CHARACTERIZATION OF ANTIOXIDANT PROPERTIES FOR DRIED ORGANIC FRUITS IN TURKISH MARKET AND DEVELOPMENT OF NOVEL STRATEGIES TO INCREASE THEIR POTENTIAL HEALTH BENEFITS

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ABSTRACT

CHARACTERIZATION OF ANTIOXIDANT PROPERTIES FOR DRIED ORGANIC FRUITS IN TURKISH MARKET AND DEVELOPMENT OF NOVEL STRATEGIES TO INCREASE THEIR POTENTIAL HEALTH BENEFITS

In this thesis antioxidant parameters including trolox equivalent antioxidant capacity (TEAC) and total phenolics (TPC) and flavonoid (TFC) contents of major organic dried fruits (raisins, figs, prunes and apricots) produced in Turkey have been determined to understand the bioactive potential of these products. Moreover, a novel method based on controlled rehydration of specified dried fruits in phenolic extracts has been developed to boost the phenolic content and antioxidant activity of dried fruits with low bioactivity and potential health benefits. The TEAC, TPC and TFC of sun dried fruits varied between 35.7 and 74.1 µmol trolox/g (d.w.), 1762 and 4062 µg gallic acid/g (d.w.) and 830 and 2559 µg catechin/g (d.w.), respectively. The TEAC and TPC of prunes were 1.7 to 2.3 fold higher than those for apricots, raisins and figs which showed quite similar TEAC and TPC values. On the other hand, the TFC of prunes and figs were similar and 1.7 to 3 fold higher than those of raisins and apricots. The rehydration studies with raisins conducted in different concentrations (0.5% or 1% (w/w)) of green tea extract (GTE) and walnut shell extract (WSE) at room temperature until reaching of final moisture content of 38.73 % (w/w) showed the possibility of increasing TEAC, TPF and TPC of raisins 1.6-1.8 fold. Similar rehydration strategy applied to figs, prunes and apricots in 1% GTE to bring their moisture content to 39.83, 36.97 and 43.81 % respectively, caused 1.1-1.6 fold increase in TEAC, TPC and TFC of these dried fruits. This work clearly showed the considerably higher bioactive potential of organic dried prunes than organic dried raisins, figs and apricots. However, the application of controlled rehydration process developed in this work enables increasing antioxidant potential and phenolic contents of figs, raisins and apricots to the level of prunes.

ÖZET

TÜRKİYE PAZARINDA BULUNAN ORGANİK KURUTULMUŞ MEYVELERİN ANTİOKSİDANT ÖZELLİKLERİNİN KARAKTERİZASYONU VE SAĞLIK ÜZERİNDEKİ POTANSİYEL YARAYIŞLILIKLARININ ARTTIRILMASI İÇİN YENİ STRATEJİLER GELİŞTİRİLMESİ

Bu tez çalışmasında Türkiye'de üretilmekte olan başlıca organik kuru meyvelerin (kuru üzüm, incir, erik ve kayısı) biyoaktif potansiyelinin belirlenmesi amacıyla bu ürünlerde troloks eşdeğeri antioksidant aktivite (TEAC), toplam fenolik madde (TPC) ve toplam flavonoit tayini (TFC) gerçekleştirilmiştir. Ayrıca, tezde biyoaktivitesi düşük olan kuru meyvelerin antioksidant fenolik çözeltiler içerisinde rehidrasyonuna dayalı yenilikçi bir yöntemle antioksidant aktivite ve fenolik madde miktarlarının dolayısıyla sağlığa yarayışlılıklarının artırılması üzerinde de durulmuştur. Gerçekleştirilen ölçümler kuru meyvelerin TEAC, TPC ve TFC gibi antioksidant parametrelerinin sırasıyla 35.7 ile 74.1 µmol trolox/g (d.w.), 1762 ile 4062 µg gallic acid/g (d.w.) ve 830 ile 2559 µg catechin/g (d.w.) değerleri arasında değiştiğini göstermiştir. Kuru eriklerin TEAC ve TPC değerleri bu parametrelerin ve dolayısıyla antioksidant potensiyelin oldukça benzer olduğu üzüm, incir ve kayısılardakinden 1.7 ile 3.0 kat kadar yüksek bulunmuştur. Kuru üzümlerin oda sıcaklığında farklı konsantrasyonda (%0.5 veya %1 (w/w)) yeşil çay ekstraktı (GTE) veya ceviz kabuğu ekstraktı (WSE) içerisinde % 38.73 (w/w) neme ulaşacak şekilde rehidre edilmeleri sonucunda TEAC, TPF ve TPC gibi parametreleri 1.6-1.8 kat artırılmıştır. İncir, erik ve kaysılarda aynı rehidrasyon prosedürünün %1 GTE içerisinde uygulanması ve bu ürünlerin nem değerlerinin sırasıyla % 39.83, 36.97 ve 43.81 değerlerine getirilmesiyle ise TEAC, TPC ve TFC değerlerinde 1.1-1.6 kat arasında artışlar sağlanabilmektedir. Bu tez çalışması organik kuru eriklerin biyoaktif özelliklerinin kuru üzüm, incir ve kayısılara göre belirgin şekilde yüksek olduğunu göstermiştir. Ancak, bu tezde geliştirilmiş olan kontrollü rehidrasyon işlemiyle üzüm, incir ve kayısıların antioksidant potensiyel ve fenolik madde miktarlarının eriklerinki düzeyine çıkartılması mümkündür.

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CHAPTER 1

INTRODUCTION

According to European Commission a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease is called a functional food. Such food is consumed as part of a normal food pattern and it is not a pill, a capsule or any form of dietary supplement (Froidmont-Görtz 2010). The total value of the global functional food market is estimated to reach 180 billion TL in 2013 and this value is expected to increase several folds in a near future. The reason for the great demand of consumers to functional food is quite simple: people desire to be healthy. Of course a healthy life requires much more than a healthy diet. However, no one can ignore the contribution of a balanced diet enriched with natural food in human health.

Organic fruits have attracted much attention as functional foods since they are free from pesticides, hormones and contaminated chemical residues, but rich in bioactive phenolic compounds and dietary fiber. In recent years, great efforts have been spent to characterize the phenolic compounds in organic fruit and show the differences between the phenolic contents and profiles of organic and conventional fruits. There is an increasing consensus now that the phenolic content and/or phenolic profile of organic fruit is different than those of conventional fruits (Lombardi-Boccia, Lucarini et al. 2004, Vian, Tomao et al. 2006, Raigon, Rodriguez-Burruezo et al. 2010, Vallverdu-Queralt, Jauregui et al. 2012). However, much more studies are needed to conclude that the bioactive properties of phenolic compounds in organic fruit are considerably higher than those of conventional fruits. On the other hand, there is a general public myth that the health benefits of organic fruit are higher than those of conventional fruit, and this myth still continues to be a driving force in boosting organic product market.

