an extraction funnel. Twenty-five millilitres of CH_2Cl_2 were added; the separatory funnel was shaken and allowed to stand for phase separation. The organic phase was removed, and extraction was repeated one more time. Methanol was partially removed under vacuum at 45 °C, and the concentrated slurries were freeze-dried for 72 h at -45 °C (Labconco Corp). Dried extracts were stored at -20 °C. The yield of each extract was then determined.

3.2.3 Determination of total phenolic content

The total phenolic content was determined according to an improved version of the procedure explained by Singleton and Rossi (1965). The Folin Ciocalteu's phenol reagent (0.5mL) was added to centrifuge tubes containing 0.5mL of methanolic extracts. Contents were mixed thoroughly and 1mL of sodium carbonate (75g/L) was added to each tube. To the mixture, 10mL of distilled water were added and mixed thoroughly. Tubes were then allowed to stand for 45min at ambient temperature. Contents were centrifuged for 5min at 4000xg (ICE Centra M5, International Equipment Co., Needham Heights, MA). Absorbance of the supernatant was read at 725nm. A blank sample for each extract was used for background subtraction. Content of total phenolics in each

extract was determined using a standard curve prepared for gallic acid. Total extracted phenolics were expressed as mg of gallic acid equivalents per gram of extract.

3.2.4 Determination of total flavonoid content

Total flavonoid content was measured by the aluminum chloride chlorimetric assay (Zhishen *et al.,* 1999). One millilitre of extracts or standard solution of quercetin (0.75, 1.5, 3 mg/mL) was added to 10mL volumetric flask containing 4 mL distilled water. To the flask, 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL 10% $AlCl_3$ was added. At the 6^{th} min, 2 mL 1M NaOH solution were added and the total volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QE)/g dry plant material. Samples were analyzed in triplicates and the results were expressed as mean ± standard deviation.

3.2.5 Determination of total anthocyanin content

The content of anthocyanins was determined by the pH-differential method of Giusti and Wrolstand (2001). Each extract (0.5 mL of) was diluted with 2.5 mL of 0.025 M potassium chloride, pH 1.0 and 0.4 M sodium acetate buffer, pH 4.5, separately. The diluted solutions were then left at temperature room for 15 min, and the absorbance of each dilution was read at 520 and 700nm against a blank cell filled with distilled water. The anthocyanin content was calculated using the following equation:

Anthocyanins content (mg/100g of dry matter) = A***MW*DF/ (ε*W)**

Where A= absorbance (A_{520nm}-A_{700nm}) pH 1.0 - (A_{520nm}-A_{700nm}) pH 4.5, MW= molecular weight of cyanidin-3-glucoside (C_{15} H_{11} O_6, 449.2), DF= dilution factor, ε= molar absorptivity (26900), and *W*= sample weight (g).

3.2.6 Measurement of total antioxidant capacity by trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay is based on scavenging of 2,2' azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical anion ($ABTS^{\bullet+}$). A solution of $ABTS^{\bullet+}$ was prepared in 2.5mM saline phosphate buffer (PH 7.4, 0.15M sodium chloride) (PBS) by mixing 2.5mM 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) with 2.0 mM $ABTS^{+\bullet}$. The solution was heated for 16 min at 60°C, protected from light and stored in the dark at room temperature until used. The radical solution was used within 2 h as the absorbance of the radical itself decreases with time. Onion extracts were dissolved in PBS at a concentration of 0.17 mg/ mL and diluted accordingly to have them fit in the range of values in the standard curve. For measuring antioxidant capacity, 40 µL of the sample were mixed with 1.96 mL of $ABTS^{\bullet+}$ solution. Absorbance of the above mixture was monitored at 734 nm over a six min period. The decrease in absorbance at 734 nm, 6 min after the addition of a test compound, was used for calculating TEAC values. A standard curve was prepared by measuring the reduction in absorbance of $ABTS^{\bullet+}$ solution at different concentrations of trolox. Appropriate blank measurements (decrease in absorption at 734 nm due to solvent without any compound added) were made and the values recorded (Van den Berg *et al.,* 1999) as modified by Siriwardhana and Shahidi (2002).

TEAC values were determined as follows:

$\Delta A_{trolox} = \{A_{t=0\ trolox} - A_{t=6\ min\ trolox}\} - \Delta A_{solvent\ (0\text{-}6\ min)}$

$\Delta A_{trolox} = m \times [trolox]$

$TEAC = \{\Delta A_{extract} / m\} \times d$

Where, ΔA= reduction in absorbance, A= absorbance at a given time, m= slope of the standard curve, [trolox] = concentration of trolox, and d= dilution factor.

3.2.7 DPPH radical scavenging capacity (DRSC) using electron paramagentic resonance (EPR)

DRSC assay was carried out using the method explained by Madhujith and Shahidi (2006). Two millilitres of 0.18 mM solution of DPPH in methanol were added to 500µl of appropriately diluted free, esterified and bound phenolics extracts in methanol. Contents were mixed well, and after 10min. the mixture was passed through the capillary tubing, which guides the sample through the sample cavity of a Bruker e-scan EPR spectrophotometer (Bruker E-scan, Bruker Biospin Co., Billercia, MA). The spectrum was recorded on Bruker E-scan food analyzer (Bruker Biospin Co.). The parameters were set as follows: 5.02×10^2 receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100.000 G sweep width, 3495.258 G centre field, 5.12 ms time constant, 9.795 GH_Z microwave frequency, 86.00 kH_Z modulation frequency, 1.86 G modulation amplitude. For quantitative measurements of radical concentration remaining after reaction with the extracts, the method of comparative determination based on the

corresponding signal intensity of first-order derivative of absorption curve was used. *DRSC* of the extracts was calculated using the following equation:

DPPH radical scavenging capacity % =

100 – (EPR signal intensity for the medium containing the additive/EPR signal intensity for the control medium) x 100.

From the standard curve plotted for the DRSC of trolox, the scavenging activity of potato extracts was determined and expressed as μmol TE /g dried onion peel/flesh.

3.2.8 Determination of Oxygen Radical Absorbance Capacity ($ORAC_{FL}$)

The determination of $ORAC_{FL}$ was carried out using a Fluostar Optima plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps with fluorecsein as the probe and AAPH as the radical generator. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), at a final reaction volumes of 200 μL in a 96-well Costar 2650 black plate (Nepean, ON, Canada). Fluorescein dissolved in a phosphate buffer (120 μL; 64 nM, final concentration) was injected into each well using the first injector pump into the wells containing the extract (20 μL of onions extract). The mixture was incubated for 20 min at 37 °C in the built-in incubator, and subsequently APPH solution (60 μL; 29 mM final concentration) equilibrated at 37 °C was rapidly injected into the wells using the second pump. The plate was shaken for 4 s after each addition at a 4 mm shaking width. To optimize the signal amplification in order to obtain maximum sensitivity, a gain adjustment was performed at the beginning by manually pipetting 200 μL of fluorescein into a designated well. No more than 35 inner wells of the

96-well plates were used due to increased cycle time. Fluorescence was determined and recorded every minute for 60 min using a Fluostar Optima plate reader, and the antioxidant activity of the extracts was calculated as trolox equivalents using a standard curve prepared with 1-10 μM (final concentration) control (trolox, buffer, fluorescein, and AAPH) and positive control (phosphate buffer and fluorescein) were used. Filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used (Madhujith and Shahidi, 2006).

3.2.9 Reducing power activity

The reducing power of extracts was determined by the method of Amarowicz et al. (1995a) and Oyaize (1986). Briefly, each extract (0.2–1.0 mg) was dissolved in 1.0 mL of distilled water to which was added 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide for determination of reducing power. The mixture was incubated in a water bath at 50 °C for 20 min. Subsequently, 2.5 ml of a 10% (w/v) solution of trichloroacetic acid were added and the mixture was subsequently centrifuged at 1750×*g* for 10 min. afterwards, a 2.5-ml of the supernatant was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride. Absorbance of the reaction mixture was read spectrophotometrically at 700 nm; the increased absorbance of the reaction mixture indicates greater reducing power. Results were expressed as μmoles trolox equivalents of the extract versus absorbance at 700 nm.

3.2.10 Measurement of Iron (II) Chelation Capacity

The chelation of ferrous ions by the extract was estimated by the method of Liyana-Pathirana *et al.* (2006) with some modifications. In brief, 0.5 mL of extract was mixed with 1.85 mL of methanol and 0.05 mL of 1 mmol/L ferrozine, followed by vigorous shaking and allowing the mixture to react at room temperature for 10 min. The absorbance was measured spectrophotometrically at 562 nm. The chelation capacities of onions were expressed as µmol ethylenediaminetetraacetic acid (EDTA) equivalents/g extract. The blank was devoid ferrozine. Iron chelation capacities of the extracts were calculated using the following equation:

Fe (II) chelation capacity, % = (1-Absorbance)* 100/ blank Absorbance

3.2.11 Supercoiled strand DNA scission by peroxyl and hydroxyl radicals

The inhibition activity of the onion extracts against supercoiled DNA strand scission induced by peroxyl radical was evaluated according to the methods of Hiramoto *et al.* (1996) and Chandrasekara and Shahidi (2011). Plasmid supercoiled DNA (pBR 322) was dissolved in 10 mM phosphate buffered saline (PBS) pH 7.4. The DNA (4 µL) was added to 2 µL of extract samples, 4 µL of AAPH (22.5 mM) dissolved in PBS. For peroxyl radical-induced oxidation, the mixture was mixed well and incubated at 37°C for 1h. Upon completion of incubation, 2 µL of the loading dye (consisting of 0.25% bromophenol blue and 0.25% xylene cyanol) was added to the extracts and loaded to a 0.7% (w/v) agarose gel. The gel was prepared in 40 mM Tris-acetic acid-EDTA buffer, 1

mM EDTA, pH 8.5. Thereafter, 5 µL SYBR Safe were added to DNA gel, setting at 85 Volt for 75 min at 4 °C. DNA strands were visualized under ultraviolet light. For hydroxyl radical-induced DNA oxidation, 2 µL of test compounds, dissolved in methanol, were added into an Eppendorf tube and the solvent was evaporated under a stream of nitrogen. To the tube, 2 µL of distilled water were added, followed by through vortexing for 1 min. The following reagents were then added to the tube in the order stated: 2 µL of PBS (pH 7.4), 2 µL of supercoiled pBR322 DNA, 2 µL of H_2O_2 and 2 µL of $FeSO_4$. The mixture (10 µl) containing 1 µM test compound, 0.1 M PBS, 10 µg/mL DNA, 0.2 mM H_2O_2 and 0.1 mM $FeSO_4$ (final concentration/assay) was incubated at 37°C for 1 hour.

The protective effect of extracts was calculated DNA retention (%) based on the following equation.

DNA retention (%) = (Supercoiled DNA content in sample/Supercoiled DNA content in control)*100

3.2.12.1 Cooked comminuted fish meat model system

Fish model systems were prepared as described by Shahidi and Pegg (1990). Ground salmon fish (80 g) was mixed with deionized water (20 mL) in Mason jars. Soluble extracts of onion (200 ppm based on phenolics content) as well as 200 ppm BHA and quercetin, respectively, were added separately to fish mixture in the Mason jars and thoroughly homogenized. A control sample containing no extract was also prepared. Samples were cooked in a thermostated water bath at 80 ± 2°C for 40 min while stirring

every 5 min with a glass rod. After cooling to room temperature, fish samples were homogenized for 30 s, transferred into plastic bags, and then stored in a refrigerator at 4°C for 7 days. Samples for the analyses of TBARS were drawn on days 0 and 7.

3.2.12.2 Determination of 2-thiobarbituric acid reactive substances (TBARS)

The TBARS were determined using a modified version of the method of Siu and Draper (1978), as described by Shahidi and Hong (1991). Samples were analyzed for TBARS on days 0 and 7. Two grams of each sample were weighed into a centrifuge tube to which 5 mL of a 10% (w/v) solution of trichloroacetic acid (TCA) were added and vortexed (Fisher Vortex Genie 2; Fisher Scientific, Nepean, ON) at high speed for 2 min. An aqueous solution (0.02 M) of TBA (5 mL) reagent was then added to each centrifuge tube, followed by further vortexing for 30 s. The samples were subsequently centrifuged at 3000x*g* for 10 min and the supernatants were filtered through a Whatman No. 3 filter paper. Filtrates were heated in a boiling water bath for 45 min, cooled to room temperature in cold water, and the absorbance of the resultant pink-coloured chromogen read at 532nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane as a precursor of the malondialdehyde (MDA; 0, 1, 2, 3 and 6 ppm). The TBARS values were then calculated using the standard curve and expressed as milligrams MDA equivalents per gram sample. Inhibition of TBARS formation was determined using the equation:

% inhibition=100 (1-TBARS value for the treated sample/TBARS value for the control sample).

3.2.13 Determination of proximate composition of salmon fish

Ground fish samples (11.0 g) were weighed into pre-weighed aluminum dishes and placed in a preheated forced-air oven (Fisher Isotemp 300, Fair Lawn, NJ). Samples were maintained at 105±1°C until a constant mass was obtained. The moisture content was then calculated as the percent ratio of the weight difference of the sample before and after drying to (AOAC, 1990).

Total fat content of the samples (25) was determined using the procedure described by Bligh and Dyer (1959). The total fat content was calculated as the percent value of the original samples.

3.2.14 Effect of Onion extracts on preventing cupric ion induced human low density lipoprotein (LDL) cholesterol peroxidation

The method of Chandrasekara and Shahidi (2011) and Andreasen *et al.* (2001) were used to measure inhibitory activities of onion extracts against human LDL cholesterol oxidation. Human LDL cholesterol (in PBS, pH 7.4, with 0.01% EDTA) was dialyzed against 10mM PBS (pH 7.4, 0.15 M NaCl) for 12h under nitrogen at 4°C, and EDTA-free LDL was subsequently diluted with PBS to obtain a 0.1 mg/mL. The diluted LDL cholesterol solution (0.8 mL) was mixed with 100 µL of extract (0.125 and 0.5 mg/mL) in an Eppendorf tube. Oxidation of LDL cholesterol was initiated by adding 0.1 mL of 100 µM $CuSO_4$ solution in distilled water. The mixture was incubated at 37°C for 20 hours. The initial absorbance (t=0) was read at 234nm immediately after mixing and

conjugated diene (CD) hydroperoxides formed at the end of 20 h were measured. The corrected absorbance at 20 h against 0 h was employed to calculate the percentage inhibition of CD formation using the following equation:

% inhibition of CD formation = $(Abs_{oxidative} - Abs_{sample})/ (Abs_{oxidative} - Abs_{native})$ X 100, where $Abs_{oxidative}$ = absorbance of LDL mixture and distilled water with $CuSO_4$ only, Abs_{sample} = absorbance of LDL with extract and $CuSO_4$, and Abs_{native} = absorbance of LDL with distilled water.

3.2.15 Determination of major phenolic compounds by HPLC/ESI-MS/MS

Reversed phase high performance liquid chromatography with tandem mass spectrometry was used to determine the major phenolic compounds present in the onion samples. A slightly modified version of the method outlined by Zheng and Wang (2001) was used. Briefly, samples were prepared by dissolving 0.5mL of free, esterified, and bound phenolic extracts of onion in 2.0 mL of 50% HPLC grade methanol and passing through a 0.45-μm syringe filter (purchased from Sigma-Aldrich Canada Ltd, Oakville, ON) before injection into a reverse phase C18 column (250 mm length, 4.6 mm i.d., 5 μm particle size, Sigma-Aldrich Canada Ltd., Oakville, ON) with a guard column. The mobile phase was methanol (A) and acidified water containing 0.01 M phosphoric acid (B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the wavelengths of detection were 280, 325, and 360 nm. HPLC of onion extracts was

analyzed online by using a mass selective detector system (LC-MSD-Trap-SL, Agilent) in electrospray ionization (ESI) mode. An external standard method using authentic compounds was used to confirm the identified compounds.

3.2.15 Statistical analysis

All experiments were carried out in triplicates and results were reported as mean ± deviation. The significance of differences among the values was determined at $p < 0.05$ using analysis of variance (ANOVA) followed by Tukey's multiple range tests (Snedecor and Cochran, 1980).

CHAPTER 4

RESULTS AND DISCUSSION: ONIONS AND BY-PRODUCTS

4.1 Total phenolic content

The total free, esterified and insoluble-bound phenolics were determined in onion samples. The content of free phenolics in tested onion varieties in the decreasing order was: pearl onion skin > red onion skin > yellow onion skin > red onion flesh > sprouted red onion flesh > white onion skin (Table 4.1). The esterified and bound forms of phenolic compounds in the onion samples followed a similar trend. White onion skin extract had the lowest amount of free, esterified, and bound phenolics compared to the others onion varieties (Table 4.1). There was no significant ($p>0.05$) difference between free phenolic content of red and yellow onion skins or between the non-sprouted and sprouted red onion flesh. However, bound phenolic content in yellow onion skin was significantly ($p<0.05$) lower than that in red and pearl onion skins (Table 4.1).

Phenolics were predominantly present in the free form both in the onion skin and flesh, except in red onion skin where bound phenolics were present in slightly higher quantities (41.30%) than the free form (38.41%). The percentage of free phenolics in the total phenolic content of pearl, red, yellow, white skin, red flesh, sprouted red flesh and green shoot were 56.85, 38.42, 44.17.70.5, 90.17, 87.0 and 95.14 %, respectively. The bound phenolics were present in higher concentrations (41.30%) than the esterified forms (20.28%) in the skin; while the flesh had higher concentrations of the esterified (9.67%) as compared to the bound form (0.73%) of phenolic compounds. However, the free

phenolics content of white onion skin was 32 times lower than those present in red onion flesh. Similar results were reported by Prakash *et al*. (2007), though they studied only the free phenolics of different onion varieties. They reported the TPC of the skin and flesh of red onions as 74.1 and 21.5 mg GAE/g dried onion which is for white onion was also reported to be much lower than that of the red variety. Furthermore, Shon *et al.* (2004) reported the phenolic content and antioxidant activity of red onion to be higher than those of yellow and white onions.