The antioxidant activity is the major health benefit of phenolic compounds and it is attributed to their capacity to show free radical scavenging, metal chelating and singlet oxygen quenching activities. The frequent and long term intake of phenolic compounds via consumption of fresh fruits and vegetables enables shifting toward to antioxidant side of pro-oxidant/antioxidant balance which plays important role in

immune system to suppress oxidative stress and its damages on cells and provides protection against diseases including cardiovascular diseases, cancer, diabetes, neurodegenerative disorders, autoimmune disorders, and aging (Blažeković, Štefan et al. 2012). Dried fruits including raisins, prunes, figs and apricots are good sources of antioxidant phenolic compounds. A recent study demonstrated that raisins are a good dietary source of flavonols (quercetin and kaempferol) and phenolic acids (caftaric and coutaric acid), and they can lower the postprandial insulin response, modulate sugar absorption (glycemic index), affect certain oxidative biomarkers, and promote satiety (Karadeniz, Durst et al. 2000, Williamson and Carughi 2010). Dried figs are also good sources of phenolic compounds such as proanthocyanidins (mainly cyanidin-3-rutinoside), flavonols (quercetin-rutinoside), phenolic acids (chlorogenic acid) and flavones (luteolin 6C-hexose-8C-pentose and apigenin-rutinoside) (Vallejo, Marín et al. 2012). On the other hand, prunes contain neochlorogenic and chlorogenic acids which play important roles in laxative action and in delaying glucose absorption (Stacewicz-Sapuntzakis, Bowen et al. 2001). The phenolic compounds in prunes are also effective on inhibiting human LDL oxidation in-vitro and they serve as preventive agents against chronic diseases such as heart disease and cancer (Stacewicz-Sapuntzakis, Bowen et al. 2001). Although the fruits are rich sources of phenolic compounds, the aqueous infusions of tea (*Camellia sinensis*) are still considered as the most abundant sources of phenolic compounds by majority of the society. The major types of tea consumed worldwide are black, oolong, and green tea (Wu and Wei 2002). However, the health benefits of green tea have been most extensively studied since it is the richest source of catechins which are known with their potent antioxidant activity. The major catechins in green tea include (−) epicatechin (EC), (−) epicatechin-3-gallate (ECG), (−) epigallocatechin (EGC), (−) epigallocatechin-3-gallate (EGCG), (+) catechin, and (+) gallocatechin (GC), but the EGCG is the most abundant catechin in green tea (Zaveri 2006). The green tea polyphenols are known as powerful antioxidants against oxidative stress and oxidative stress related diseases (Zaveri 2006). Besides green tea, walnuts are also among the most potent sources of antioxidant phenolic compounds. A walnut extract generally contains different phenolic compounds including ellagic acid, ellagitanins, gallic acid and flavonoids (Fukuda, Ito et al. 2003). It was reported that the walnuts are potent sources of ellagic acid and ellagitanins which are potent cancer chemopreventive agents (Arcan and Yemenicioğlu 2009).

In this thesis antioxidant parameters including trolox equivalent antioxidant capacity (TEAC) and total phenolics (TPC) and flavonoid (TFC) contents of major organic dried fruits (raisins, figs, prunes and apricots) produced in Turkey have been determined to understand the bioactive potential of these products. Moreover, a novel method based on controlled rehydration of specified dried fruits in green tea and walnut shell extracts has been developed to boost the phenolic content and antioxidant activity of dried fruits.

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CHAPTER 2

PHENOLIC SUBSTANCES

Phenolic substances are a compounds having one or more aromatic ring being attached at least one hydroxyl group (Jaganath and Crozier 2010). Phenolic compounds have been of interest in many researchers due to their nutritional and health effect. Phenolic compounds also have great antioxidant effect and their showing antioxidant effect depending on the number and position of the OH groups and on the pH. Up to now, so many phenolic compounds have been identified (Figure 2.1) (Belitz, Grosch et al. 2009). Phenolic compounds are divided into two main groups as nonflavonoid phenolic compounds and flavonoids (Jaganath and Crozier 2010).

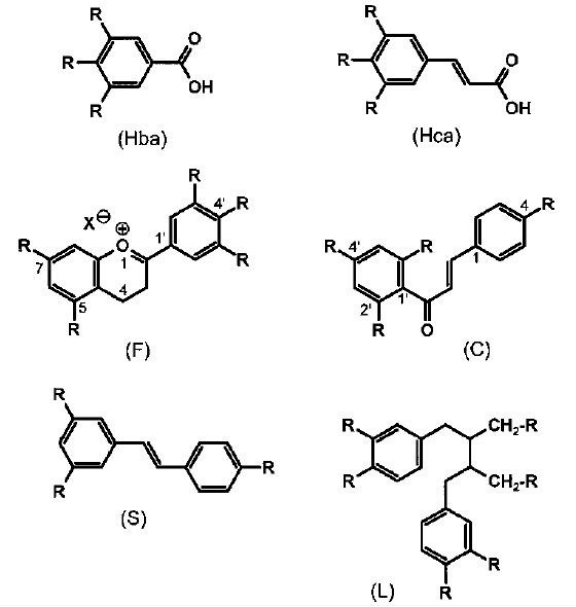


Figure 2.1. Chemical structures of polyphenols; Hydroxybenzoic acids (Hba), Hydroxycinammic acids (Hca), Flavonoids (F), Chalcones (C), Stilbenes, Lignans (L), R: H, OH or OCH_3. (Source: Belitz, Grosch et al. 2009)

2.1. Nonflavonoid Phenolic Compounds

The main nonflavonoid phenolic compounds are the C_6-C_1 phenolic acids, the C_6–C_3 hydroxycinammates and their conjugated derivatives, and the polyphenolic C_6–C_2–C_6 stilbenes (Jaganath and Crozier 2010).

2.1.1. Phenolic Acids

Phenolic acids present in various fruits as derivatives of sugars and organic acids and generally are known as hydroxybenzoic acids. Blackberry, black currant, raspberry, red currant, strawberry, and white currant can be given as examples of fruits that contain phenolic acids. They have eight important unit as salicylic acid (2-hydroxybenzoic acid), 4-hydroxybenzoic acid, gentisic acid (2,4-dihydroxybenzoic acid), protocatechuic acid (3,4-dihydroxybenzoic acid), gallic acid (3,4,5-trihydroxybenzoic acid), vanillic acid (3- methoxy-4-hydroxybenzoic acid) and ellagic acid, the dilactone of hexahydroxydiphenic acid (Figure 2.2). These compounds can be found as free and bounded form in different types of grains such as sorghum, millet, barley, wheat, rice, oat, and rye (Belitz, Grosch et al. 2009).