The phenolic content in the red onion skin and flesh were analyzed to determine the distribution of phenolics in onions in general. The red onions were also sprouted to study the changes in the phenolics during germination and its content in the green shoots that emerged during germination. As expected, the phenolic composition of the red onion flesh was 1.4 times lower than those found in the surrounding skin, but higher than those in the sprouted red onion flesh samples and green shoots. It is also interesting that the free and esterified forms of phenolics were present at a higher concentration in the red flesh and the sprouted flesh; green shoot had a higher content of bound phenolics. It is clear that the phenolics in the flesh decreased during germination. Though no such similar studies have been done for onions, Tian *et al.* (2004; 2005) studied the changes in phenolic constituents of brown rice during germination. They observed a reduction of approximately 70% in the concentration of feruloylsucrose and sinapoylsucrose, with an increase in the content of ferulic and sinapic acids in the light brown pericarp of rice grains during germination. They speculated that this reduction was probably caused by the hydrolysis, indicating that germination caused the metabolism of phenolic

compounds. In addition, the total content of insoluble phenolic compounds increased from 18.47 mg/100 g of flour in brown rice to 24.78 mg/100 g of flour in germinated brown rice, similar to that observed for sprouted onions. In the cell wall, phenolic compounds, particularly hydroxycinnamates, are ester linked to insoluble fibres, polysaccharides, and lignin components. The increase in phenolic compounds during germination could be explained as an increase in the free forms with alkaline hydrolysis, due to dismantling of the cell walls during germination. It is speculated that, during germination, as product moisture increases, there is a potential for injury by oxidation and/or microorganism infiltration. Induced saccharolytic enzymes (carbohydrases) hydrolyze starch and other sugars, hence would produce free phenolic compounds having more effective antioxidant activity than hydroxycinnamate sucrose esters. As a result, the content of hydroxycinnamate sucrose esters decreased, whereas that of free phenolic compounds increased (Tian *et al.*, 2004). This may be due to the changes taking place in the sprouted red onion flesh; however, this explanation requires experimental verification.

4.2 Total flavonoids content

In vitro and *in vivo* studies have demonstrated that flavonoids exhibit a variety of biological activities including antioxidative effects (Boyle *et al.*, 2000), reduction of cardiovascular disease and reduction of the risk of rheumatoid arthritis (Pattison *et al.*, 2004). Flavonoids are important group of compounds present in large amounts in onions (Ly *et al.*, 2005; Prakash *et al.*, 2007).

In the present study, red onion skin exhibited the highest free flavonoid content (20.22 ± 0.39 mg/g sample), followed by pearl onion skin (19.64 ± 0.2 mg/g sample). There was, however, no significant difference ($p>0.05$) between the free, esterified and bound flavonoids of red and pearl onion skin. White onion skin contained the lowest free flavonoids content (0.08 ± 0.08 mg/g). Bonaccorsi *et al.* (2008) also made a similar observation and found that the flavonol content in white onion bulb was about 7 mg/kg against 600-700 mg/kg in red and gold onion varieties. It is also clear that the free flavonoids were present in highest quantities in all varieties compared to the esterified and bound flavonoids (Table 4.1). As expected, the flavonoid content in red onion skin was also higher than those in red onion flesh. Price *et al.* (1996, 1997) and Boyles (2011) have also indicated that the outer layers of onions are rich in flavonoids compared to the whole onion bulb or edible part. Moreover, there is evidence of decreasing trend in the content of some flavonoids from the dry skin to the inner rings (Patil and Pike, 1995). Meanwhile USDA (2007) reported that the range of quercetin in different onions varied from 33.43 ± 2.38 mg/g in red onion bulb to 7.29 ± 1.27 mg/100g in young green of onion (USDA, 2007). These results are similar to those of the present study for the free form of phenolics from sprouted red flesh onion and red flesh onion (Table 4.1).

Among the different varieties of onion, the highest level of quercetin has been reported in fleshy scales of yellow/brown onion (170-1200 mg/kg fresh weight) and in red onion (190-1900 mg/kg fresh weight), while lower levels were found in white onion (50-650 mg/kg fresh weight) (Tusushida and Suzuki 1995; Crozier *et al.*, 1997; Price *et al.*, 1997; Price and Rhodes, 1997; Lugasi and Hovari, 2000). The compounds quercetin-

4′-glucoside and quercetin-3,4′-diglucoside are two main derivatives of quercetin found in red onion (Leighton *et al.*, 1992). High concentration of quercetin has been found in dry red onion skin. This part of onion contains five-fold higher content of quercetin in comparison to the flesh layers (Patil and Pike, 1995). Dry onion skin also has a different content of quercetin derivatives compared to the flesh layers where as much as 53% of total quercetin was present in the free form (Wiczkowski *et al.*, 2003). This high amount of quercetin in the outer layers of onion bulb is probably a consequence of exposure to sunlight (sunlight may promote rapid synthesis of quercetin) after the harvest (Hirota *et al.*, 1998; Harborn and Williams, 2000; Lee *et al.*, 2008).

4.3 Determination of total anthocyanins

Total anthocyanins content was determined in the soluble phenolics extracts as mg cyanidin-3-O-glucoside equivalents per gram of dry onion peel/flesh. Anthocyanins content in different varieties of onion in the decreasing order were: red onion skin > pearl onion skin > red onion flesh > sprouted red onion flesh > yellow onion skin > white onion skin > green shoots (Table 4.1). This is in agreement with the report of Gorinstein *et al.* (2008) who found that white onion had a low content of anthocyanins compared to coloured onions, both for skin and flesh. The highest content of anthocyanins was present in red onion skin (10.04 ± 0.90 mg/100g), while anthocyanin content in white onion skin was 0.06±0.01 mg/100g. This result is in agreement with that of Lauro and Francis (2000) who reported that the total anthocyanins in red onions was 7-21 mg/100g

sample. There was also no significant difference ($p>0.05$) between green shoot of sprouted red onion and white onion skin.

More than 20 derivatives of anthocyanins have been identified in red onions (Slimestad *et al.*, 2007). Cyanidin glucosides and acylated glucosides of cyanidins were the main anthocyanins of red onions (Fossen *et al.*, 1996; Donner *et al.* 1997). Moreover, cyanidin-3-(6′′-malonyl)-glucoside represents more than 50% of the total anthocyanins in different cultivars of red onion, and there is 20-250 mg/kg of anthocyanins in fresh weight of red onion (Fossen *et al.,* 1996). Anthocyanins constitute about 10% of the total flavonoid content of red onions (Rhodes and Price, 1996). Makris (2010) reported the utilization of waste from more pigmented onions as well as consideration of factors such as temperature and particle size that could make onion wastes a promising source of water-soluble anthocyanin pigments. Rhodes and Price (1996) found that anthocyanins are heavily concentrated in the skin and in the outer fleshy layers, whereas in the edible tissue they are restricted to a single layer of cells in the epidermal tissue. Gennaro *et al.* (2002) reported that the dry skin of onions is rich in anthocyanins and flavonols, with high percentage of glycone forms that corresponds to 2% of the total weight in the portion that cannot be eaten and is generally discarded. Therefore, ~63% of total red onion anthocyanins are present in the dry skin and outer fleshy layers that accounts for 15% of the total weight. Ferreres *et al.* (1996) detected cyanidin 3-glucoside and cyanidin 3-arabinoside and their malonated derivatives in red onions. Therefore, the skin of coloured onions can serve as an excellent source of natural cyanidin derivatives.

4.4.1 Measurement of total antioxidant capacity by trolox equivalent antioxidant capacity (TEAC) assay

The TEAC method is frequently used for determination of antioxidant activity. TEAC assay is based on the scavenging of 2,2'-azinobis-((3-ethylbenzothiaoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$) by the antioxidant over a period of 6 min. The TEAC values of extracts were determined and compared with trolox. Trolox reacts instantly with $ABTS^{\bullet+}$ and the reaction is completed within one minute. However, due to the biphasic nature of most antioxidative compounds, 6 min which includes a greater part of the slow-biphasic reaction (Van den Berg *et al.*, 1999) has shown good results. Therefore, 6 min was used as the time point in the present study. Generally, samples with higher phenolic content were most effective as free radical scavengers. The free phenolics in the skin showed higher activity than the esterified and bound phenolics in the skin and flesh as was the trend for their phenolic content. Table 4.2 lists the TEAC values of skin and flesh extracts of onions. Though pearl onions had a higher free phenolic content than red and yellow onions, they showed lower TEAC activity possibly because of the existing difference in the chemical nature of their phenolic constituents and hence activity differences. Esterified phenolic extracts showed low TEAC values, ranging from 0.05 (white skin) to 1.92 (pearl skin) mmoles TE/g dried sample. TEAC values of bound phenolic extracts varied from 0.04 mmoles TE/g for white onion skin to 3.40 mmoles TE/g for red onion skin. Red onion skin had the highest TEAC value, 384.25 times higher than that of white onion skin in the free, esterified, and bound phenolics. TEAC method is useful in screening antioxidants, but antioxidant

effectiveness must also be studied by other methods because their activity in foods is dependent on a variety of factors, including polarity, solubility, metal-chelating capacity and the system used for their evaluation. Others have measured the TEAC activity (μM TE/g of DW) of different onions, (though not separately studying the activity of the free, esterified and bound phenolics) as 15.6 (Sellappan and Akon, 2002), 29.02 (Bahorun *et al.,* 2004) and 64.11 μM TE/g of DW (Proteggente *et al*., 2002).

4.4.2 DPPH radical scavenging capacity (DRSC) using electron paramagentic resonance (EPR)

Red onion skin showed the highest DRSC activity, followed by, yellow onion skin, pearl onion skin, red onion flesh, sprouted red onion flesh and white onion skin (Table 4.3). This trend is similar to those obtained for the phenolic and flavonoid contents of the samples and clearly indicates that samples with higher phenolic content exhibit higher antioxidant activity. However, pearl onion skin that had a higher phenolic content than red onion skin showed a slightly lower, although insignificant ($p>0.05$), DRSC, possibly because the existing differences in the chemical constituents contributing to the scavenging activity. Velioglu *et al.* (1998) and Shahidi and Naczk (2004) reported that the antioxidant activity of a given food or food product depends on the chemical nature of its constituents and, not always their quantities, as the efficacies of compounds present varies considerably. Furthermore, DRSC of the sprouted red

Table 4.1. Total phenolics, flavonoids and anthocyanin content of freeze dried onion samples[1]

Onion sample	Total phenolics (mg GAE/g dried onion)			Total flavonoids (mg quercetin/g dried onion)			Total anthocyanins (mg anthocyanidin-3-glucoside/100g dried onion)
	Free phenolics	Esterified phenolics	Bound phenolics	Free flavonoids	Esterified flavonoids	Bound flavonoids	Soluble phenolics
Pearl skin	62.65 ±0.60^{a}	22.62 ± 0.54^{a}	24.92 ± 0.84 ^{a}	19.64 ± 0.2^{a}	2.6 ± 0.66^{a}	4.62 ± 1.16^{a}	6.32 ± 0.06^{a}
Red skin	23.67 ±0.16^{b}	12.50 ± 0.30^{b}	25.45 ± 0.74 ^{a}	20.22 ±0.39^{a}	2.57 ± 0.54^{a}	4.69 ± 0.97^{a}	10.04 ± 0.91^{b}
Yellow skin	22.71 ±2.86^{b}	10.75 ± 1.12^{c}	17.96 ± 0.43^{b}	10.69 ±0.40^{b}	0.56 ± 0.13^{b}	1.25 ± 0.06^{b}	0.21 ± 0.008^{c}
White skin	0.54 ± 0.03^{c}	0.013±0.003^{d}	0.213 ± 0.006^{c}	0.08 ± 0.08^{c}	0.006±0.005^{c}	0.01 ± 0.01^{c}	0.06 ± 0.01^{d}
Red flesh	17.33 ±0.98^{d}	1.86 ± 0.16^{e}	0.14 ± 0.09^{c}	0.14 ± 0.06 ^{d}	0.017±0.027^{d}	0.001 ± 0.001^{c}	0.69 ± 0.03^{e}
Sprouted Red flesh	15.66 ±1.34^{d}	1.81 ± 0.006^{e}	0.58 ± 0.01^{c}	0.42 ± 0.07^{d}	0.03 ± 0.012^{d}	0.003 ± 0.007^{c}	0.86 ± 0.06^{e}
Green shoot	10.57±0.289^{e}	0.34 ± 0.082^{d}	0.20±0.001^{c}	0.14 ± 0.05 ^{d}	0.024 ± 0.02^{c}	0.003 ± 0.005^{c}	0.05 ± 0.03^{d}

[1]Data are expressed as means ± SD (n=3). Values with the same letter in the each column are not significantly different ($p > 0.05$).

onion flesh was mainly contributed by its esterified phenolics, while in the red onion flesh free phenolics displayed the highest activity. It is also interesting that the free phenolics in the onion samples were stronger DPPH radical scavengers as compared to the bound and esterified forms except in the case of white onion peels and sprouted red onion flesh, where the esterified phenolics were more abundant and had a higher activity. In the DRSC assay, the onion extracts were able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. Thus, the DRSC activity of onion extracts may be mostly related to their phenolic hydroxyl group. Red onion skin extracts possessed the highest activity (more than 5.63 times higher than those of red onion flesh), the red onion being again more active than the yellow onion. Prakash *et al*. (2007) and Gorinstein *et al*. (2008) also found a similar trend in the DRSC of red onion skin and flesh.

4.4.3 Reducing power activity

Reducing power of the onion extracts was determined by the method of Amarowicz *et al*. (2002). Compounds that have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which then react with ferric chloride to form a complex that has an absorption maximum at 700nm. These compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so they can act as primary and secondary antioxidants (Jayanthi and Lalitha, 2011).

Table 4.3 shows the reducing power of the free, esterified and bound phenolics of the extracts using the potassium ferricyanide reduction method. The reducing power of the different varieties followed the same trend as other antioxidant activity tests with free phenolics of pearl onion skin showing the highest (3.58 ± 0.01 mmoles TE/g sample) and white onion skin the lowest (1.36 ± 0.07 mmoles TE/g sample) activity. However, the reducing power of the bound phenolics were comparable to that of the free phenolics, unlike those for DPPH and TEAC assays. In general, it can be concluded that free phenolics are the dominant form of phenolics and contribute most to the antioxidant activity of onions followed by bound and esterified phenolics. There was no significant difference between free phenolics of red pearl onion skin and yellow onion skin.

4.4.4 Measurement of Iron (II) Chelation Capacity

Metals such as iron, copper, manganese, nickel and cobalt at their higher valence state are known to participate in direct initiation of lipid oxidation via electron transfer and lipid alkyl radical formation while lower valence metals can directly initiate lipid oxidation via the formation of reactive oxygen species (ROS) (Kanner, 1986). So, chelation of free iron can prevent the formation of free radicals as well as preventing the impairment of vital organ function *in vivo*. The formation of a complex is formed between antioxidant and the metal renders metal ions inactive so that they cannot any longer act as initiator of lipid oxidation (Shahidi and Zhong, 2007). In the determination of iron chelating capacity of onion extracts, the iron chelating activity of free phenolics of onion skin, expressed as μmol EDTA eq/100g sample, indicated very strong activity.

Additionally, the free phenolics of pearl onion skin showed the highest iron chelating activity (2991.45 ± 403.30 μmoles EDTA eq /100 g dried onion), although not significantly different from that of red and yellow onion skin. As expected the extracts from the red onions flesh and white onion skin. There were no significant differences ($p > 0.05$) among the iron chelating activities of white onion skin, green shoot and red onion flesh samples. These results, presented in Table 4.3, follow the same trend as those observed for other antioxidant activity tests employed in this study.

There were no significant differences ($p > 0.05$) among the iron chelating activities of white onion skin, green shoot and red onion flesh samples. These results, presented in Table 4.3, follow the same trend as those observed for other antioxidant activity tests employed in this study.

Phenolic compounds are the major fractions that chelate metal ions although non-phenolic constituents in the crude extracts may also participate in sequestering of metal ions (Wettasinghe and Shahidi, 2002). Onions are a rich source of flavonoids which can effectively deactivate prooxidant metal ions and thus prevent or retard metal ion-induced lipid oxidation. Quercetin, a dominant flavonoid in onions, is well known as a strong metal ion chelator (Prakash *et al.*, 2007).

Table 4.2. Antioxidant capacity (TEAC and DPPH) of crude extracts and corresponding fractions prepared from dried, frozen onion skin and corresponding fractions

Onion sample	TEAC (mmoles trolox eq/g freeze dried onion)			DPPH radical scavenging activity (mmoles trolox eq/g freeze dried onion)		
	Free phenolics	Esterified phenolics	Bound phenolics	Free phenolics	Esterified phenolics	Bound phenolics
Pearl skin	12.42 ± 0.85 [a]	1.92 ± 0.5 [a]	3.34 ± 0.05 [a]	0.149 ± 0.005 [a]	0.047 ± 0.001 [a]	0.045 ± 0.002 [a]
Red skin	15.37 ± 0.24 [a]	1.55 ± 0.10 [a]	3.40 ± 0.77 [a]	0.152 ±0.004 [a]	0.054 ± 0.007 [a]	0.047 ± 0.009 [a]
Yellow skin	14.23 ± 0.24 [a]	0.84 ±0.08 [a]	1.83 ± 0.13 [b]	0.086 ± 0.004 [b]	0.004 ± 0.000 [b]	0.014 ±0.002 [b]
White skin	0.04 ± 0.005 [b]	0.05 ± 0.002 [b]	0.04 ± 0.004 [c]	0.001 ± 0.000 [c]	0.024 ± 0.003 [c]	0.002 ± 0.000 [c]
Red flesh	2.18 ± 0.05 [c]	0.39 ± 0.01 [c]	0.13 ± 0.001 [d]	0.027 ± 0.001 [d]	0.003 ± 0.000 [b]	0.014 ± 0.001 [b]
Sprouted Red flesh	2.94 ± 0.44 [c]	0.13 ± 0.01 [c]	0.16 ± 0.004 [d]	0.002 ±0.000 [c]	0.014 ±0.002 [c]	0.004 ± 0.000 [c]
Green shoot	1.96 ± 0.44 [c]	0.05 ± 0.02 [b]	0.07 ± 0.02 [c]	0.012 ± 0.000 [e]	0.001 ± 0.000 [b]	0.002 ± 0.000 [c]

[1]Data are expressed as means ± SD (n=3). Values with the same letter in the each column are not significantly different ($p > 0.05$).