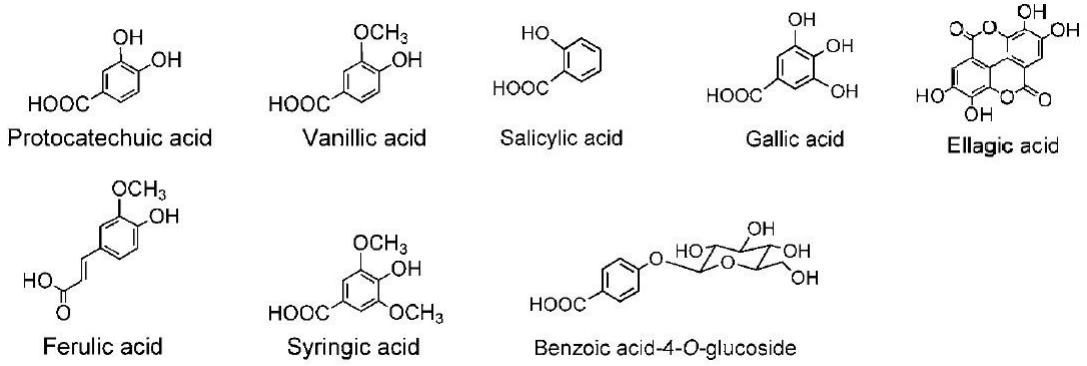


Figure 2.2. Some phenolic acids found in plant foods
(Source: Jaganath and Crozier 2010)

Complexes of phenolic acids form lignins and hydrolyzable tannins, and these complex structures are generally present in the bound form. Gallic acid is the component of gallotannins, in addition to that, parts of gallic acid and hexahydroxydiphenoyl are both components of the ellagitannins, and these are classified as hydrolsable tannins (Jaganath and Crozier 2010).

2.1.2. Hydroxycinammates

Hydroxycinnamates being known as hydroxycinnamic acids are generally found as coumaric, ferulic, caffeic and sinapic acids in fruits and vegetables. Hydroxycinnamic acids form ester bonds with D-quinic and D-glucose. Quinic acid having four –OH groups and caffeic acid conjugates form 3-, 4-, and 5-*O*- caffeoylquinic acid derivatives (Figure 2.4), belonging to chlorogenic acid family and form other chlorogenic acid derivatives such as neochlorogenic acid and cryptochlorogenic acid (Figure 2.3) (Belitz, Grosch et al. 2009, Jaganath and Crozier 2010).

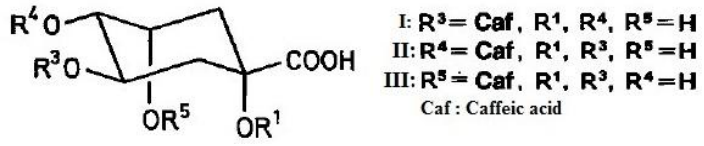


Figure 2.3. Quinic acid structure; neochlorogenic acid (I), cryptochlorogenic acid (II), chlorogenic acid (III) (Source: Belitz, Grosch et al. 2009)

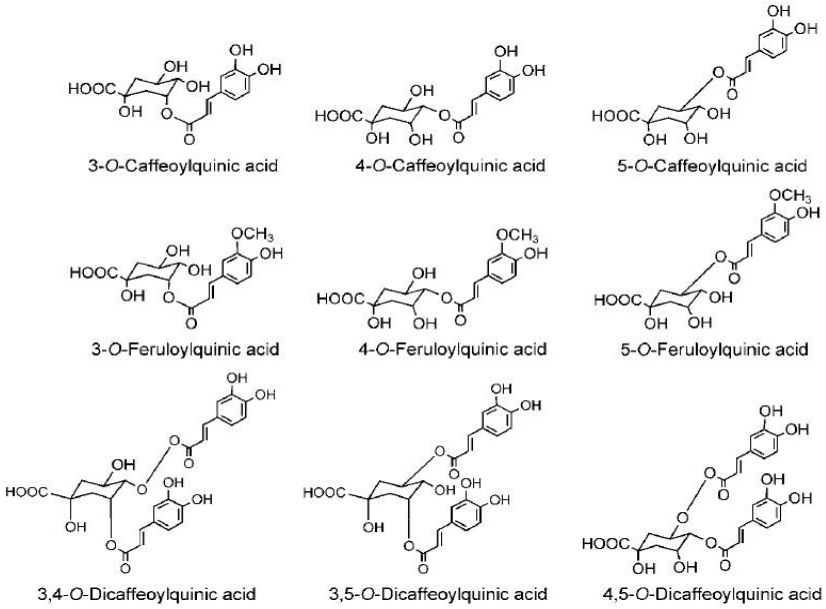


Figure 2.4. Main chlorogenic acids in some plant foods (Source: Jaganath and Crozier 2010)

In order to D-quinic acid and D-glucose, hydroxycinnamic acids can esterified with other alcoholic components including shikimic, malic and tartaric acids and meso-inositol (Belitz, Grosch et al. 2009).

2.1.3. Stilbenes

Stilbenes are nonflavonoid polyphenolic compounds having C_6-C_2-C_6 basic structure and they are also phytoallexins produced by plants in case of disease, injury, and stress. Stilbenes are also known as resveratrol (3,5,4'-trihdroxystilbene), and resveratrol are generally found as *cis* and *trans* isomers in diets (Figure 2.5) (Jaganath and Crozier 2010). Human diet contains low amounts of stilbenes. Until now, lots of stilbenes have been isolated from plants being more than 70 species (D'Archivio, Filesi et al. 2007). Peanuts, berries, red cabbage, spinach, and certain herbs can be given as examples of these plant species. Trans-resveratrol plays important role to inhibit or delay the cardiovascular illnesses and cancer. They also improve resistance to stress and provide longevity (Jaganath and Crozier 2010). It is reported that trans-resveratrol and its derivatives encompass apoptosis, cell-cycle arrest, and inhibition of lymphendothelial gap formation in vitro (Madlener, Saiko et al. 2010).