There were no significant differences ($p > 0.05$) among the iron chelating activities of white onion skin, green shoot and red onion flesh samples. These results, presented in Table 4.3, follow the same trend as those observed for other antioxidant activity tests employed in this study.

Phenolic compounds are the major fractions that chelate metal ions although non-phenolic constituents in the crude extracts may also participate in sequestering of metal ions (Wettasinghe and Shahidi, 2002). Onions are a rich source of flavonoids which can effectively deactivate prooxidant metal ions and thus prevent or retard metal ion-induced lipid oxidation. Quercetin, a dominant flavonoid in onions, is well known as a strong metal ion chelator (Prakash *et al*., 2007).

4.4.5 Determination of Oxygen Radical Absorbance Capacity ($ORAC_{FL}$)

Table 4.4 presents $ORAC_{FL}$ values of free, esterified, and bound phenolic extracts; the ranges of values were 1347.46-6232.59, 255.16-3549.66, and 235.63-2116.78 μmoles trolox equivalent/g freeze dried onion, respectively. As observed from the DRSC data, the contribution of free phenolics of onion extracts towards the total $ORAC_{FL}$ was significantly higher than bound and esterified phenolics. Free phenolics of sprouted red onion flesh exhibited the highest total $ORAC_{FL}$ followed by pearl onion skin, yellow onion skin, red onion skin, red flesh onion, green shoot of sprouted red flesh onion, and white onion skin. $ORAC_{FL}$ value is based on the inhibition of the peroxyl radical-induced oxidation initiated by thermal decomposition of azo compounds such as 2,2′-azinobis [3-ethylbenzthiazoline-6- sulphonic acid] (AAPH). $ORAC_{FL}$ is the only

assay that combines both inhibition time and degree of inhibition into a single quantity (Prior *et al.*, 2005).

Fluorescein (FL) is employed as the probe resulting in the loss of fluorescence, which is detected with a fluorometer (Shahidi and Zhong, 2007). The ORAC assay measures the radical chain breaking ability of antioxidants by monitoring the inhibition of peroxyl radical, and the fluorescence decay indicates its reaction with peroxyl radical. In the presence of antioxidative compounds FL decay is inhibited, and the intensity can be measured at 485 nm excitation and 525 nm emission. The results of this study demonstrated that free, esterified, and bound phenolics from onion extracts scavenge peroxyl radicals effectively, although the trend of scavenging differed among varieties tested (Table 4.4). The ORAC values of free and bound phenolic extracts of onion ranged from 1347.46 to 6232.59 μmol trolox eq/g of the skin and flesh onion extract, and from 255.16 to 3549.66 μmol trolox eq/g of the esterified extract, and from 235.63 to 2116.78 μmol trolox eq/g of the bound phenolic extracts. The ability of free phenolic extracts to scavenge peroxyl radicals was in the order of: sprouted red flesh onion > pearl onion skin > yellow onion skin > red onion flesh > red onion skin > green shoots > white onion skin. Red onion skin esterified phenolic extracts showed the highest peroxyl radical scavenging activity, followed by yellow onion skin, pearl onion skin, green shoots, red onion flesh, sprouted red flesh onion, and white onion skin. Bound phenolic extracts of red onion skin showed the highest peroxyl radical scavenging activity, followed by yellow onion skin, green shoots, pearl onion skin, white onion skin, red flesh onion, and sprouted red onion flesh. Interestingly, in the present study free phenolics in the extract of

sprouted red onion flesh exhibited ORAC values superior to those of other cultivars such as purple onion and white onion as reported by Ou *et al*. (2002). This could be attributed to the high phenolic content in the skin compared to the flesh. However, the importance of size different in onion tested as well as existing differences between the skins which are dried and the flesh which has moisture content cannot be ignored. Cao *et al*. (1996) showed that the ORAC values of onion indicated their strong antioxidant activity. The ORAC assay is based on the hydrogen atom transfer (HAT) reaction, and thus hydrogen-donating ability of phenolics in onion to scavenge peroxyl radical was demonstrated. Peroxyl radicals are intermediate species generated during oxidation of membrane lipids. Thus, the scavenging efficacy of peroxyl radicals by phenolic extracts of onion skin and flesh as a source of natural antioxidants to manage disease conditions is important. No significant difference ($p>0.05$) existed between free phenolics of pearl onion skin and that of the sprouted red onion flesh. Similarly, no such difference existed between esterified and bound phenolics of red onion skin and yellow onion skin (Table 4.4).

Table 4.3. Antioxidant capacity (Reducing power and iron chelation) of crude extracts and corresponding fractions prepared from dried, frozen onion skin and corresponding fractions

Onion sample	Reducing power (mmoles trolox eq/g freeze dried onion)			Iron chelating activity (µmoles EDTA eq/100 g dried onion)		
	Free phenolics	Esterified phenolics	Bound phenolics	Free phenolics	Esterified phenolics	Bound phenolics
Pearl skin	3.58 ± 0.01 [a]	0.79 ± 0.14 [a]	2.01 ± 0.11 [a]	2991.45 ± 403.30 [a]	560.89 ± 113.32 [a]	961.54 ± 160.26 [a, b]
Red skin	3.45 ± 0.18 [a]	0.62 ± 0.01 [a]	1.68 ± 0.13 [b]	2938.03 ± 403.30 [a]	1228.63 ± 185.05 [b]	1282.05 ± 160.26 [a, b]
Yellow skin	3.37 ± 0.36 [a]	0.77 ± 0.15 [a]	1.50 ± 0.12 [b]	2617.52 ± 244.79 [a]	1121.79 ± 183.26 [b]	1495.73 ± 185.05 [a]
White skin	1.36 ± 0.07 [b]	0.43 ± 0.01 [b]	0.20 ± 0.007 [c]	1282.05 ± 160.26 [b]	16.03 ± 1.14 [c]	1442.31 ± 277.57 [a, b]
Red flesh	1.51 ± 0.14 [b]	0.31 ± 0.09 [b]	0.45 ± 0.005 [d]	1068.38 ± 333.60 [b]	1121.79 ± 170.50 [b]	1228.63 ± 244.80 [a, b]
Sprouted Red flesh	1.05 ± 0.12 [c]	0.18 ± 0.03 [c]	0.39 ±0.01 [d]	694.44 ± 185.05 [b]	961.53 ± 105.80 [b]	908.12 ± 244.80 [b]
Green shoot	0.94 ± 0.03 [c]	0.18 ± 0.01 [c]	0.19 ± 0.03 [c]	961.53 ± 135.67 [b]	1442.30 ± 131.21 [b]	1362.18 ± 113.32 [a, b]

[1]Data are expressed as means ± SD (n=3). Values with the same letter in the each column are not significantly different ($p > 0.05$).

4.5 Inhibition of oxidation in fish model system

During lipid oxidation, malondialdehyde (MDA), a minor secondary oxidation product of fatty acids with 3 or more double bonds, is formed. MDA reacts with 2-thiobarbituric acid (TBA) to form a pink TBA-MDA complex that is measured spectrophotometrically at its absorption maximum at 532 nm (Shahidi and Zhong, 2007).

The muscle of salmon used for the analysis contained 12.73 ± 0.27% total lipids, in agreement with 12.7-17.9% value reported by Morkore *et al.*(2001). The moisture content in salmon was 62.18 ± 0.65%.

The TBARS values of antioxidant-treated fish meat samples stored at 4°C over 7 days are shown in Table 3. The soluble onion extracts were added at 0.1% and the reference antioxidants, BHA and chlorogenic acid were each added at 200 ppm. The extracts were effective in inhibiting the oxidation of cooked salmon in comparison with the control, which showed the highest TBARS value at the end of the 7 days of storage period. The samples arranged in the order of their effectiveness in inhibiting the formation of TBARS (%) was as follows: red onion skin (68.46%) > pearl onion skin (60.48%) > green shoot (53.29%) > BHA (51.89%) > red onion flesh (50.30%) > yellow onion skin (48.71%) > white onion skin (46.51%) > quercetin (36.72%) > sprouted red onion flesh (9.78%) > Control. This trend is similar to that obtained in other assays conducted in this study. Thus, onions are highly effective in inhibiting oxidation in a cooked fish meat system, especially the extracts from the skin of red and pearl onions and green shoot, which were found to be better than BHA. There was no significant

difference between quercetin, white skin, and green shoot on day 0 and day 7 (Table 4.5). Quercetin alone showed a lower inhibition activity, probably because the onion extracts contained other antioxidant compounds, the synergistic effects of which improved its efficiency in inhibiting fish meat oxidation.

4.6 Effect of onion extracts in preventing cupric ion induced human low density lipoprotein (LDL) peroxidation

Natural antioxidants from dietary sources that may inhibit LDL cholesterol oxidation are of great importance in the prevention of atherosclerosis and associated cardiovascular diseases. It has been shown that the uptake of oxidized LDL by macrophages and smooth muscle cells leads to the formation of fatty streaks or vascular lesions which further accumulate lipids (Decker *et al.*, 2001). In addition, phenolic compounds can protect endogenous antioxidants such as tocopherol, β-carotene, lycopene and ubiquinol in LDL cholesterol molecule, or inhibit enzymes such as xanthine oxidase involved in the initiation of oxidation or cell-mediated LDL cholesterol oxidation (Chandrasekara and Shahidi, 2011).

In this study, the protective activity of extracts of skin and flesh phenolics of onions for chelating cupric ions and thus reducing metal catalyzed oxidation of LDL cholesterol was demonstrated. It is noteworthy that at the beginning the rate of conjugated diene (CD) formation was slow as LDL cholesterol molecules contain antioxidant compounds such as tocopherol, β-carotene and lycopene. The rapid oxidation started after the depletion of endogenous antioxidants of LDL cholesterol molecules.

Figure 4.4 shows the inhibitory activities of onion extracts at a concentration of 0.5 mg/mL against human LDL cholesterol oxidation induced by cupric ion.

The ability of phenolic compounds to inhibit copper ion-mediated LDL cholesterol oxidation may be attributed to their capacity to remove cupric ions from the medium (Decker *et al.*, 2001). It was noted that red onion flesh had a low LDL cholesterol oxidation inhibition, accounting for 22.5±7.12%, whereas pearl onion skin and red onion skin extracts exhibited high inhibitory activities against LDL cholesterol oxidation, accounting for 46.45 and 43.6%, respectively (Table 4.6). The results also showed no significant difference ($p>0.05$) between pearl onion skin extract and red onion skin extract and between yellow onion skin extract and red onion flesh extract. Pearl onion skin that also has a high total phenolic content showed the highest inhibition of LDL cholesterol oxidation of 46.45% after 22 h of incubation (Figure 4.1). This result is in agreement with that of Vinson *et al.* (1998) who reported that red and yellow onions had high antioxidant activity toward inhibition of LDL oxidation. In this study, onions have been found to contain high amounts of flavonoids which constitute the largest and most studied group of plant phenolics. Flavonoids are powerful antioxidants and their activity is related to chemical structures (Rice-Evan *et al.*, 1995; Rice-Evan *et al.*, 1996). Plant flavonoids are multifunctional and can act as reducing agents, as hydrogen atom-donating antioxidants, and as singlet oxygen quenchers. Certain flavonoids also act as antioxidants via their metal ion chelation properties (Brown *et al.*, 1998), thereby reducing the metal's capacity to generate free radicals. Flavonoids can act as potent inhibitors of LDL oxidation via several mechanisms like protection of the LDL-

associated antioxidants α-tochopherol (vitamin E) and carotenoids from oxidation (Spencer *et al.,* 2003). The protection of LDL against copper ion or free radical-induced oxidation by flavonoids depends on their response to copper ion, their partitioning between the aqueous and the lipophilic compartments within the LDL particle, and their hydrogen donating antioxidant properties (Brown *et al*., 1998).

Table 4.4. Oxygen radical absorbance capacity ($ORAC_{FL}$) of free, esterified, and bound phenolics of onion extracts.

Onion types	ORAC (μmoles trolox eq/g freeze dried onion)		
	Free	Esterified	Soluble
Pearl Skin	5803.46±97.73[a]	1907.52±287.66[a]	1193.75±183.95[a]
Red skin	4889.47±474.14[b]	3549.66±985.69[b]	2116.78±721.10[b]
Yellow skin	5433.86±0.0[c]	3158.29±735.08[b]	2009.27±384.70[b]
White skin	1347.46±19.69[d]	255.16±156.22[c]	329.06±48.82[c]
Sprouted red flesh	6232.59±1.26[a]	415.19±101.80[c]	235.63±198.57[d]
Red flesh	4889.57±2091.27[b]	848.03±663.43[d]	291.11±82.28[d]
Green shoots	3489.70±1045.27[d]	877.63±114.42[d]	1199.15±533.72[a]

*Values in each column having the same letter are not significantly different ($p < 0.05$).

Table 4.5. Effect of extracts from onion samples on the formation of malondialdehyde in a cooked fish model system[1]

Soluble extracts added to Fish	TBARS (mg MDA eq/kg Fish)	
	Day 0	Day 7
Control	2.40 ± 0.05	5.01 ± 0.56
Quercetin	2.66 ± 0.59	3.17 ± 0.81
BHA	1.43 ± 0.06	2.41 ± 0.37
Pearl onion skin	0.20 ± 0.08c	1.98 ± 0.09
Red onion skin	0.14 ±0.05c	1.58 ± 0.40
White onion skin	2.19 ± 0.23	2.68 ± 0.53
Yellow onion skin	0.87 ± 0.04	2.82 ± 0.45
Red onion flesh	2.55 ± 0.27	2.49 ± 0.05
Sprouted Red onion flesh	2.40 ± 0.06	4.52 ± 0.32
Green shoot	2.74 ± 0.16	2.34 ± 0.20

[1]Data are expressed as means ± SD (n=3). Values with the same letter, in the same row or column, are not significantly different ($p > 0.05$). [2]Soluble extracts from onion skin and flesh were added to the fish at 0.1% level. The concentration of sample is eqalent to 200 ppm gallic acid equivalents calculated on the basis of total phenolic content.

Table 4.6. Effect of Onion extracts on preventing cupric ion induced human low density lipoprotein (LDL) peroxidation

Onion Samples	Inhibition (%)
Pearl skin	46.45 ± 7.12^{a}
Red skin	43.60 ± 2.40^{a}
Yellow skin	25.66 ± 5.22^{b}
Red flesh	22.50 ± 2.91^{b}

Values with the same letter, in the same row or column, are not significantly different ($p > 0.05$).

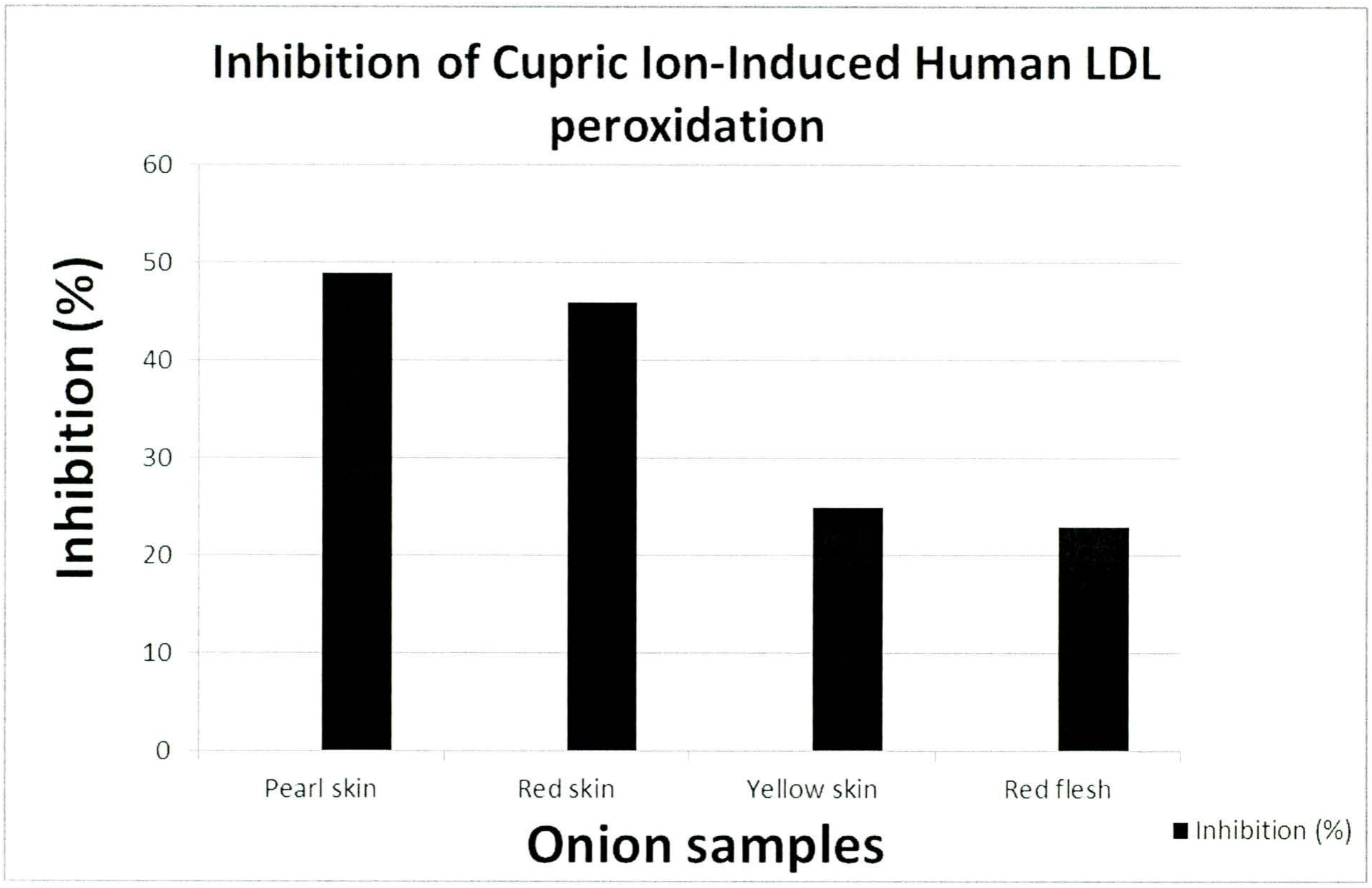


Figure 4.1. Inhibition of cupric ion-induced human low density lipoprotein (LDL) cholesterol oxidation by Onions. LDL (0.2 mg of 0.1 mg protein/mL) was oxidized in PBS (pH 7.4) at 37 °C with 4 μM $CuSO_4$, and absorbance was continuously monitored at 232 nm. Each column shows the individual % of different soluble samples (all 0.5 mg/mL). The control is LDL and $CuSO_4$ only.

4.7 Supercoiled strand DNA scission by peroxyl and hydroxyl radicals

Oxidants produced as by-products of mitochondrial electron transport and products from lipid peroxidation that escape the numerous antioxidant defense systems can cause damage to cellular macromolecules, including DNA, and such damages can lead to mutation and cancer initiation (Ames and Shigenaga, 1993). Oxidation damage of DNA results in a wide range of scission products, which include strand breaks and sister formation, chromatid exchange, DNA-DNA, and DNA-protein cross-links as well as base modification (Ames and Shigenaga, 1993). DNA damage is often measured as single strand-breaks, double strand-breaks, or chromosomal aberrations (Breimer, 1990). In the present study, soluble (free and soluble esters) and bound phenolics of onion extracts were evaluated for their capacity in inhibiting peroxyl and hydroxyl radical-induced DNA supercoiled (form I) strand scission. Figures 4.1A-C show the activity of soluble onion extracts for inhibiting peroxyl and hydroxyl radical-induced DNA supercoiled (form I) strand scission.

DNA molecules are easily attacked by free radicals that induce base modification and strand scission; they lead to mutagenesis and possibly cancer. Thus, the effectiveness of the extracts to prevent the scission of the DNA strands is a reflection of their positive effects against many diseases in the biological systems. Peroxyl radicals, which are used in the present study, are known to exert oxidative damage in biological systems due to their comparatively long half-life and thus greater affinity to diffuse into biological fluids in cells (Hu *et al.,* 2001). Soluble extracts from different onion varieties were dissolved

in PBS at a concentration of 3.5 mg/mL before mixing them with the DNA. Figure 4.3A shows the percentage of supercoiled DNA strands retained after incubation with peroxyl radicals generated by AAPH. Soluble phenolic extracts from red onion skin were most effective showing a DNA strand scission inhibition of 94.45% followed by pearl onion skin (91.45%) and yellow onion skin (84.26%), while extracts from white onion skin, red onion flesh and sprouted red onion flesh showed a low activity of approximately 10%.

Radicals cleave supercoiled pBR 322 plasmid DNA (form I) to nicked circular DNA (form II) as shown in Fig.4.2B. Lane 1 represents the native DNA without AAPH and antioxidant additives and lane 2 represents the blank, where the reaction mixture does not contain any antioxidant. The presence of a high intensity form II (nicked) band and the disappearance of form I (supercoiled) band in lane 2 indicate that the DNA was completely nicked. The onion extracts which were added in the remaining wells showed good strand scission inhibiting activity as already described. In the absence of any antioxidant, it may be expected that the peroxyl radical abstracts a hydrogen atom from the nearby DNA to generate a new DNA radical, which in turn evokes a free radical chain reaction resulting in the cleavage of the DNA molecule. However, in the presence of antioxidants, this chain reaction is terminated by abstracting a hydrogen atom from the antioxidant molecule (Hu and Kitts, 2000).

In the present study, soluble onion extracts exhibited inhibition of hydroxyl radical- induced DNA nicking in both site-specific and non-site-specific models. However, no prooxidant effects were observed in the range of concentrations used in is this study. The concept of site-specific effect of hydroxyl radical was described by

Gutteridge (1984). In the absence of EDTA, iron ions bind to deoxyribose molecules and bring about a site-specific reaction in the molecule. However, in the presence of EDTA, iron ion is removed from binding site to form EDTA metal complex and produce hydroxyl radical that can be removed by hydroxyl radical scavenging.

Onion extracts showed radical scavenging and antioxidant activities. The onion extracts have phenolic hydroxyl groups in their structures and these have been recognized to function as electron or hydrogen donors (Shahidi and Wanasundara, 1992). The antioxidants have attracted much interest with respect to their protective effect against free radical damage that may be the cause of many diseases, including cancer (Nakama *et al.*, 1993). The antioxidative effect of onion extract is mainly due to its phenolic components, such as flavonoids (Pietta *et al.*, 1998). Some flavonoid and non-flavonoid compounds have been reported to show alkyl and peroxyl radical scavenging activity, thus reducing radical-mediated pathogenesis, e.g. carcinogenesis (Sawa *et al.*, 1999). Ethanolic extracts of onion also contain lipophilic antioxidants such as onion oil which contains dialkyl disulphides, their oxides and thiols, which can trap electrons from other systems. Thus it scavenges different free radicals including hydroxyl radicals (Klanns-Dieter, 1983).

Hydroxyl radicals generated by Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms. At a concentration of 20μg/mL, free radical scavenging effect of 70% methanol/acetone/water solvents of soluble phenolic extracts of different onion skin and flesh were studied (Figure 4.2C) on plasmid DNA damage. The extracts of soluble pearl onion skin and red

onion skin (lanes 3 and 4) showed a significant reduction in the formation of nicked DNA and increased native form of DNA. The protection offered by yellow onion skin extract was close to that of red onion skin extract (lane 5). The red onion flesh (lane 7) and sprouted red onion flesh extract (lane 8) showed moderate, while white onion skin (lane 6) and green shoot of sprouted red onion flesh (lane 9) showed comparatively low protection. The pearl onion skin and red onion skin extracts with high phenolic content showed better protection compared to the others (Fig.4.3B), indicating that protection was directly proportional to the concentration of total phenolics present. Quercetin effectively protected DNA strand scission from *tert*-butylhydroperoxide (Prakash *et al.*, 2007). Therefore, in the pearl onion skin and red onion skin extracts presence of high quantities of quercetin might be responsible for better protection of DNA. Polyphenols are potential protecting agents against the lethal effects of oxidative stress and offer protection to DNA by chelating redox-active transition metal ions.

Onions are widely used all over the world and produce a large amount of waste, mainly skins. In the present study, it was found that pearl onion skin and red onion skin were rich sources of phenols with promising antioxidant and free radical scavenging activities and ability to provide protection against DNA damage caused by reactive oxygen species. Thus, they may be used in foods in order to protect them from spoilage and possible in supplements to render health benefits.

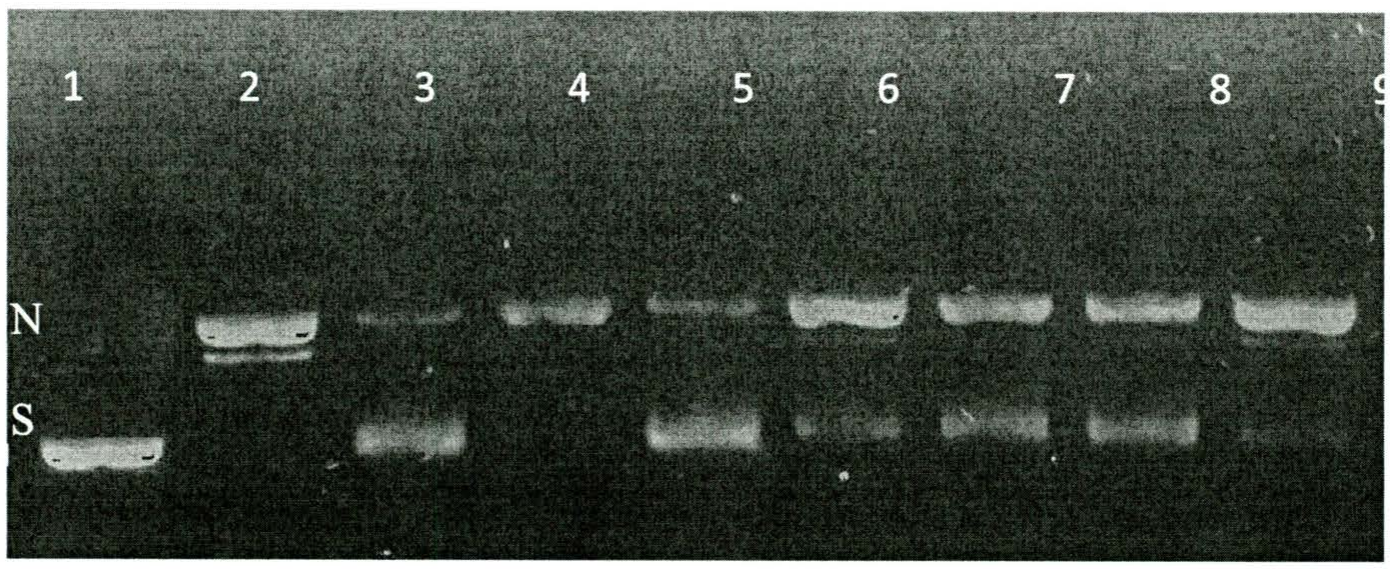


Figure 4.2A. Effect of addition of onion skin and flesh phenolic extracts in peroxyl radical treated DNA system.

Lane 1: Control (DNA only); Lane 2: Blank (DNA and AAPH); Lane 3: soluble Pearl onion skin extract; Lane 4: soluble white onion skin extract; Lane 5: Soluble red onion skin extract; Lane 6: soluble yellow onion skin extract; Lane 7: Soluble sprouted Red onion flesh extract; Lane 8: soluble Red onion flesh extract; Lane 9: soluble green shoot extract, S=supercoiled plasmid DNA strands; and N= nicked DNA strands.

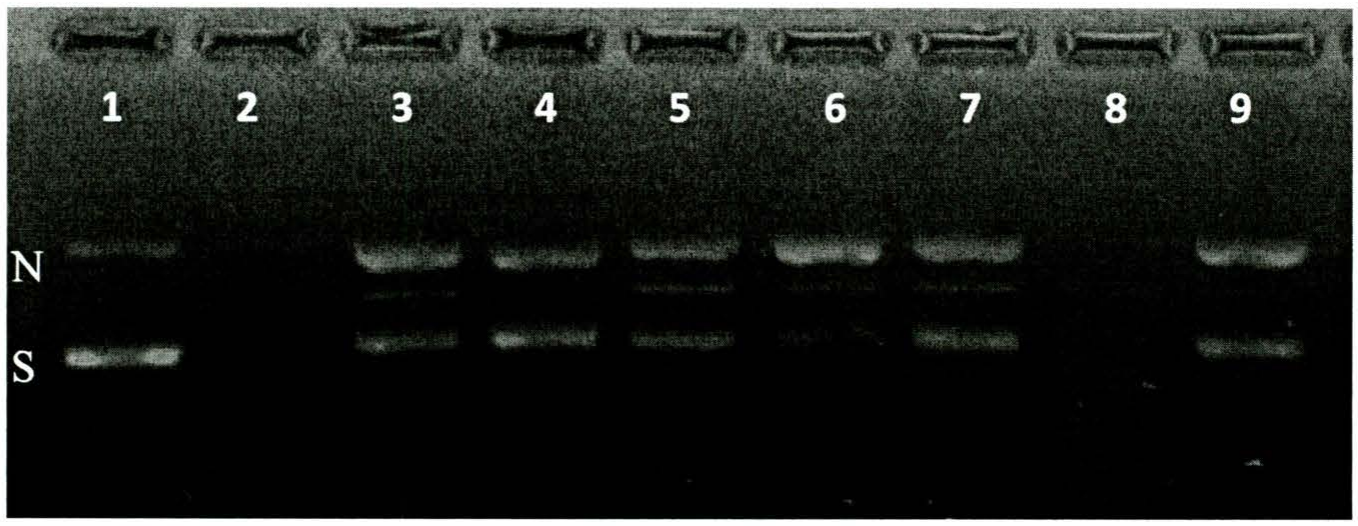


Figure 4.2B. Representative figure to illustrate the effect of soluble onion extracts in preventing hydroxyl radical induced DNA scission.

Lane1: DNA+PBS; Lane2= DNA+hydroxyl radical; Lane3= DNA+hydroxyl radical+Pearl skin; Lane4= DNA+hydroxyl radical+red skin; Lane5= DNA+hydroxyl radical+yellow skin; Lane6= DNA+hydroxyl radical+white skin; Lane7= DNA+hydroxyl radical+prouted red flesh; Lane8= DNA+hydroxyl radical+red flesh; and Lane9= DNA+hydroxyl radical+green shoot.

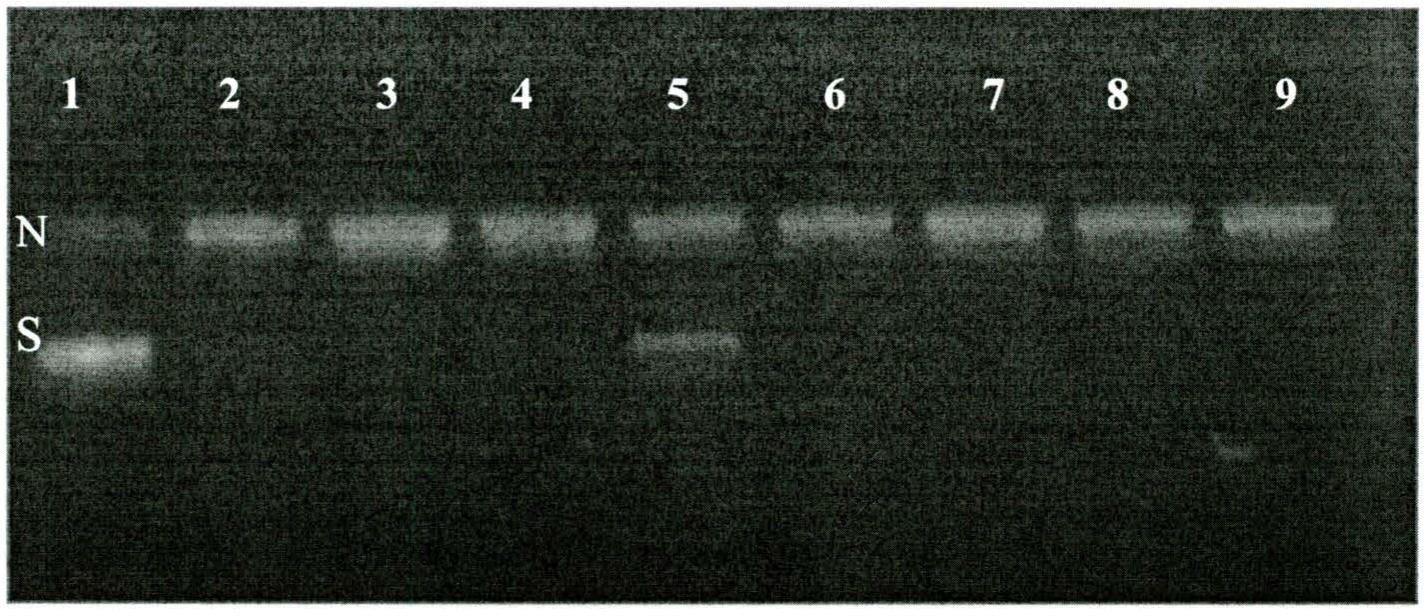


Figure 4.2C. Effect of bound onion extracts in preventing peroxyl radical induced DNA scission.

Lane1: DNA+PBS; Lane2= DNA+1mM AAPH; Lane3= DNA+1mM AAPH +red onion skin; Lane4= DNA+1mM AAPH+ yellow onion skin; Lane5= DNA+1mM AAPH+pearl onion skin; Lane6= DNA+1mM AAPH +white onion skin; Lane7= DNA+1mM AAPH +sprouted red onion flesh; Lane8= DNA+1mM AAPH +red onion flesh; and Lane9= DNA+1mM AAPH +green shoot.