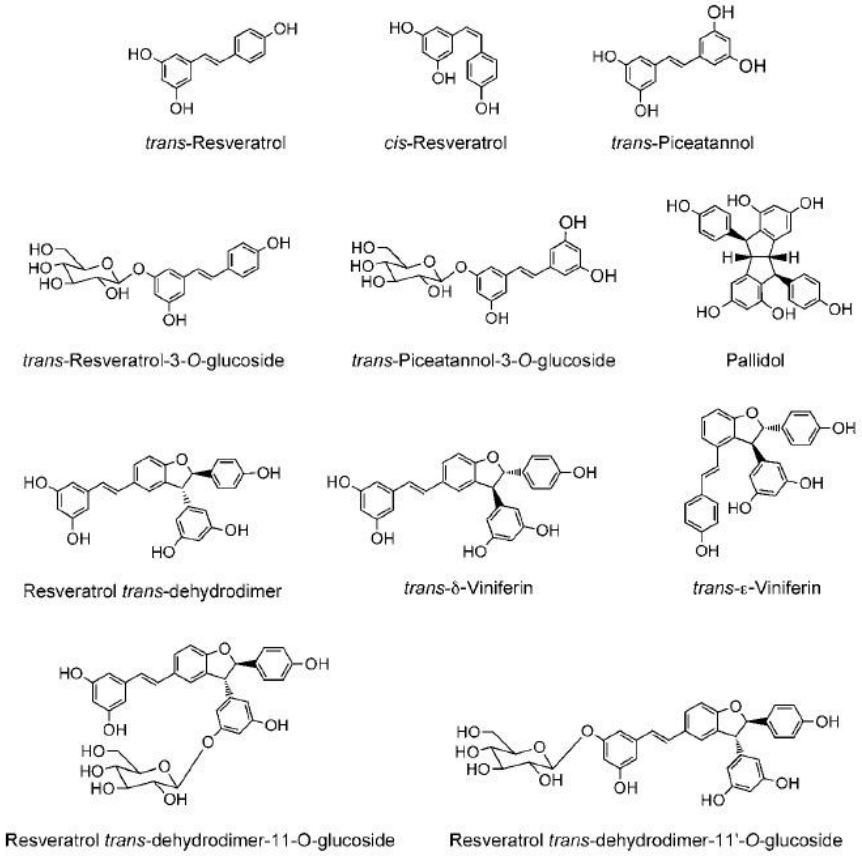


Figure 2.5. The *trans*- and *cis*- resveratrol and other stilbene structures (Source: Jaganath and Crozier 2010)

2.2. Flavonoids

Flavonoids, which have two heterocyclic C rings that are interconnected with a C_3 bridge and C_6-C_3-C_6 form, are polyphenolic compounds. Since the understanding of their nutritional and health benefits, flavonoids have attracted interest of many researchers, and studies about flavonoids increase continuously. In light of these studies, thousands of different flavonoids have been isolated, determined and described (Jaganath and Crozier 2010). Change in the C_3 bridge determines classification of flavonoids (Vermerris and Nicholson 2008). Flavonoids include 6 main subgroups as flavones, flavonols, flavanones, flavanols (flavan-3-ol) or catechins, anthocyanidins, isoflavones (Figure 2.6) (Jaganath and Crozier 2010).

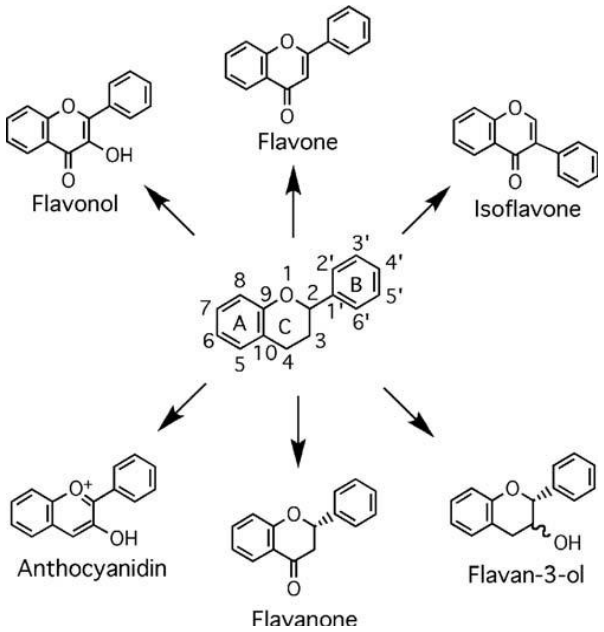


Figure 2.6. Structures of the main flavonoid subgroups.
(Source: Jaganath and Crozier 2010)

Flavonoids also contain some minor dietary components as dihydroflavones, flavan-3,4-diols, coumarins, and aurones (Jaganath and Crozier 2010). Other flavonoid groups are chalcones and dihydrochalcones (Figure 2.7). They have linear C_3 chain which connecting two heterocyclic C rings (Vermerris and Nicholson 2008).

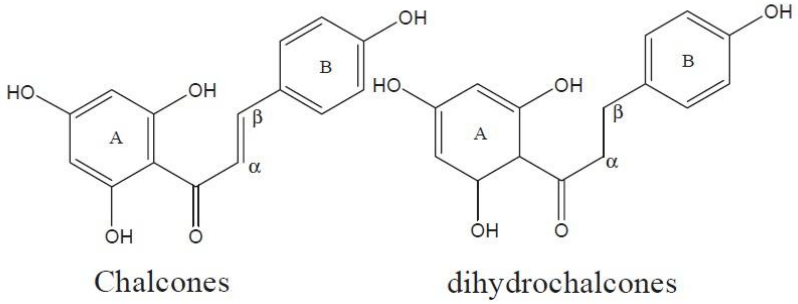
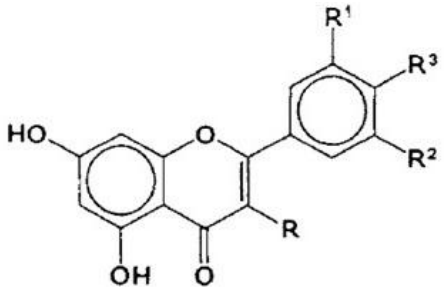


Figure 2.7. Structures of the chalcones and dihydrochalcones
(Source: Vermerris and Nicholson 2008)

2.2.1. Flavones

Flavones represent a ketone group with two heterocyclic C-ring and a carbonyl group. They have an unsaturated carbon-carbon bond (Vermerris and Nicholson 2008). Apigenin, luteolin, diosmetin, and chrysoeriol are known as flavones (Figure 2.8) (Belitz, Grosch et al. 2009). Flavones and their glycosidic derivatives (such as 3-glycosides and, less frequently, 7-glycosides) found in fruits, citrus fruits, and tropical fruits (Belitz, Grosch et al. 2009, Jaganath and Crozier 2010). Methoxylated flavones increase not only metabolic stability and membrane transport in the intestine/liver but also oral bioavailability. Also, compared with unmethylated flavones, methoxyflavones have more cancer chemopreventive properties (Jaganath and Crozier 2010).