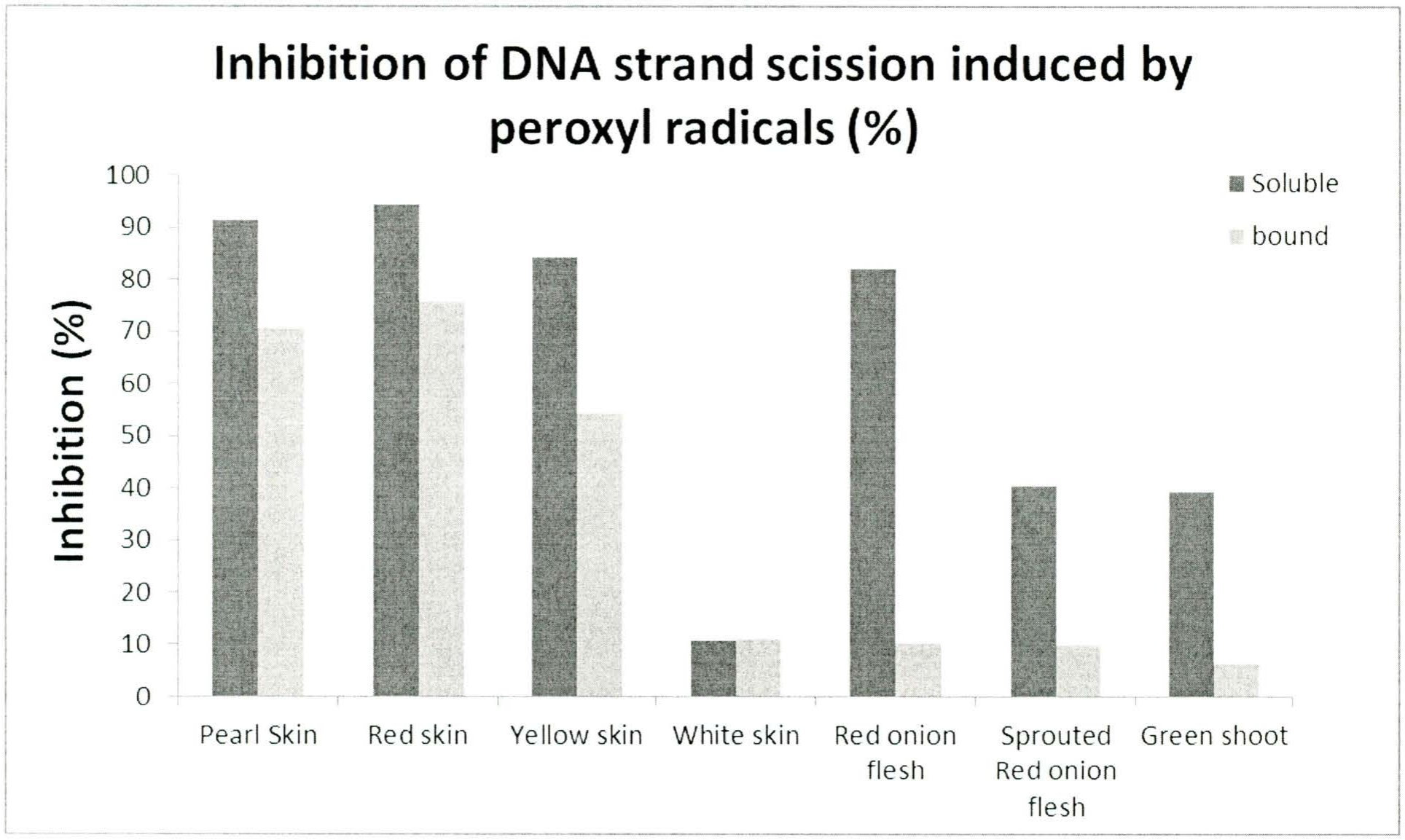


Figure 4.3A. Inhibition percentage of supercoiled pBR 322 plasmid DNA scission in peroxyl radical-mediated systems with extracts from different onion samples.

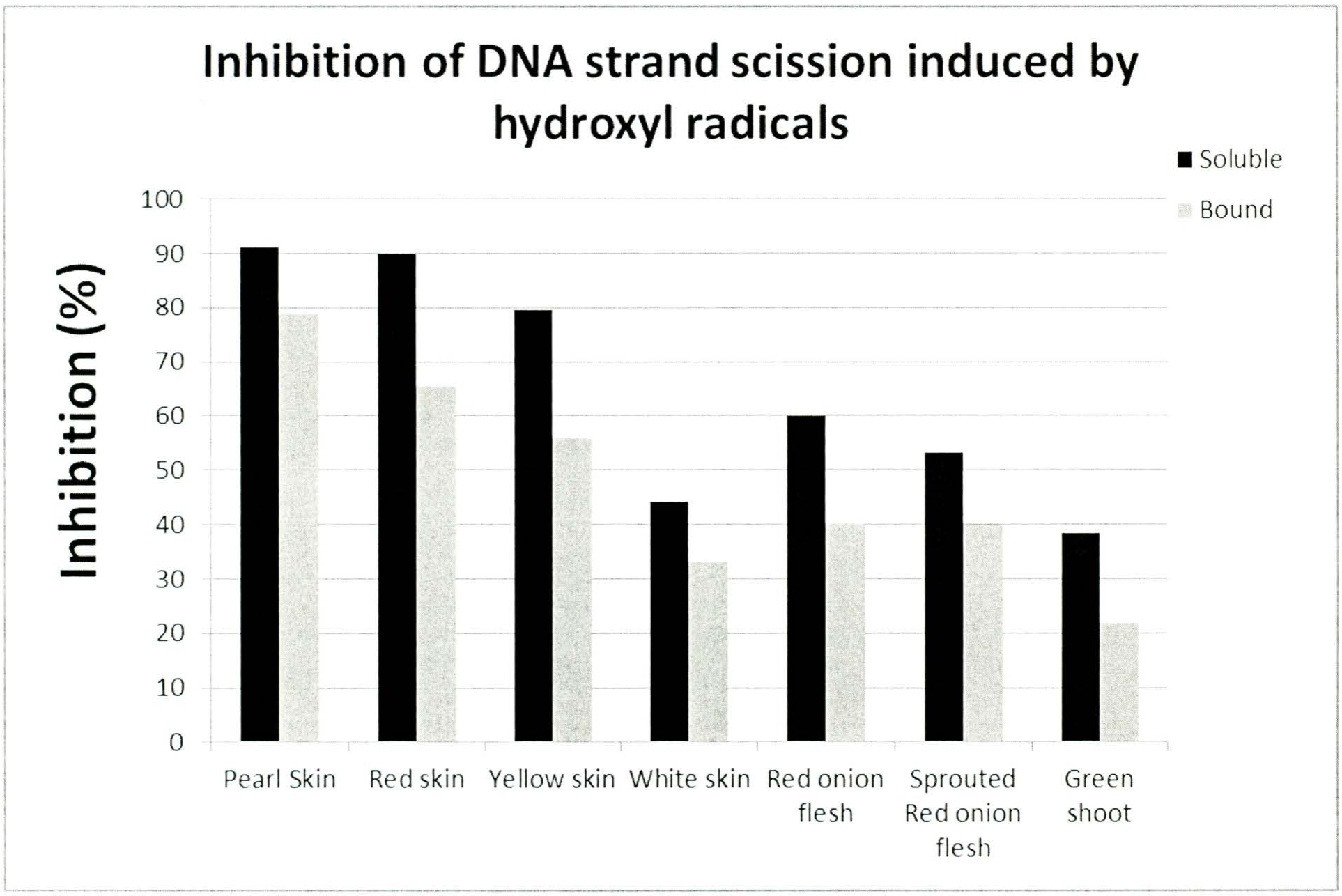


Figure 4.3B. Inhibition percentage of supercoiled pBR 322 plasmid DNA scission in hydroxyl radical-mediated systems with extracts from different onion samples.

4.8 Determination of major phenolic compounds by HPLC/ESI-MS/MS

The identity of soluble and bound phenolic compounds were ascertained using HPLC-DAD and HPLC-MS analyses and by comparison of their retention times and mass spectral data with those of the available standards and reports in the literature. The predominant phenolic acids and flavonoids present in the onion samples that were identified and quantified (mg/g dried onion sample) using HPLC are listed in Table 4.7.

Quercetin, quercetin 3,4′-diglucoside and kaempferol were predominant in the free form in all onion samples; quercetin glucoside being the most abundant (Figure 4.4).

Quercetin and kaempferol could easily be identified with standards. However, quercetin 3,4′-diglucoside was identified by its mass spectral data. The use of fragmentation in mass spectrometry allowed us to observe the corresponding flavonol aglycone as fragments of the molecular ion. Quercetin 3,4′-diglucoside at a retention time of 19.5 min showed molecular ion $[M-H]^-$ with *m/z* value of 625. The fragmentation (MS^2) in the negative mode of the ion with *m/z* 625 resulted in a fragment with *m/z* 463, by loss of 162 amu corresponding to the loss of a glucose moiety and *m/z* 301 corresponding to yet another glucose moiety. Fragmentation by MS^3 of the aglycone obtained (*m/z* 301) originated fragments common to those from the fragmentation of quercetin (m/z 151, 179). Thus, from the respective fragmentation patterns we concluded that the peak at Rt 19.5 min corresponded to quercetin 3,4′-diglucoside.

The high level of antioxidant activity in onions is attributed to their flavonoid constituents, namely quercetin, kaempferol, myricetin, and catechin (Patil *et al.*, 1995; Cook and Samman, 1996). Two major components quercetin monoglucoside and quercetin diglucoside account for 80% of the total flavonoids in onions (Rhodes, and Price, 1996, Bonaccorsi *et al.*, 2008) with levels of quercetin glucosides being much higher in onion than those in other vegetables (Proteggent *et al.*, 2002; Sellappan and Akoh, 2002; Shahidi and Naczk, 2004). Similarly, Price and Rhodes (1997) reported that quercetin 3,4′-*O*-glucoside and quercetin monoglucoside (quercetin 4′-*O*-glucoside) were

the major flavonols in edible portions of onions, however, they were mostly concentrated in the skin. These conclusions correspond to those that can be drawn in this study.

Additionally, in the present study, bound and esterified phenolic fractions of onion samples were present in much lower concentrations as compared to the free form. None of the phenolic compounds could be detected in the esterified and bound fractions of the white onion peels, but quercetin and quercetin glucoside were detected at lower concentrations in the esterified or bound fractions of all other onion peels. As expected, both the red onion and sprouted red onion flesh had much lower concentrations of phenolics as compared to the skin. However, surprisingly, the sprouted red onion flesh was found to contain higher concentrations of both quercetin and quercetin glucoside as compared to the ones not sprouted. As also determined through the Folin total phenolics test, pearl onion skin was found to contain the highest amount of total phenolic acids, followed by red and yellow onions, sprouted red onion flesh, red onion flesh and white onion skin. This result is in agreement with that of Patil *et al.* (1995) who reported that the red, pink, and yellow onions had higher amounts of quercetin than white varieties. Prakash *et al.* (2007) also reported that the content of quercetin decreased in all varieties from outer to inner fleshy layers.

Kaempferol, another flavonoid, was detected at much lower levels in the esterified and bound forms of onion skin and flesh; the highest amount was observed in the free form of pearl onion skin followed by red onion skin, yellow onion skin, red onion flesh, sprouted red onion flesh, green shoot of sprouted red onion flesh and white onion skin. Sellappan and Akoh (2002) reported that the kaempferol in onions were found to be

in minor quantities in comparison to quercetin and kaempferol 3- and 4-glucosides. Onions grown in the United States were reported to have kaempferol at 0.68 g/kg in the outer dry skin and 3-7 mg/kg in outer and inner skins of the bulb (Bilyk *et al.*, 1984), whereas onions grown in the United Kingdom did not have any detectable quantities of kaempferol (Crozier *et al.,* 1997). These variations may be due to many factors including variety, climatic conditions and maturity (Sellappan and Akoh, 2002).

Another set of experiments were carried out to test the efficiency of the solvent extraction method for dechlorophyllization of green shoots (to reduce the interference of pro-oxidants) using spectrophotometry and HPLC data. This was done by measuring the reduction in the absorbance of the solvent extracted sample at 660 nm (λ_{max} for chlorophyll). The efficiency of the extraction was confirmed using HPLC analysis which showed the successful removal of chlorophyll (data not shown) without affecting the phenolic composition of the extract (Table 4.7).

Table 4.7. Content of prominent flavonoids (mg/g freeze dried sample) in the skin and flesh of four onion varieties

Onion sample	Quercetin glucoside			Quercetin			Kaempferol		
	Free	Esterified	Bound	Free	Esterified	Bound	Free	Esterified	Bound
Pearl	9.59	2.54	1.44	8.33	0.03	0.23	1.36	0.01	0.01
Red skin	5.59	0.50	0.43	2.99	0.01	0.151	1.15	_	0.005
Yellow	2.57	0.16	0.19	3.11	0.007	0.01	1.13	_	0.006
White	0.004	_	_	0.004	_	_	0.003	_	_
Red flesh	2.54	0.05	0.01	0.15	_	_	0.01	_	_
Sprouted Red onion flesh	2.91	0.09	0.03	0.19	0.003	_	0.03	_	_
Green shoot (with chlorophyll)	2.32	0.06	0.01	0.14	_	_	0.03	_	_
Green shoot (dechlorophyllized)	2.11	0.08	0.02	0.11	0.001	-	0.02	-	-

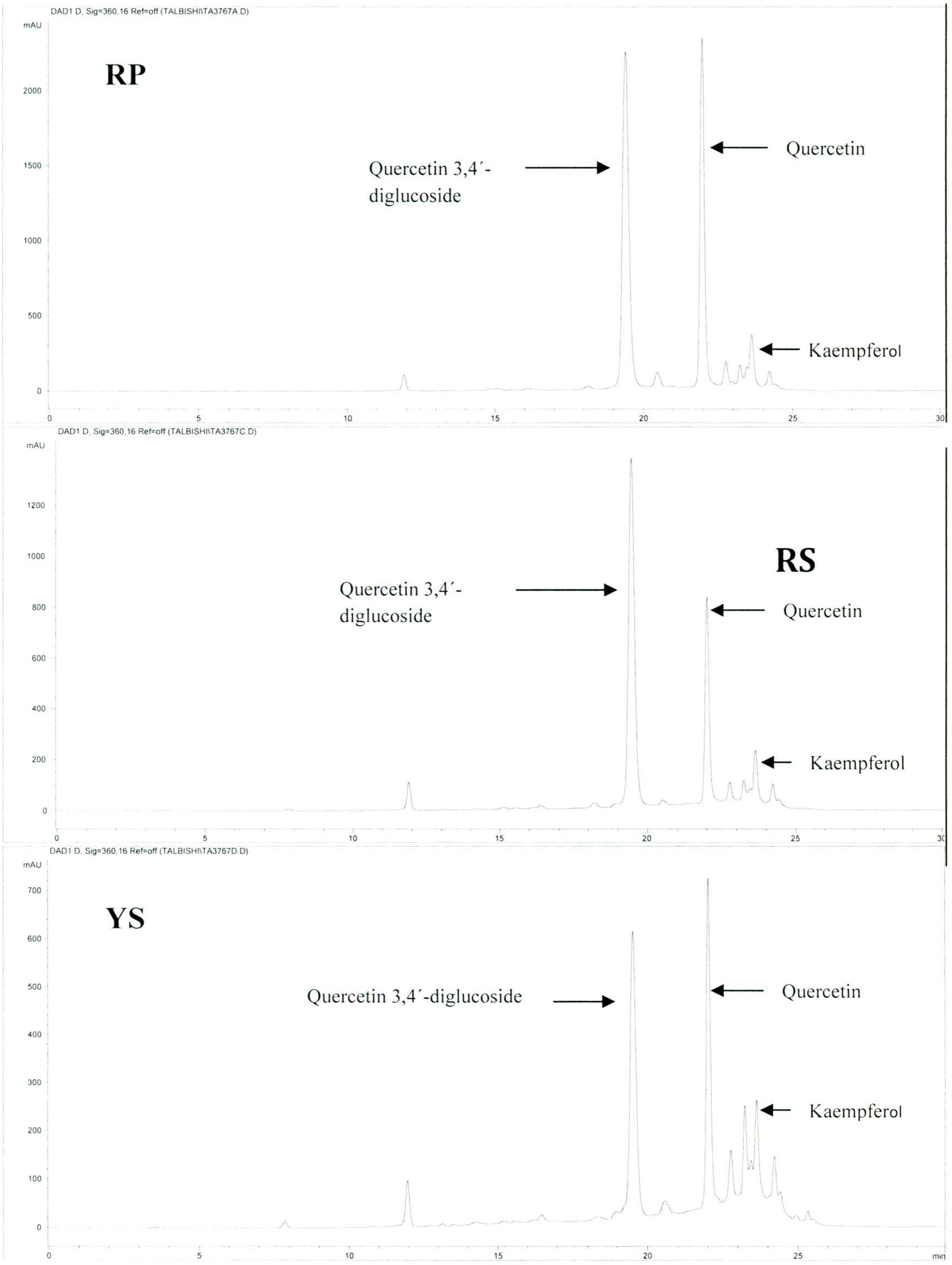


Figure 4.4. HPLC chromatograms at 360 nm of free flavonoids extracted from skin of (PS) Pearl (RS), Red onion, and (YS) Yellow onion varieties.

4.9 Active compounds in onion extracts

Flavonoids

Flavonoids in fruits, vegetables, and other plant foods have been linked to reduced risk of chronic diseases including cancer and cardiovascular disease (CVD. Flavonoids in nature occur as conjugates in glycosylated or esterified forms but can occur as aglycones, especially upon food processing.

High performance liquid chromatographic (HPLC) analysis of onion extracts showed the presence of quercetin, keampferol, and myricetin. Each compound was tentatively identified by its retention time and by comparison with standards under the same conditions. The chromatograms obtained for HPLC analysis are illustrated in Figure 4.4.

4.9.1 Quercetin

Quercetin is the major flavonol in onions. Levels of quercetin glucosides were much higher in onion than those in other vegetables (Sellappan and Akoh, 2002). Hertog *et al*. (1992) reported that quercetin in white onion was present at 37.3 times higher level than that in different varieties of spanich tomato. Price and Rhodes (1997) reported that quercetin 3,4′-*O*-glucoside and quercetin monoglucoside (quercetin 4′-*O*-glucoside) were the major flavonols in edible portions of onions, however, they were mostly concentrated in the skin.

In the present study, the quercetins identified in the free form of onion extracts were quercetin and quercetin 3-glugoside. The highest amount of quercetin was found in

the free phenolics of extracts of pearl onion skin (9.585 mg/g), followed by red onion skin, yellow onion skin, sprouted red flesh onion, red flesh onion, and green shoots (dechlorophillized), respectively (Table 4.7). In addition, quercetin was found in both bound and esterified forms in lesser amounts than those in the free form, compared to those of myrcetin and kaempferol. The skin of pearl onion and red onion were the richest source of quercetin, myrcetin, and kaempferol. This result is in agreement with the findings of Patil *et al.* (1995) who reported that red, pink, and yellow onions had higher amounts of quercetin than white varieties. Prakash *et al.* (2007) reported that the content of quercetin was decreased in all varieties from outer to inner fleshy layers. Proteggent *et al.* (2002) further demonstrated that the concentration of quercetin glycosides in onion extracts was higher compared to that found in different vegetables in the same study. Quercetin represented approximately 80% of the total flavonol content of onions (Rhodes and Price, 1996).