Flavones	R_1	R_2	R_3	R
Apigenin	H	H	OH	H
luteolin	OH	OH	H	H
diosmetin	OH	H	OCH_3	H
chrysoeriol	OCH_3	H	OH	H

Figure 2.8. Mainly flavone structures
(Source: Belitz 2009)

2.2.2. Flavonols

Flavonols are structurally similar to flavones. The differences between flavonols and flavones, while flavones have atomic H group at the third position in the middle of the ring, flavonols have OH group. Flavonols are the most commonly present in plant food and their colors are diverse from white to yellow. The main flavonols are quercetin, kaempferol, myricetin and methylated derivative isorhamnetin (Figure 2.9). Flavonols are almost always present in the glycosylated conjugates form in plant tissues and the most common flavonol is quercetin (Figure 2.10) (Cemeroğlu 2004, Jaganath and Crozier 2010). Makris reported that, Grapes of *Vitis vinifera*, grape products and wines have lots of flavonols. These are quercetin, myricetin, kaempferol, isorhamnetin, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-glucuronide, kaempferol 3-O-glucoside, kaempferol 3-O-galactoside, myricetin 3-Oglucoside, and myricetin 3-O-glucuronide. Their amount in these products are listed below (Table 2.1) (Makris, Kallithraka et al. 2006).

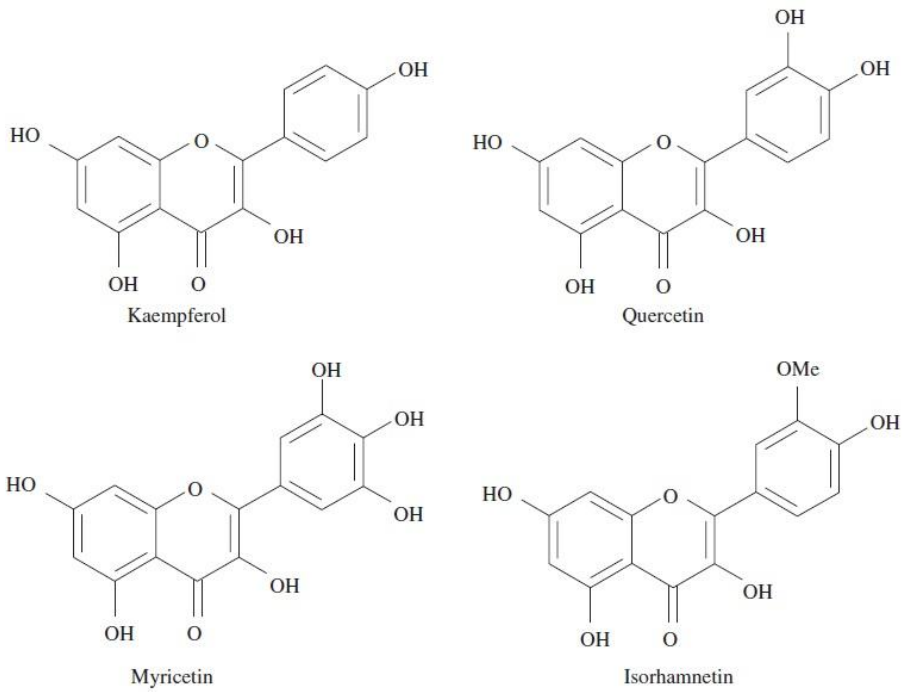


Figure 2.9. Structures of four common flavonol aglycones encountered in plant tissues. (Source: Makris, Kallithraka et al. 2006)

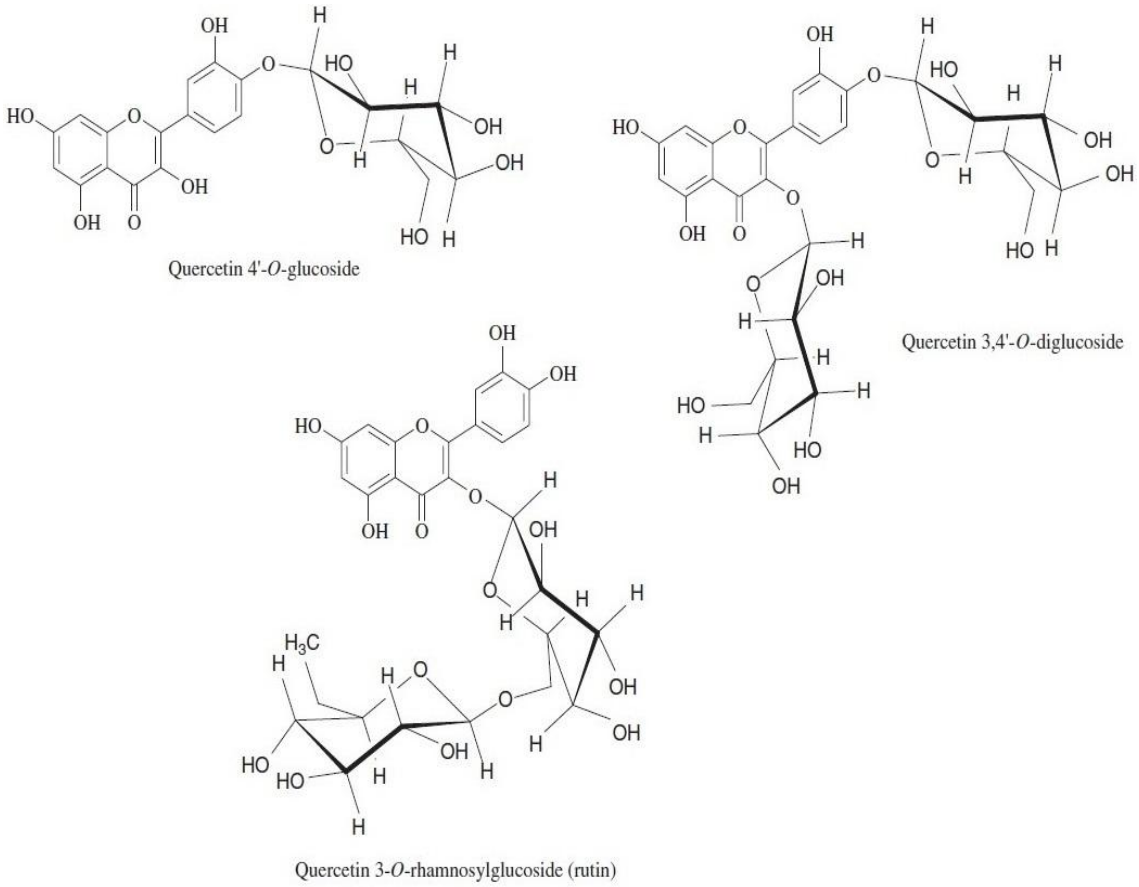


Figure 2.10. Three characteristic flavonol glycosides illustrating various combinations of sugar attachment on the flavonol skeleton. (Source: Makris, Kallithraka et al. 2006)