From HPLC analysis, it was determined that quercetin represented the major flavonoid in onion skin and flesh, however the amount of quercetin in pearl onion skin and red onion skin was higher than other types and may be related to anthocyanins present in the red onion *Allium Cepa*. Geetha *et al.* (2012) reported that quercetin and cyanidin were the major flavonoids in big red onion peels.

4.9.2 Anthocyanins

Anthocyanins are polyphenolic pigments responsible for most of the colour diversity found in plants. *In vivo*, colour expression and the stability of anthocyanins are

interpreted by extrapolation of the results acquired *in vitro* with model solutions of pigments obtained through extraction or laboratory synthesis. The colour of anthocyanins is due to their chromophore units and may be influenced by some constituents of the plant cells (Brouillard *et al.*, 1997).

Anthocyanins are stored in an organized aqueous medium in the cell vacuoles, where there is a slightly acidic environment and is rich in inorganic ions. Polyphenols are essential for transformations of these pigments that enable the formation of molecular complexes and subsequent colour changes and stabilization (Brouillard and Dangles, 1993).

Red onion contains a number of anthocyanins that are mostly concentrated in the skin and outer fleshy layer, specifically, cyanidin derivatives which constitute over 50% of total anthocyanins and delphinidin derivatives which comprise about 30% of total anthocyanins in whole red onions (Gennaro *et al.*, 2002). Ferreres *et al.* (1996) detected cyanidin 3-glucoside and cyanidin 3-arabinoside and their malonated derivatives in red onions. Fuleki (1971) and Herrmann (1976) identified cyanidin 3-glucoside in the outer layers of onions.

The four main anthocyanins of red onion have previously been identified as 3-(3″-glucosyl-6″-malonylglucoside), 3-(6″-malonylglucoside), 3-(3″-glucosylglucoside) and 3-glucoside of cyanidin, respectively (Fossen *et al.*, 1996; Terahara *et al.*, 1994). In addition, some minor anthocyanin pigments have been detected, namely 3-(3″,6″-dimalonylglucoside), 3-(3″-malonylglucoside) and 3,5-diglucoside of cyanidin, 3-

glucoside, 3,5-diglucoside and 3-malonylglucoside of peonidin (Donner *et al.*, 1997; Fossen *et al.*, 1996).

Several studies have suggested that the anthocyanin content and their corresponding antioxidant activity contribute to the protective effect of fruits and vegetables against degenerative and chronic diseases (Heinonen *et al.*, 1998; Record *et al.*, 2001). Some plants and fruit extracts with high phenolic content have been reported to act as inhibitors of mutagenesis and carcinogenesis (Kumpulainen and Salonen, 1998; Macheix *et al.*, 1990).

In the present study, total anthocyanins content in different onion varieties was determined as mg of cyanidin 3-glucoside/100g DW of freeze-dried samples (section 4.3). Both red onion skin and pearl onion skin contained the highest amounts of anthocyanins compared to those present in red onion flesh and sprouted red onion flesh, yellow onion skin, white onion skin, and green shoots of red onion flesh. These results demonstrate the ability of red onion skin and pearl onion skin as natural antioxidant, or for using extracted anthocyanins as natural pigments to increase the antioxidants capacity in order to prevent food quality deterioration.

4.9.3 Kaempferol

Quantitative data of the amount of kaempferol in various skins and flesh of onion cultivars are summarized in Table 4.7. A much smaller amount of kaempferol was found in the esterified and bound forms in onion skin and flesh, and the highest kaempferol content occurred in the free form in pearl onion skin followed by red onion skin > yellow

onion skin > red onion flesh > sprouted red onion flesh > green shoot of sprouted red onion flesh > white onion skin. No kaempferol was detected in the esterified and bound forms in red flesh onion, white onion skin, and green shoot of sprouted red onion flesh. Ewald *et al*. (1999) found that kaempferol was lost during blanching of the raw onions, to even a larger extract than that for quercetin (64 vs 39%). Sellappan and Akoh (2002) reported that the kaempferol in onions occurred in minor quantities in comparison to quercetin and kaempferol 3- and 4-glucosides. Onions grown in the United States were reported to have kaempferol at 0.68 g/kg in the outer dry skin and 3-7 mg/kg in outer and inner skins of the bulb (Bilyk *et al*., 1984), whereas onions grown in the United Kingdom did not have any detectable amount of kaempferol (Crozier *et al*., 1997). These variations may be due to many factors including variety, climatic conditions and maturity (Sellappan and Akoh, 2002).

CHAPTER 5

RESULTS AND DISCUSSION: POTATO AND BY-PRODUCTS

5.1 Total phenolic content and fractions thereof

The free, esterified and insoluble-bound phenolics in the extracts of potato peel and flesh samples are given in Table 5.1. Results indicate that purple potato peel contained the highest amount of free (7.2 mg GAE/g peel) and esterified (4.74 mg GAE/g peel) phenolics among all varieties analyzed, followed by Innovator potato peel, Russet potato peel, yellow potato peel, Russet potato flesh, purple potato flesh and yellow potato flesh, arranged in the decreasing order of their free phenolics. It is also interesting to note that the phenolics were predominantly found in the bound form in the peels of both the Innovator (51.07% of the total phenolics) and Russet (45.95% of total phenolics) varieties while the free and esterified phenolics were the predominant forms in both the purple and yellow potatoes (Table 5.1). As expected, the contents of free, esterified and insoluble-bound phenolics in flesh were significantly ($p \leq 0.05$) lower than those in the peels. These findings are comparable to those reported for total phenolics in the existing literature. Chlorogenic acid has been reported as being the predominant phenolic in potato tuber, constituting up to 90% to the total amount (Shahidi and Naczk, 2004). Friedman *et al.* (1997) reported that approximately 50% of chlorogenic acid isomers were found in potato skin (peel) and adjoining tissues, while in the tuber cortex the level of chlorogenic acids gradually decreased from outside towards the centre of the potato tuber. There is also clear evidence that the total amount of phenolics in potato varies significantly among different varieties (Brandl and Herrmann, 1984), as it was also

observed in the present study. Significant difference among varieties may be attributed to the genotypes and harvest location which influence the accumulation of phenolic compounds by synthesizing different quantities and/or types of phenolics present (Shahidi and Naczk, 1995; Hesam *et al.*, 2012).

Al-Weshahy and Rao (2009) reported that the total phenolic content in six varieties of potatoes, including purple and yellow potatoes, ranged from 1.51 to 3.32 mg GAE/g dry potato peel. These values are much lesser than the total phenolic content (sum of free, esterified and bound phenolics) in our study which was in the range of 4.64-13.85 mg GAE/g dried potato peel. This clearly highlights the importance of including the esterified and bound phenolics in the determination of total phenolics. Some important factors such as sample treatment and extraction conditions may also affect the phenolic content of potatoes. Mohagheghi Samarin *et al.* (2008) found that ultrasound treatment can enhance the extraction of phenolics from potato peels. Thus, higher yield of total phenolics in their study compared to those in our work may be due to the use of ultrasound and vigorous shaking in their extraction process, however, effects of growing conditions and cultivation area on the content of phenolics cannot be ruled out.

5.3 Determination of total anthocyanins

In the present study, soluble extracts of purple, Russet, and yellow potato peel and flesh, as well as Innovator potato peel were evaluated for their total anthocyanin content, presented as mg cyanidin-3-O-glucoside equivalents (Table 5.1). The purple potato peel (6.84 ± 4.03 mg/100g) contained a higher amount of anthocyanins compared to that of its

flesh counterpart (0.64 ± 0.1 7mg/100g), reflecting a 10.69 times higher content in the peel compared to that of the flesh. These values were followed by those for Russet potato peel > yellow potato peel > Innovator potato peel > yellow potato flesh > Russet potato flesh. No significant difference ($p > 0.05$) existed between yellow potato peel and Innovator potato peel. These results are in agreement with those of Brown *et al*. (2008) who found that pigmented potatoes (red and purple) had higher content of monomeric anthocyanins than those of non-pigmented varieties. An important attribute of these pigments is that they serve as potent dietary antioxidants (Brown *et al*., 2003; Brown et al., 2004) and are known to protect against oxidants, free radicals and LDL cholesterol oxidation (Hung *et al*., 1997). Acylated pigments constitute more than 98% of the total anthocyanins present in pigmented potatoes. Pigmented potatoes displayed two to three times higher antioxidant potential than their white-fleshed counterparts (Brown, 2004). Studies have also confirmed that the red- and/or purple-fleshed potatoes have significantly higher antioxidant values than white and yellow potatoes (Brown, 2004; Lachman and Hamouz, 2005). Lachman and Hamouz (2005) found that purple potatoes contained acylated anthocyanins and pigmented potatoes displayed two to three times higher antioxidant potential in comparison with white-fleshed potatoes, in agreement with the results obtained in the present study.

5.4 Antioxidant activities of potato extracts

Plant polyphenols are known to have multifunctional properties by acting as reducing agents, hydrogen donating antioxidants, iron chelators and singlet oxygen

quenchers. The most important property is their capacity to act as antioxidants, thus protecting the body against reactive oxygen species and may have an additive or synergistic effect with endogenous antioxidants (Shahidi and Naczk, 1995). Due to the different mechanisms involved in the antioxidative processes, best conclusions could be drawn when at least two methods are used in a study. Taking this into consideration, in the present study, the antioxidant activities of the extracts were measured using different assays such as TEAC, DRSC (Table 5.2), reducing power and ORAC. There are two main mechanisms by which antioxidants can scavenge free radicals, hydrogen atom transfer (HAT) and single electron transfer (SET), however very few reactions exclusively follow one or the other (Prior *et al.,* 2005). From the antioxidant activity assays used in this study the only one that is believed to occur exclusively by HAT is ORAC. Reducing power measures antioxidant activity by a SET mechanism and $DPPH^{\cdot}$ scavenging and TEAC assays follow a mix of both HAT and SET. With respect to the antioxidant activity tests carried out, samples which had the highest phenolic content were most effective as free radical scavengers (Tables 5.2 and 5.3). The extract from purple potato peel, which had the highest content of free phenolics among all varieties tested, exhibited the highest antioxidant activity in all tests except DRSC. In DRSC assay, the Innovator and Russet varieties showed the highest scavenging activity. Again, the activities in the flesh were much lower than those exhibited by the peel. It is also noteworthy that the antioxidant activities of the bound phenolics in the peels of Russet and Innovator varieties were higher than those of the free phenolics, while the esterified phenolics made an almost equal contribution to free phenolics in the antioxidant activities

of the purple potato peels which again correlated with the relative content of phenolics. In addition, though the purple potato peel showed much higher radical scavenging activity compared to other samples, its reducing power was comparable to those of Innovator and Russet varieties, thus demonstrating a better contribution to antioxidant activity via radical scavenging than the reducing mechanism.

Table 5.1. Total phenolics and anthocyanins in freeze dried potato samples[1]

Potato sample	Potato variety	Total phenolics (mg GAE[2]/g freeze dried sample)			Total anthocyanins (mg/100g)
		Free phenolics	Esterified phenolics	Bound phenolics	Soluble phenolics
Peel	Russet	3.14 ± 0.05[a]	1.42 ± 0.0.2[a]	4.76 ± 0.13[a]	0.40±0.33[b]
	Innovator	3.23 ± 0.39[a]	2.97 ± 0.07[b]	5.27 ± 0.17[b]	0.24±0.05[c]
	Purple	7.20 ± 0.10[b]	4.74 ± 0.07[c]	1.91 ± 0.30[c]	6.84±4.03[a]
	Yellow	2.15 ± 0.89[a]	1.79 ± 0.05[a]	0.60 ± 0.01[d]	0.27±0.08[c]
Flesh	Russet	1.18 ± 0.03[d]	0.58 ± 0.14[b]	0.33 ± 0.01[d]	0.007±0.006[e]
	Purple	0.78 ± 0.06[c]	0.82 ± 0.06[e]	0.45 ± 0.16[d]	0.64±0.17[d]
	Yellow	0.45 ± 0.03[c]	0.41 ± 0.05[b]	0.50 ± 0.16[d]	0.02±0.02[f]

[1]Data are expressed as means ± SD (n=3). Values in each column having the same letter are not significantly different ($p > 0.05$). [2]GAE, gallic acid equivalents.

Hale (2003) noted that the flesh and skin of purple potato genotypes had higher antioxidant activity, possibly due to the presence of anthocyanins that serve as major contributors to antioxidant activity. Reyes *et al.* (2005) also reported that the ORAC of purple potatoes were two and a half folds higher than those of their white fleshed counterparts. This fundamental difference in antioxidant activity of purple and non-pigmented varieties is related to their anthocyanin content (Brown *et al.*, 2007; Lachman *et al.* 2008, 2009). Thus, it is clear that depending on the variety of potato, bound and esterified phenolics contribute as much or even more than free phenolics to the antioxidant activity of the peels; the extracts from purple variety exhibiting the highest activity.

In order to shed light on the contribution of phenolics to antioxidant activity measured by different methods, the correlations between the free, esterified and bound phenolic content and total antioxidant activities were analyzed using the Pearson correlation test; correlation coefficients are summarized in Table 5.4. The total bound phenolic content positively and strongly correlated with TEAC ($R^2 = 0.88$, $p < 0.01$), DRSC ($R^2 = 0.97$, $p < 0.01$), ORAC ($R^2 = 0.99$, $p < 0.01$) and reducing power ($R^2 = 0.96$, $p < 0.01$). As shown in Table 5.4, the total free and esterified phenolics also showed a strong linear relationship with TEAC, ORAC and reducing power. The positive correlation indicates that the higher phenolic content resulted in a higher antioxidant activity; the strongest correlation existed between bound phenolics and different antioxidant activity assays employed. However, in contrast to the earlier report by Hesam *et al.* (2012), no significant linear relationship existed between total free or esterified

phenolic content and DRSC (R^2 = 0.56 and 0.54, respectively, $p > 0.05$) in this study. This may indicate the presence of components other than phenolics in potatoes that can directly react with radicals. Further analysis of potato extracts is necessary to clearly explain the different observation of the correlation between TPC and DRSC.

5.5 Inhibition of oxidation in fish meat model system

Lipid oxidation is a major cause of food quality deterioration, in general, and in muscle foods, in particular, thus leading to the formation of a number of products which are responsible for off-odour and off-flavour development (Shahidi and Zhong, 2010). Secondary oxidation products are a reliable indicator of flavour deterioration in fish products (Shahidi 1998) and hence the TBARS assay was used in this study to assess the efficacy of extracts from different varieties of potato peel and flesh to retard the development of oxidative rancidity in fish. Natural sources of antioxidants and preferred to synthetic antioxidants due to possible toxicity and carcinogenic potential of the latter (Barlow, 1990; Prior and Cao, 2000; Kaur and Kapoor, 2001). The muscle of fish used contained 62.18 ± 0.65% moisture and 12.73 ± 0.27% total lipids. The TBARS values of antioxidant-treated fish samples stored at 4°C over 7 days are shown in Table 5.5. The soluble potato extracts were added at 1% level which is approximately equal to 200 ppm gallic acid (GA). BHA and chlorogenic acid were used as positive controls at 200 ppm. The extracts were effective in inhibiting the oxidation of cooked salmon in comparison with the control which showed the highest TBARS values at the end of the 7 day storage period. The order of effectiveness in inhibiting the formation of TBARS was: Russet

potato peel > BHA > purple potato peel > Innovator potato peel > chlorogenic acid > purple potato flesh > yellow potato peel > Russet potato flesh > yellow potato flesh > control. However, no significant ($p > 0.05$) difference existed for the efficacy of the Russet potato flesh, yellow potato flesh and the control. At the end of day 7 of storage, Russet, purple, Innovator and yellow potato peel extracts inhibited the formation of TBARS by 83.4, 39.7, 31.4 and 9.48%, respectively, while purple potato flesh inhibited oxidation by 14.7%. Furthermore, BHA and chlorogenic acid at 200 ppm inhibited TBARS formation by 45.0 and 31.0%, respectively. The better efficacy of Russet potato peel compared to BHA in lowering TBARS values is of interest. Russet, purple and Innovator varieties also exhibited better antioxidant activity, as reflected in lower TBARS values than chlorogenic acid, possibly due to synergistic activity of different phenolics present in the extracts.

5.6 Supercoiled strand DNA scission by peroxyl and hydroxyl radicals

DNA molecules are easily attacked by free radicals that induce base modification and strand scission, leading to mutagenesis and possibly cancer. Thus, the effectiveness of the extracts to prevent the scission of DNA strands is a reflection of their positive effects against many diseases. Peroxyl radicals, used in the present study, are known to exert oxidative damage in biological systems due to their comparatively long half-life and thus greater affinity to diffuse into biological fluids in cells (Hu and Kitts, 2001). Soluble extracts from different potato varieties were dissolved in PBS at a concentration of 17.8 mg/mL before mixing them with the DNA. Figure 5.2 shows the percentage of

supercoiled DNA strands retained after incubation with peroxyl radicals generated by AAPH. Phenolic extracts from purple potato peels were most effective showing a DNA strand scission inhibition of 91.02%, while extracts from yellow flesh potato were least effective with 34.42% inhibition.

Radicals cleave supercoiled pBR 322 plasmid DNA (form I) to nicked circular DNA (form II) as shown in Figure. 5.1A. Lane 1 represents the native DNA without any additives and lane 2 represents the blank, where the reaction mixture does not contain any antioxidant. The presence of a high intensity form II band and the disappearance of form I band in lane 2 indicate that the DNA was completely nicked. Potato extracts which were added in the remaining wells showed good strand scission inhibiting activity as greater intensity of form I band or the supercoiled plasmid DNA was clearly displayed. The DNA scission inhibitory effects of potato extracts in the present study may be due to their ability to scavenge peroxyl radicals as shown in ORAC and TEAC assays (Tables 5.2 and 5.3).