Flavonol composition of grapes, grape juices and grape products and by-products

Product	Number of samples (*n*)	Compound(s)	Range[a]	Average[a]
Red grapes	2	Quercetin 3-*O*-galactoside, Quercetin 3-*O*-glucoside	22.1–47.8	34.95
Red grapes	11	—	1.4–33.5[b]	12.89[b]
Red grapes	1	Quercetin 3-*O*-glucoside, Quercetin 3-*O*-glucuronide	—	21.6
Red grapes	4	Quercetin, Myricetin, Kaempferol, Isorhamnetin, Glycosides thereof	84.6–327.9[c]	162.43[c]
Red grapes[d]	5	Quercetin, Myricetin, Kaempferol	1.6–3.5[e]	2.4[e]
White grapes	3	—	4.8–10.4[b]	8.2[b]
White grape pomace	1	Quercetin 3-*O*-glucoside, Quercetin 3-*O*-glucuronide, Kaempferol 3-*O*-glucoside, Kaempferol 3-*O*-galactoside	—	—
White grapes[d]	5	Quercetin, Myricetin, Kaempferol	3.3–7.4[e]	6.5[e]
White grape juice	—	Quercetin glycosides	7.2–9[f]	—
White grape juice	2	—	5.7–8.6	7.15
White grape juice	18	Quercetin 3-*O*-glucuronide	—	0.5
White grape juice[d]	1	Quercetin, Myricetin, Kaempferol	—	9.9
Red grape juice	2	—	21.1–24.6	22.85
Red grape juice[d]	2	Quercetin, Myricetin, Kaempferol	13.4–100.9	57.15
Red grape stems	1	Quercetin 3-*O*-glucuronide, Quercetin 3-*O*-glucoside, Kaempferol 3-*O*-glucoside, Myricetin 3-*O*-glucoside, Myricetin 3-*O*-glucuronide	—	218
Vinegar	92	Quercetin, Isoquercitrin	0–3.1	1.53
Raisins	3	Quercetin glycosides, Kaempferol glycosides	82.1–121.8	105.4
Grape molasses	2	Quercetin	—	1.69

[a]For grapes, pomace and stems concentration is expressed as mg kg^{-1}. For juices concentration is expressed as mg L^{-1}.
[b]Concentration is referred to whole grape extract and is expressed as mg L^{-1}.
[c]Content expressed as nmol g^{-1}.
[d]Varieties belonging to *V. rotundifolia*.
[e]Concentration is expressed as mg per 100 g fresh weight.
[f]Concentration is expressed as μg L^{-1}.

Table 2.1. Flavonol composition of grapes, grape juices and grape products and by-products. (Source: Makris, Kallithraka et al. 2006)

2.2.3. Flavan-3-ols

Flavan-3-ols are the most common members of flavonoid being consumed with diet. Fruits include large amounts of flavan-3-ols and they affect some properties of food such as astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation (Aron and Kennedy 2008, Jaganath and Crozier 2010). Flavan-3-ols include (-)-epicatechin, (+)-catechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate, theaflavin-3-gallate, theaflavin-3'-

gallate, theaflavin-3,3'-digallate, proanthocyanidin B_2 dimer, proanthocyanidin B_5 dimer, and proanthocyanidin A_2 dimer (Figure 2.11). They also include less commonly (-)-epiafzelechin and (+)-afzelechin (Figure 2.12) (Jaganath and Crozier 2010).

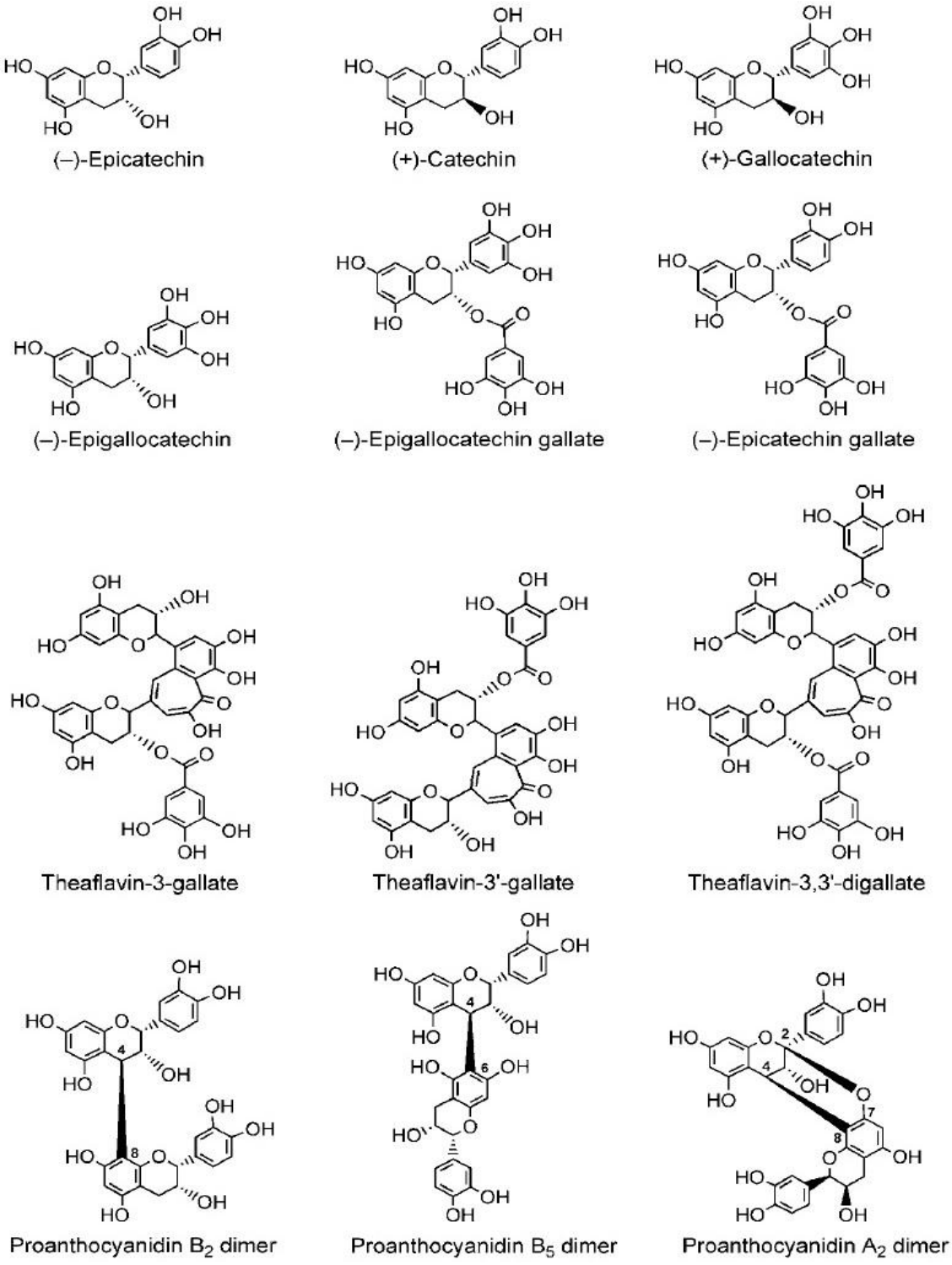


Figure 2.11. Structures of flavan-3-ol.
(Source: Jaganath and Crozier 2010)