Hydroxyl radicals generated by Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield open circular or relaxed forms. The concentration of phenolic extracts of different potato peel and flesh was (2 μg/mL) effect of methanol/acetone/water (7:7:6, v/v/v) soluble. In Figure 5.1B, the soluble extracts of purple potato peel and russet potato peel (lanes 7 and 8) showed significant reduction (89.7 and 79.1%, respectively) in the formation of nicked DNA and increased retention of the native form of DNA. The protection offered by Innovator potato peel (lane 9) (75.9%) was close to that of the Russet potato peel. The purple potato flesh (lane 3,

74.2%) and yellow potato peel (lane 6, 65.4%) and Russet potato flesh (lane4, 60.2%) showed moderate, while yellow potato flesh (lane 5, 53.22%) showed comparatively low protection. The purple potato peel and Russet potato peel with high phenolic content showed better protection compared to the others, indicating that protection was directly proportional to the total phenolic content. Chlorogenic acid has been found to be the predominant phenolic acid in potato and constitutes up to 90% of total phenolics in potato tuber, and approximately 50% of chlorogenic acid are found in the skin (Friedman, 1997; Shahidi and Naczk, 2004; Rice-Evans *et al*., 1996). Further, chlorogenic acid can inhibit DNA damage in vitro (Margreet *et al*., 2001). Margreet *et al*. (2001) showed that one third of chlorogenic acid and almost all of the caffeic acid were absorbed in the small intestine of humans. This implies that part of chlorogenic acid from foods will enter into the blood circulation, but most will reach the colon. Therefore, in the purple potato peel and Russet potato peel presence of high quantities of chlorogenic acid might be responsible for better protection of DNA. In biological systems metal binding can occur on DNA leading to partial site-specificity of hydroxyl radical formation. Polyphenols are potential protecting agents against the lethal effects of oxidative stress and offer protection to DNA by chelating redox-active transition metal ions.

Potato is widely used all over the world and their skin goes to the waste. In the present study, it was found that soluble portion of purple potato peel, Russet potato peel, and Innovator potato peel is rich in phenols which scavenge free radicals and provide protection against DNA damage caused by reactive oxygen species. Furthermore, bound phenolic extract (Figure 5.2A) of purple potato peel (lane 4) showed highest inhibition of

78.3% followed by Russet potato peel at 67.5% > Innovator potato peel at 66.2% > bound yellow potato peel at 55.5% (Figure 5.2B).

Table 5.2. DPPH radical scavenging activity and trolox equivalent antioxidant capacities of freeze dried flesh and peel from different potato varieties[1]

Potato sample	Potato variety	TEAC[2] (μmoles trolox eq/g freeze dried potato)			DPPH radical scavenging activity (μmoles trolox eq/g freeze dried potato)		
		Free phenolics	Esterified phenolics	Bound phenolics	Free phenolics	Esterified phenolics	Bound phenolics
Peel	Russet	348.27 ± 45.57^{a}	76.32 ± 4.94^{a}	679.08 ± 8.27^{c}	9.96 ± 2.28^{a}	8.44 ± 0.27^{a}	9.61 ± 0.76^{a}
	Innovator	301.82 ± 58.98^{a}	149.78 ± 43.47^{b}	1412.50 ± 52.84^{b}	10.81 ± 0.65^{a}	11.96 ± 0.56^{b}	9.98 ± 0.68^{a}
	Purple	835.59 ± 48.72^{b}	706.7 ± 10.95^{c}	594.41 ± 4.12^{c}	6.38 ± 0.92^{b}	5.08 ± 0.56^{c}	5.64 ± 0.74^{b}
	Yellow	203.22 ± 33.14^{a}	23.10 ± 7.20^{d}	471.97 ± 27.34^{d}	5.56 ± 1.03^{b}	6.43 ± 1.19^{c}	1.89 ± 0.28^{c}
Flesh	Russet	52.66 ± 17.93^{c}	90.61 ± 17.93^{a}	175.73 ± 7.27^{a}	0.69 ± 0.30^{c}	0.13 ± 0.05^{d}	0.08 ± 0.01^{d}
	Purple	79.33 ± 13.41^{d}	88.20 ± 19.2^{a}	183.88 ± 14.10^{a}	2.74 ± 0.60^{d}	2.30 ± 0.61^{e}	1.79 ± 0.28^{c}
	Yellow	83.00 ± 17.52^{d}	82.81 ± 11.23^{a}	110.03 ± 15.57^{b}	0.86 ± 0.04^{c}	0.32 ± 0.10^{d}	0.04 ± 0.02^{d}

[1]Data are expressed as means ± SD (n=3). Values in each column having the same letter are not significantly different ($p < 0.05$). [2] TEAC, trolox equivalent antioxidant capacity.

Table 5.3. ORAC and Reducing powers of freeze dried flesh and peel from different potato varieties[1]

Potato sample	Potato variety	ORAC[2] (μmoles trolox eq/g freeze dried potato)			Reducing power (μmoles trolox eq/g freeze dried potato)		
		Free phenolics	Esterified phenolics	Bound phenolics	Free phenolics	Esterified phenolics	Bound phenolics
Peel	Russet	882.45 ±125.56^{a}	237.59 ± 2.68^{a}	1689.64 ± 28.75^{a}	602.41 ± 25.99^{a}	145.54 ± 24.63^{a}	785.93 ± 40.39^{a}
	Innovator	1211.14 ± 149.67^{b}	1100.21 ± 14.42^{b}	1834.86 ± 171.19^{a}	694.89 ± 43.87^{a}	276.36 ± 26.95^{b}	936.01 ± 43.19^{b}
	Purple	1832.92 ± 170.29^{c}	916.96 ± 26.16^{c}	417.36 ± 92.39^{b}	674.74 ± 52.88^{a}	346.87 ± 20.72^{c}	158.15 ± 3.40^{c}
	Yellow	415.52 ± 15.59^{d}	187.10 ± 12.79^{d}	275.65 ± 19.72^{b}	223.72 ± 12.74^{b}	81.79 ± 13.26^{d}	255.42 ± 39.69^{d}
Flesh	Russet	158.97 ± 6.79^{d}	99.64 ± 4.89^{e}	71.72 ± 8.00^{c}	129.86 ± 10.59^{c}	107.92 ± 2.89^{a}	38.89 ± 0.10^{e}
	Purple	186.45 ± 43.70^{d}	193.56 ± 16.31^{d}	87.95 ± 7.12^{c}	128.73 ± 15.32^{c}	62.21 ± 20.82^{d}	136.61 ± 11.62^{c}
	Yellow	150.13 ± 24.20^{d}	128.96 ± 5.13^{e}	95.92 ± 7.35^{c}	121.20 ± 4.25^{c}	89.60 ± 3.14^{d}	117.56 ± 4.51^{c}

[1]Data are expressed as means ± SD (n=3). *Values in each column having the same letter are not significantly different ($p < 0.05$). [2] ORAC, oxygen radical absorbance capacity.

Table 5.4. Correlation analysis of total free, esterified, bound phenolics and antioxidant activities[a]

Antioxidant activities	Total phenolic content		
	Free	Esterified	Bound
TEAC	0.99**	0.85*	0.88**
DPPH radical scavenging activity	0.56	0.54	0.97**
ORAC	0.96**	0.86*	0.99**
Reducing power	0.82*	0.93**	0.96**

[a] Correlation coefficient R^2

* Significantly different $p \leq 0.05$

** Significantly different $p \leq 0.01$

Table 5.5. TBARS values as malondialdehyde (MDA) equivalents of soluble extracts of potato peel and flesh on days 0 and 7 of storage at 4°C[1]

Samples added to salmon[2]	TBARS (mg MDA eq./kg fish)	
	Day 0	Day 7
Control	2.15 ± 0.04^{a}	4.64 ± 0.11^{a}
BHA	1.35 ± 0.03^{b}	2.55 ± 0.50^{b}
Chloregenic acid	2.52 ± 0.70^{aZ}	3.20 ± 0.72^{cZ}
Innovator peel	1.47 ± 0.02^{c}	3.18 ± 0.04^{d}
Russet potato peel	1.56 ± 0.19^{d}	0.77 ± 0.04^{e}
Yellow peel	2.15 ± 0.29^{a}	4.20 ± 0.70^{f}
Purple peel	0.83 ± 0.05^{e}	2.80 ± 0.52^{g}
Russet flesh	1.6 ± 0.10^{f}	4.62 ± 0.85^{a}
Yellow flesh	2.79 ± 0.37^{a}	4.98 ± 0.08^{a}
Purple potato flesh	2.69 ± 0.16^{a}	3.96 ± 0.23^{h}

[1]Data are expressed as means ± SD (n=3).
[2]Soluble extracts from potato peel and flesh were added to fish meat at 1% level.

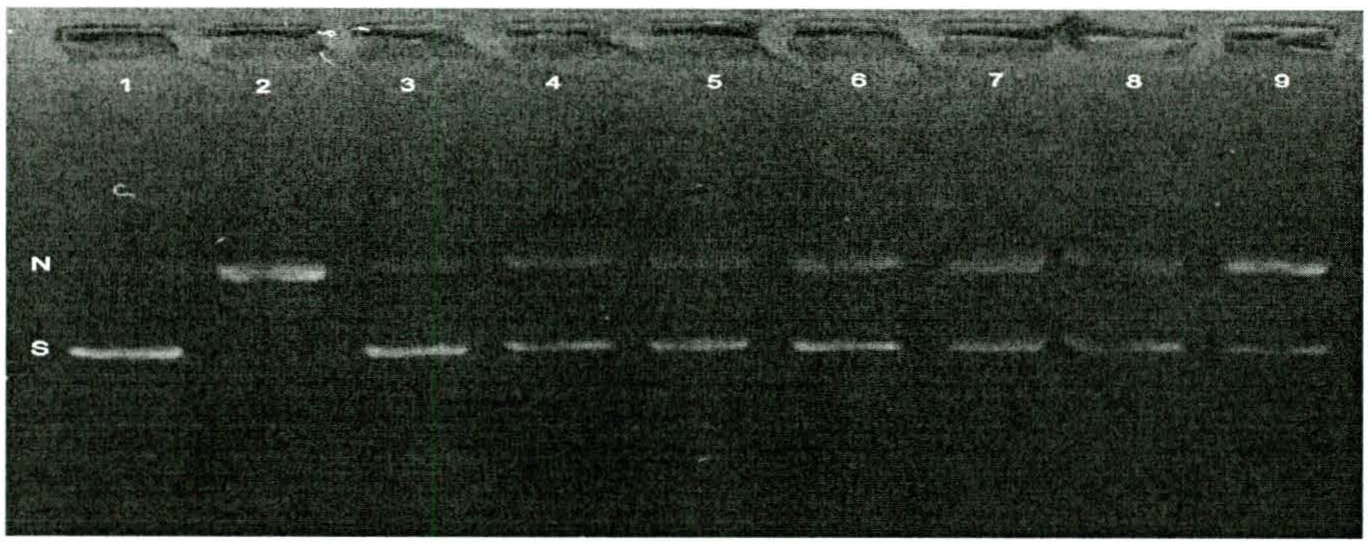


Figure 5.1.A .Effect of addition of palm leaf and date seed phenolic extracts in peroxyl radical treated DNA system.

Lane 1: Control (DNA only); Lane 2: Blank (DNA and AAPH); Lane 3: soluble Purple potato skin extract; Lane 4: soluble Russet potato peel extract; Lane 5: Soluble Yellow potato peel extract; Lane 6: soluble Innovator peel extract; Lane 7: Soluble purple potato flesh extract; Lane 8: soluble Russet potato flesh extract; Lane 9: soluble Yellow potato flesh extract; S, supercoiled plasmid DNA strands; and N, nicked DNA strands

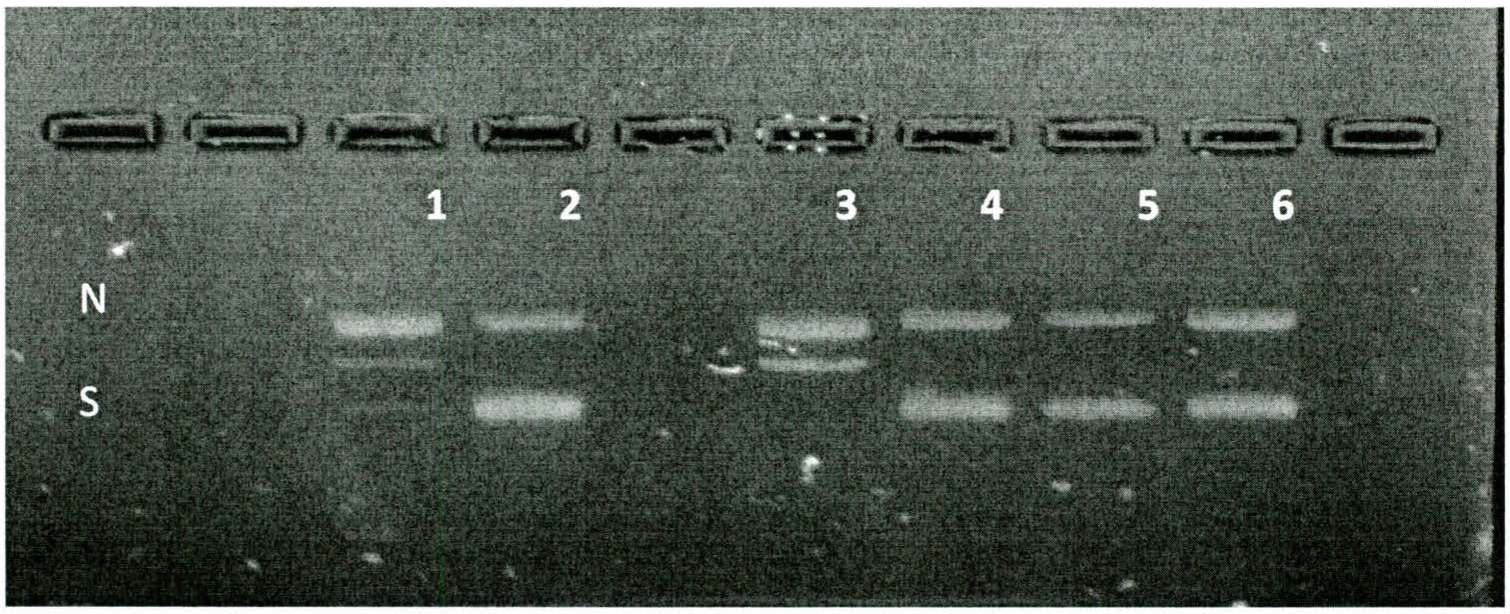


Figure 5.1.B Effect of addition of bound phenolic potato peel extracts in hydroxyl radical treated DNA system.

Lane 1: Blank (DNA and hidroxyl radical); Lane 2: Control (DNA only); Lane3: bound YP extract; Lane 4: bound PP extract; Lane 5: bound RP extract; Lane 6: bound IP extract; S: Supercoiled plasmid DNA strands; N, nicked DNA strands. Abbreviated: PF: Purple potato flesh, PP: Purple potato peel, RP: Russet potato peel, and IP: Innovator potato peel

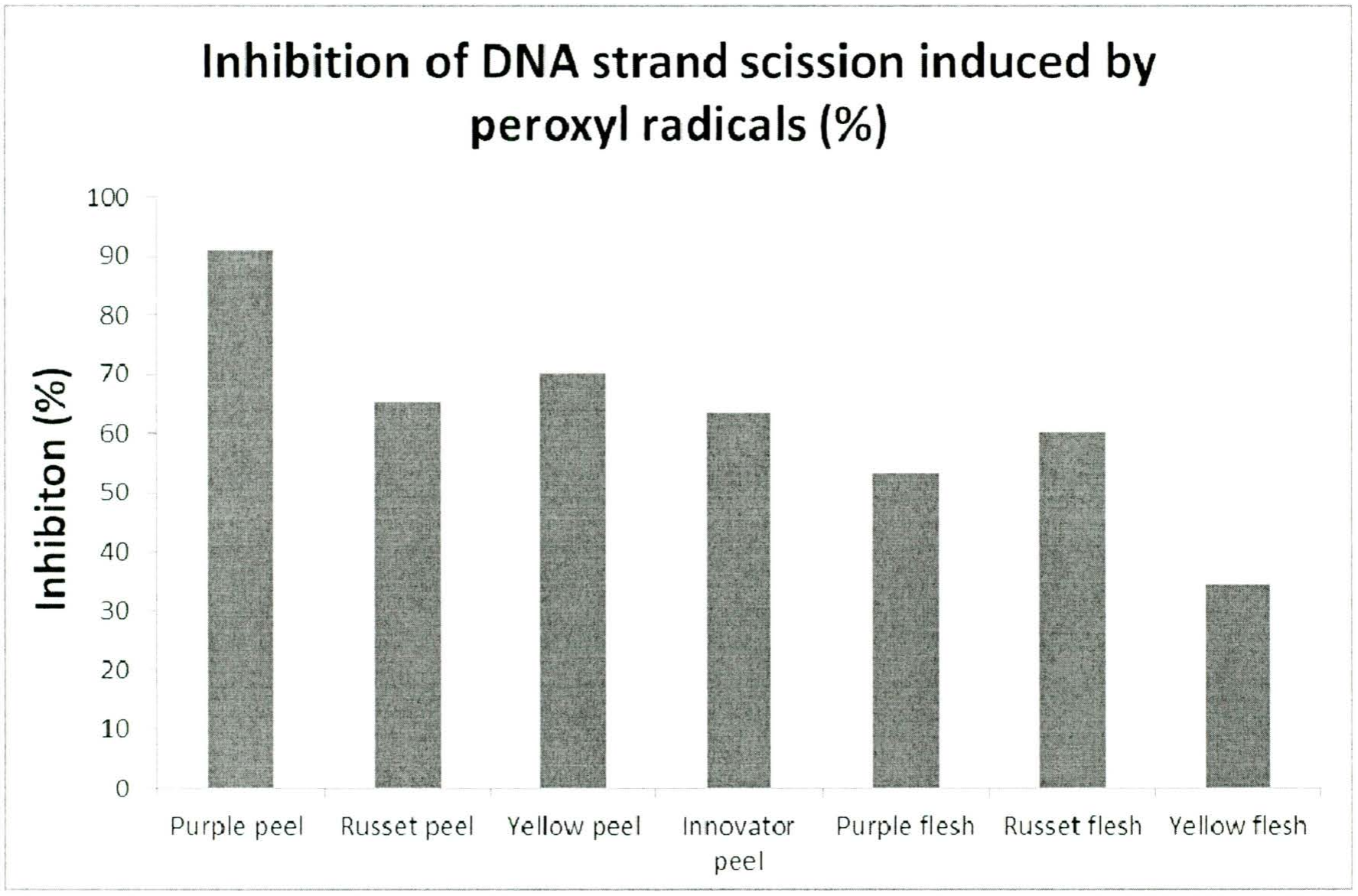


Figure 5.2. Inhibition percentage of supercoiled pBR 322 plasmid DNA scission in peroxyl radical-mediated systems with soluble phenolic extracts from different potato samples.

Figure 5.2. Inhibition percentage of supercoiled pBR 322 plasmid DNA scission in hydroxyl radical-mediated systems with soluble phenolic extracts from different potato samples.

5.7 Inhibition of Cupric Ion-induced Human LDL Cholesterol Oxidation

Oxidation of polyunsaturated lipid components of LDL cholesterol by reactive oxygen species plays a key role in the pathogenesis of atherosclerosis (Esterbauer, 1993). Furthermore, transition metal ions may promote oxidative modification of LDL

cholesterol through hydroperoxides (Decker *et al.*, 2001; Chandrasekara and Shahidi, 2011a). According to Decker *et al.* (2001) both free radical scavenging and copper chelating activity of antioxidants are responsible for inhibition of LDL cholesterol oxidation.

In the present study, the antioxidant activity of potato extracts was determined by measuring the concentration of conjugated dienes (CD) formed during copper-catalyzed human LDL cholesterol oxidation *in vitro*, and the progression of oxidation of LDL over 22 h. All samples showed a rise in CD after 22h. Although the exact reason for this observation is not clear, there is a possibility that phenolic compounds at high concentrations may complex with protein moieties of the LDL cholesterol molecules, thus making them unavailable to inhibit oxidation of cholesterol (Chandrasekara and Shahidi, 2011b; Riedl *et al.*, 2001). Table 5.6 presents the results for the effectiveness of potato peel extracts in inhibition LDL cholesterol oxidation. The results show that purple potato peel extract was the strongest in inhibiting LDL cholesterol oxidation, followed by Russet potato extract, Innovator potato peel extract, and yellow potato peel extract.

Table 5.6. Effect of potatoes peel extracts on preventing cupric ion induced human low density lipoprotein (LDL) oxidation[1]

Potato Sample	Inhibition (%)
Purple peel	26.43±0.54[a]
Russet peel	24.43±0.56[b]
Innovator peel	21.88±1.33[c]
Yellow peel	17.06±5.20[d]

[1]Data are expressed as means ± SD (n=3). *Values in each column having the same letter are not significantly different ($p < 0.05$). [2] ORAC, oxygen radical absorbance capacity.

5.8 HPLC analysis of phenolic compounds

The predominant phenolic acids and flavonoids in the potato samples that were identified and quantified by HPLC are listed in Table 5.6. The chromatograms of the major phenolic compounds in potato samples are shown in Figure 5.1. The retention times of the standards, namely gallic, protocatechuic, chlorogenic, caffeic, *p*-coumaric and ferluic acids were 7.38, 11.35, 12.53, 15.11, 15.54 min, respectively. Phenolic acids (chlorogenic, caffeic, *p*-coumaric and ferulic acids) predominated in the potato peel samples. Potato peels have been reported to be rich in phenolic acids, especially chlorogenic, gallic, cinnamic, ferulic, protocatechuic and caffeic acids (Onyeneho and Hettiarachchy, 1993; Im *et al.*, 2008). In agreement with the present results, chlorogenic acid was by far the most abundant phenolic compound present followed by caffeic acid, as has previously been reported for potato (Malmberg, 1984; Im *et al.*, 2008; Al-Weshahy

and Rao, 2009; Al-Weshahy *et al.*, 2011). Chlorogenic acids are esters of *trans*-cinnamic acids and quinic acid, and exist in multiple forms. Phenolic acids such as chlorogenic acid, caffeic acid and ferulic acid are generally found in the UV-vis spectrum, with a maximum absorption at 325 nm. The peel extract exhibited an absorption maximum close to 325nm due to the presence of cinnamic acid. In the present study, caffeic and ferulic acids were present in all three fractions (free, esterified and insoluble-bound) of potato peels. Chlorogenic acid was the most abundant phenolic acid in the Innovator, Russet and purple potato peels in the free form only; while caffeic acid was the most abundant phenolic acid in the yellow potato in the free form. The amount of chlorogenic acid found in potato varieties varies considerably (Nara *et al.*, 2006).

Ferulic acid was the major phenolic acid in the bound form in all potato peel samples, followed by caffeic acid. Nara *et al.* (2006) also identified ferulic acid to be the prominent bound phenolic acid in potato peels. Chlorogenic acid was not detected in the bound and esterified forms, while *p*-coumaric acid was only detected in the bound fraction of Russet potato peels. The amount of total phenolics, using HPLC, correlates with the results obtained using the Folin-Ciocalteau assay where purple potato peels contained the highest amounts of total phenolic acids, followed by Innovator, Russet and yellow varieties.

Table 5.7. Content of prominent phenolic acids (mg/g freeze dried sample) in the extracts of potato peels quantified using HPLC

Potato sample	Chlorogenic acid			Caffeic acid			*p*-Coumaric acid			Ferulic acid		
	Free	Esterified	Bound	Free	Esterified	Bound	Free	Esterified	Bound	Free	Esterified	Bound
Innovator [P]	1.289	_	_	0.233	0.344	0.517	0.038	0.015	_	0.028	0.027	0.793
Russet [P]	1.349	_	_	0.257	0.187	0.541	0.017	0.019	0.051	0.011	0.030	0.528
Yellow [P]	0.163	0.006	_	0.268	0.004	0.025	0.026	_	_	0.031	0.034	0.060
Purple [P]	3.074	0.575	_	0.658	0.103	0.159	_	0.05	0.024	_	0.014	0.055
Purple [F]	0.144	_	_	_	0.003	0.001	_	0.008	0.001	_	0.004	_
Russet [F]	0.150	_	_	0.001	0.022	0.001	-	0.002	0.001	0.001	0.028	0.003
Yellow [F]	0.079	_	_	0.001	0.037	0.001	-	0.002	0.002	0.006	0.034	0.002

([P]): Peel, ([F]): Flesh

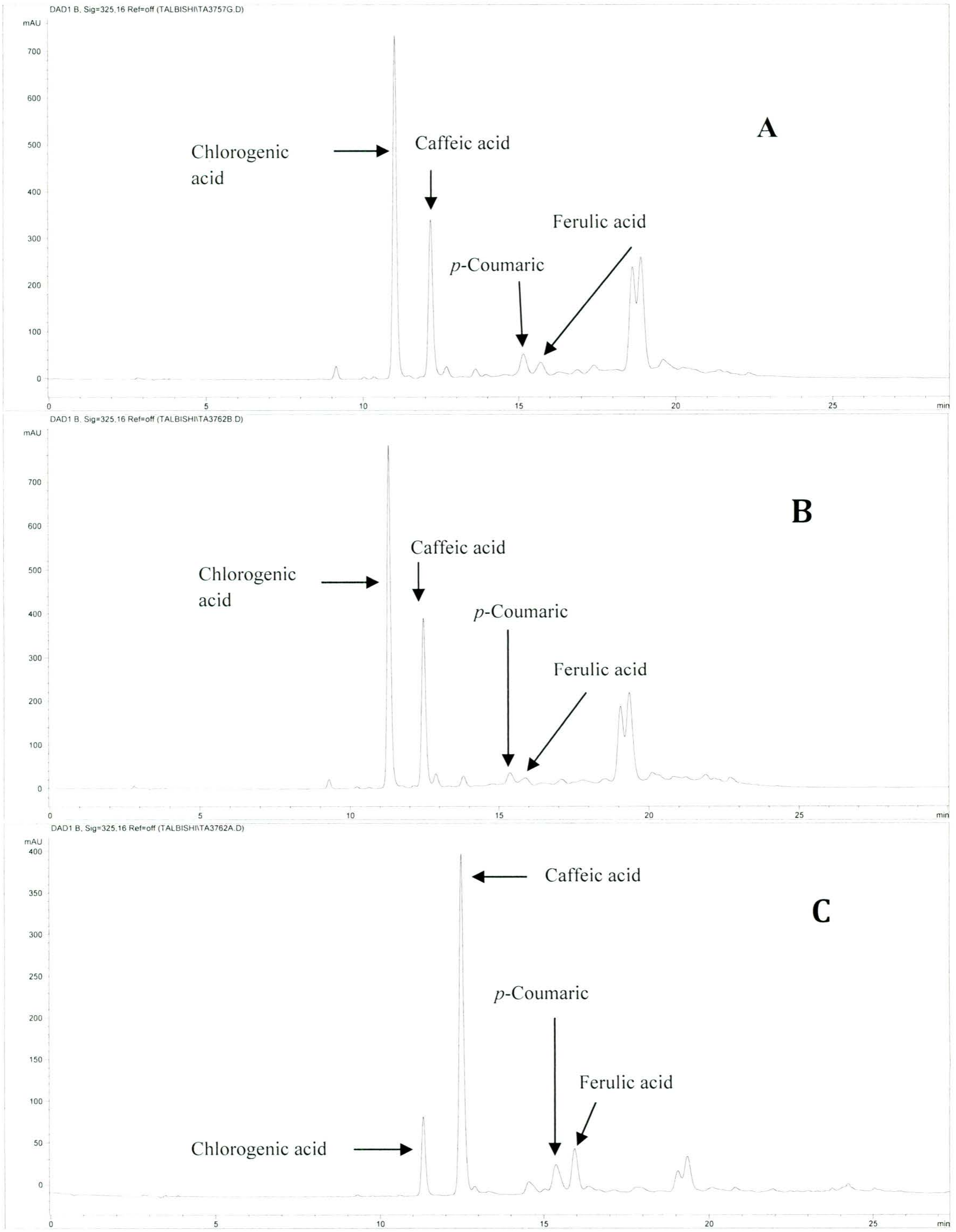


Figure 5.4. HPLC chromatograms at 325 nm of free phenolics extracted from peels of (A) Innovator, (B) Russet, and (C) Yellow potato varieties.

CHAPTER 6

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

6.1 Summary

Onion skins are an excellent source of beneficial functional ingredients including antioxidant polyphenols. The present study compared four different varieties of onions with respect to their total phenolic content and antioxidant activity in order to demonstrate their potential as a source of natural antioxidants. Their antioxidant potential and efficacy in a food and biological model systems was further investigated. The phenolic constituents of onion skin and flesh were fractionated into their respective free, esterified, and bound forms to provide a complete picture of their phenolic composition. The green shoot from one of the sprouted onions was also evaluated along with the flesh to understand the changes that take place in the phenolic constituents during sprouting. The content of free phenolics in tested onion varieties was, in decreasing order, pearl onion skin > red onion skin > yellow onion skin > red onion flesh > sprouted red onion flesh > white onion skin. A similar trend was also followed by the esterified and bound forms of phenolic compounds in the onion samples. Phenolics were predominantly present in the free form both in the onion skin and flesh. With respect to the antioxidant activity tests carried out – trolox equivalent antioxidant capacity (TEAC) and DPPH radical scavenging capacity (DRSC), and reducing power, samples which had the highest phenolic and/or flavonoid content were most effective as free radical scavengers. Red onion skin showed the highest TEAC and DRSC radical scavenging activity, followed by red pearl onion skin, yellow onion skin, red onion flesh, sprouted red onion flesh and

white onion skin. The extracts were also effective in inhibiting the oxidation of cooked salmon in comparison with the control which showed the highest TBARS values at the end of a 7-day storage period. The samples arranged in the order of their effectiveness in inhibiting the formation of TBARS and reported as malondialdehyde (MDA) equivalents (%) were as follows: red onion skin (68.46%) > pearl onion skin (60.48%) > green shoots from red onion (53.29%) > BHA (51.89%) > red onion flesh (50.30%) > yellow onion skin (48.71%) > white onion skin (46.51%) > quercetin (36.72%) > sprouted red onion flesh (9.78%) > control. Similarly, in a biological model system, soluble phenolic extracts from red onion skin was most effective in inhibiting DNA strand scission by 94.45%, followed by pearl onion skin (91.45%) and yellow onion skin (84.26%) while extracts white onion skin, red onion and sprouted red onion flesh exhibited low activity of around 10%. The HPLC analysis of samples examined showed that gallic, protocatechuic and *p*-hydroxybenzoic acids were the most abundant phenolic acids. Quercetin, quercetin 3 glucoside and kaempferol were predominant flavonoids in the free form in all the onion samples; quercetin being the most abundant. The highest content of anthocyanins was found in the coloured skin of coloured onions, while white onion skin had the lowest anthocyanin content.

Potato peels are the by-products of the potato processing industry and are an excellent source for the recovery of phenolic compounds. Nearly 50% of phenolics are located in the peel and adjoining tissues of potatoes and their content decreases towards the centre of the tuber. In the present study, the phenolic constituents of four different potato varieties (Russet, Innovator, Purple and Yellow) potato peel and flesh were

fractionated into their respective free, esterified, and bound forms and the relative proportions of the various phenolic acids present were determined using chemical tests and high-performance liquid chromatography (HPLC). The antioxidant capacities of their extracts were also studied in *in-vitro* food and biological model systems. Examination of the total free, esterified and bound phenolics indicated that purple potato peel contained the highest amount of free (7.2 mg GAE/g peel) and esterified (4.74 mg GAE/g peel) phenolics among all varieties analyzed. It is also interesting to note that the phenolics compounds were predominantly present in the bound form in the peels of both the Innovator (51.07% of the total phenolics) and Russet (45.95% of total phenolics) varieties; while the free and esterified phenolics were the predominant forms in both the purple and yellow potatoes. As expected, the flesh contained much lesser quantities of the phenolics as compared to the peels. With respect to the antioxidant activity tests carried out, extracts from purple potato peel, which had the highest phenolic content among all the varieties tested, exhibited the highest antioxidant activity in all tests except DRSC radical scavenging activity, where the Innovator potato peel and Russet potato peel varieties showed the highest scavenging activity for DRSC. Again, the activities in the flesh were much lower than those exhibited by the peel. It is also noteworthy that the antioxidant activities of the bound phenolics in the peels of Russet and Innovator varieties were higher than those of the free phenolics; while the esterified phenolics made an almost equal contribution as the free phenolics to the antioxidant activities of the purple potato peel which again correlated with their relative contents in the peel. In addition, though the purple potato peel showed much higher radical scavenging activities

compared to the other samples, its reducing power was comparable to those of Innovator and Russet varieties. Thus, it is clear that depending on the variety of potato, bound and esterified phenolics contribute as much or even more than free phenolics to the antioxidant activity of the peels; with extracts from purple variety showing the highest activity. The extracts were also effective in inhibiting the oxidation of cooked salmon in comparison with the control which showed the highest TBARS values at the end of a 7-day storage period. The samples arranged in the order of their effectiveness in inhibiting TBARS formation were as follows: Russet potato peel > BHA > purple potato peel > Innovator potato peel > chlorogenic acid > purple potato flesh > yellow potato peel > Russet potato flesh > yellow potato flesh > control. In a biological model system assay, phenolic extracts from purple potato peel were most effective in inhibiting DNA strand scission by 91.02%, while extracts from yellow potato flesh were least effective with 34.42% inhibition. The HPLC analysis revealed that phenolic acids (chlorogenic, caffeic, *p*-coumaric and ferulic acids) were predominant in the potato peel samples. Chlorogenic acid was the most abundant phenolic acid in the Innovator and Russet peels in the free form only; caffeic acid was the most abundant phenolic acid in yellow potato in the free form. Ferulic acid was the major phenolic acid in the bound form in all potato peel samples, followed by caffeic acid. Soluble extract of purple potato peel and flesh, Russet potato peel and flesh, yellow potato peel and flesh, and Innovator potato peel were evaluated for their total anthocyanin. A higher concentration of anthocyanins, 10.9 times, was found in the purple potato peel compared to that in purple potato flesh, followed by Russet potato peel > yellow potato peel > Innovator potato peel > yellow potato flesh >

Russet potato flesh. Thus, anthocyanins in pigmented potatoes display higher antioxidant potential in comparison with other potato peels and flesh.

6.2 Conclusions and suggestions for future research

Onion skin especially the darker coloured ones, are rich in a number of phenolic compounds that display antioxidative properties. The study reported here has clearly established that onion skin serves as a promising source of natural antioxidants for the development of nutraceuticals or value-added products. Moreover, the *in vitro* studies carried out further provides strong biochemical rationale for performing further *in vivo* animal and human clinical studies to demonstrate the benefit of onion-based diet and to confirm safe use of such products, as such or as functional food ingredient. Absorption, accessibility and metabolism of phenolics involved should also be studied. Similarly, the studies reported here also demonstrated potato peels contain a number of antioxidant compounds which can effectively scavenge various reactive oxygen species / free radicals under *in vitro* conditions. The broad range of activities of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity of potato peel extracts and clearly indicates their potential application as food ingredients or specialty chemicals. For the first time, it extensively examined all three forms of phenolics, namely free-, esterified- and insoluble-bound phenolics along with their contribution to antioxidant activity and clearly highlighted the importance of including the esterified and bound phenolics in the analysis and reporting of total phenolics content. The qualitative/quantitative analysis of the extracts for phenolic acids showed the presence of

chlorogenic acid, caffeic acid *p*-coumaric and ferulic acids in potato peels and flesh, consistent with the earlier reports. However, further *in vivo* studies are needed to demonstrate the absorption and metabolism of potato peel phenolics. In addition, to in vivo studies, application of these as antioxidant food preservateves should be added. Also, while the potato peel may be a good source of phenolic acids, recovery of these from the industrial doenstream waste require further study as it is very tedious.

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