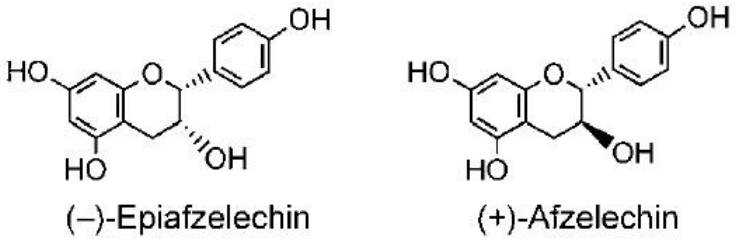


Figure 2.12. Less common flavan-3-ol monomers.
(Source: Jaganath and Crozier 2010)

Esterification of gallic acid and flavan-3-ols produces catechin gallates, and gallocatechins are produced through hydroxylation reactions of catechin gallates (Jaganath and Crozier 2010).

2.2.4. Flavanones

Flavanones, which possess the two heterocyclic ring in their structure, form a ketone group. The carbon-carbon bonds of flavanones are saturated (Vermerris and Nicholson 2008). Flavanones are generally glycosylated from seventh position of the carbon with disaccharides and they form flavanone glycosides. Citrus fruits generally comprise flavanones as flavanone glycosides. Naringenin, hesperetin, eriodictyol, sakuranetin and isosakuranetin are mainly flavanones which are found in diet (Figure 2.13) (Jaganath and Crozier 2010).

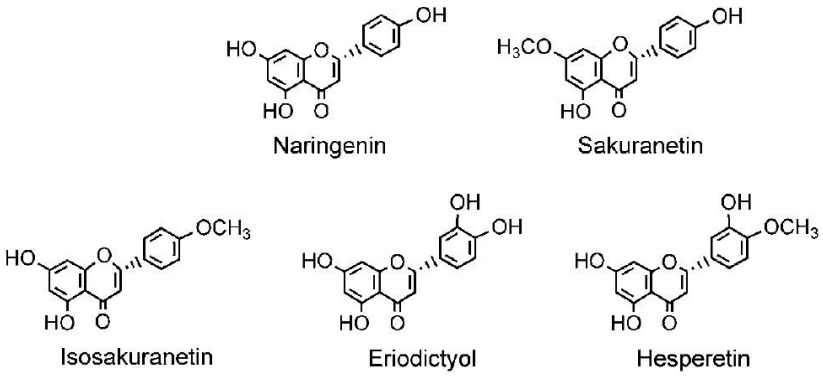


Figure 2.13. Mainly flavanones.
(Source: Jaganath and Crozier 2010)

2.2.5. Anthocyanidins/Anthocyanins

There are lots of different types of anthocyanins, water soluble and generally functioning as color pigment of plant, being isolated from plants and these anthocyanins possess the flavylium cation in their basic structure. Seven different side groups can be attached to the flavylium backbone according to different combinations (Figure 2.14). These side groups can be hydrogen, hydroxide or methoxy group, which determines the types of anthocyanin and plant color. The colors formed by anthocyanins also depend on the pH of the plant originated foods and when going to low pH to high pH these colors show diversity from red to blue (WEB_1 2013). Anthocyanins have lots of beneficial functions such as showing antioxidant properties, plant protection from UV light, attracting pollinating insects (Jaganath and Crozier 2010, WEB_1 2013).

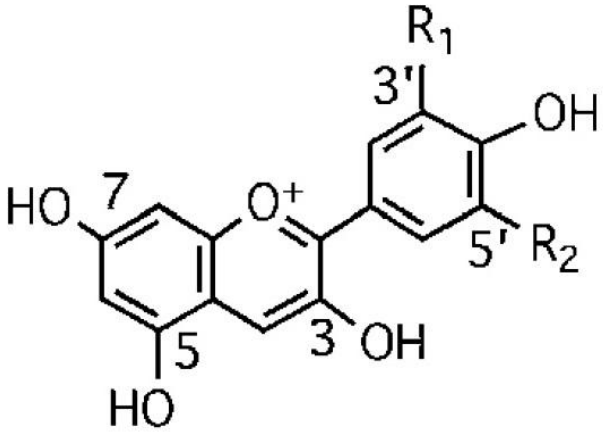


Figure 2.14. Basic structure of an anthocyanidin (flavylium cation).
(Source: Jaganath and Crozier 2010)

Anthocyanins occur when anthocyanidins form glycosidic bond with sugars, and also anthocyanin structures can contain anthocyanidin aglycones and anthocyanin glycosides (WEB_1 2013). Sugar moieties generally attached at the 3-position on the C ring or 5-position on the A ring (Jaganath and Crozier 2010). The most common glycosylation type of anthocyanidins is 3-position glycosylation on the C ring and cyanidin-3-glocoside is the most common anthocyanin in fruits (Jaganath and Crozier 2010). There are six common anthocyanidins in nature and these anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Figure 2.15) (Vermerris and Nicholson 2008).

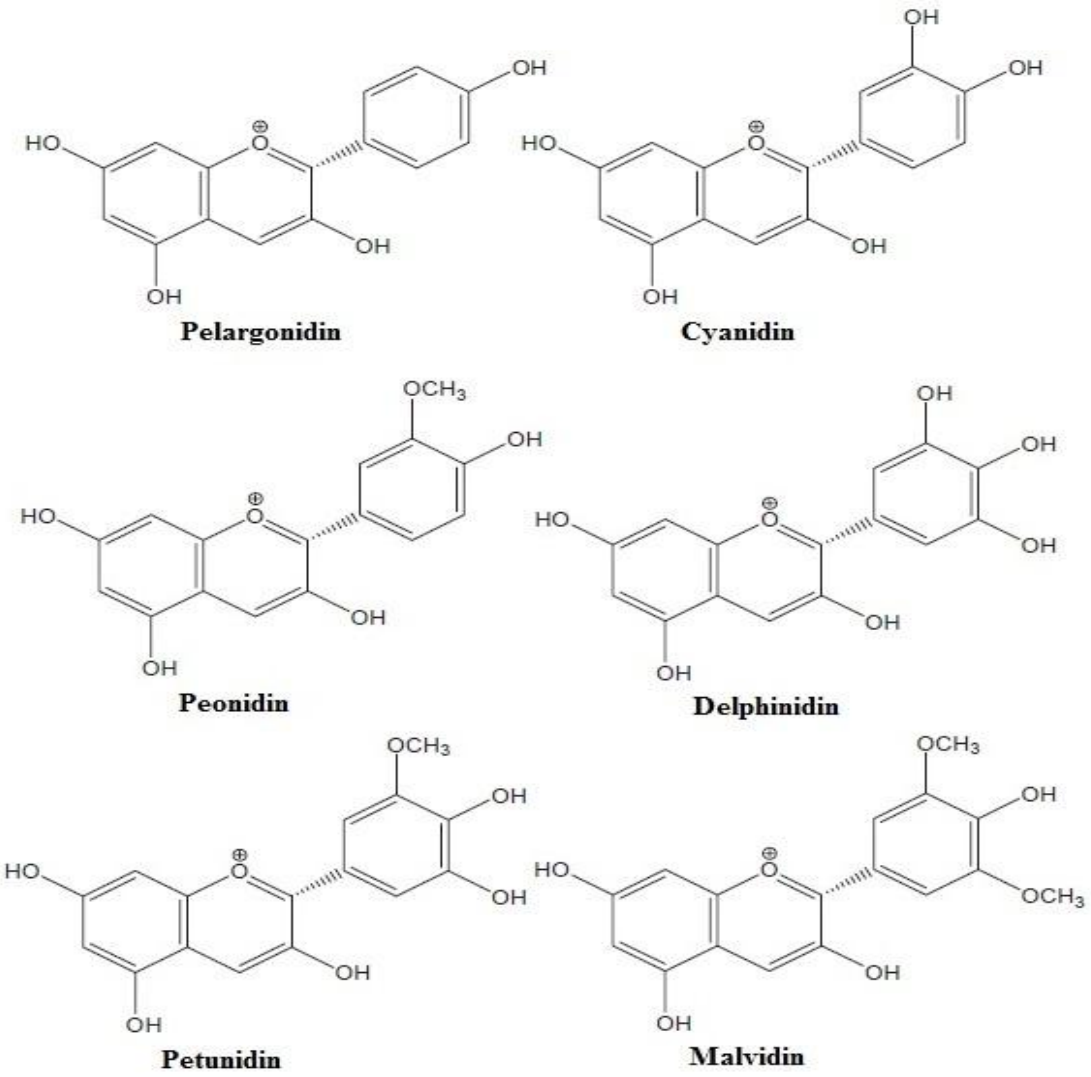


Figure 2.15. Structures of the major anthocyanidins
(Source: Vermerris and Nicholson 2008)

2.2.6. Isoflavones

Unlike other flavonoids, isoflavones possess a B-ring attached at C_3 position in place of the C_2 position. Isoflavones are found very limited amount in the plants and they are only found as sufficient amount in leguminous species. The significant dietary source of isoflavones is soybeans. Daidzein, genistein, glycitein, formononetin, and biochanin a represent commonly found isoflavones (Figure 2.16). These compounds, are polar and water soluble, generally occur as β-glucosides, acetyl-β-glucosides and malonyl-β-glucosides (Jaganath and Crozier 2010). Isoflavones show low antioxidant activity when they are compared to the flavonols, flavones, flavonones, and chalcones

(D. L. Madhavi 1996, Calabrese, Butterfield et al. 2008). Isoflavones have ability to bind to estrogen receptor and thus they show estrogenic activity. Also, isoflavones have preventative properties of breast cancer and osteoporosis (Jaganath and Crozier 2010).

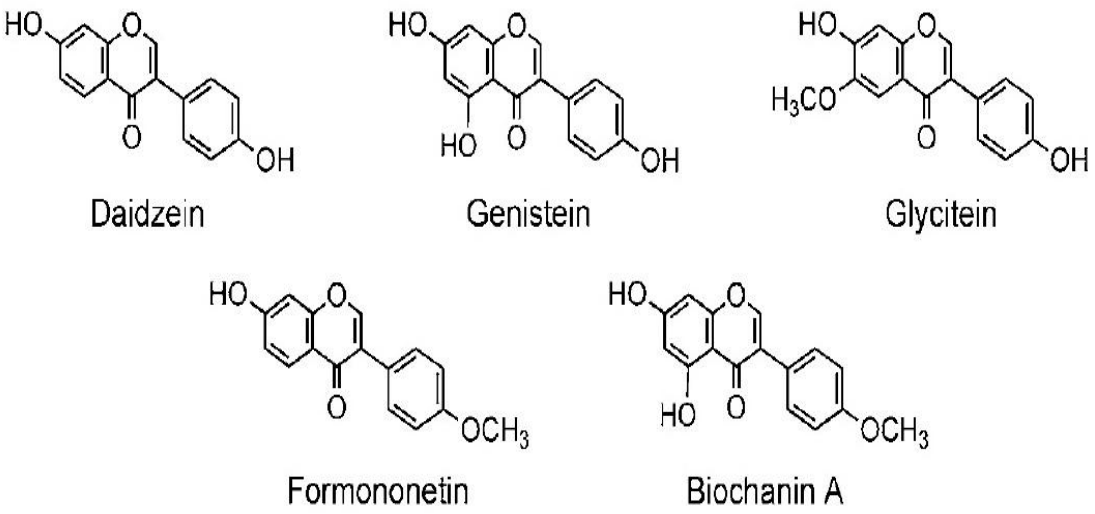


Figure 2.16. Structure of the isoflavones aglycones
(Source: Jaganath and Crozier 2010)

2.3. Biosynthesis of Phenolic Compounds

There are two main pathway, shikimate and polyketide pathway, playing important role in biosynthesis of phenolic compounds. Commonly known phenolic compounds (e.g. flavonoids, quinones, stilbenes, pyrones and xanthones) are produced through these two patways. The shikimate pathway has seven steps and begins phosphoenolpyruvate and erythrose-4-phosphate and ends with chorismate (Figure 2.17) (Mustafa and Verpoorte 2007). Chorismate is a precursor for the biosynthesis of the aromatic amino acids tryptophan, phenylalanine and tyrosine. The precursors of phenolic compounds and the phenylpropanoids are phenylalanine and tyrosine (Vermerris and Nicholson 2008). In this pathway, phenylalanine is converted to hydroxycinnamic acid by enzymes such as phenylalanineammonia lyase, cinnamic acid 4-hydroxylase, phenolases, and methyl transferases (Figure 2.18). The hydroxybenzoic acids are also produced from hydroxycinnamic acids through β-oxidation and reduction of the benzoic acid carboxyl groups (Figure 2.19). For example, vanillin and vanillyl alcohol produced from ferulic acid (Figure 2.20) (Belitz, Grosch et al. 2009).

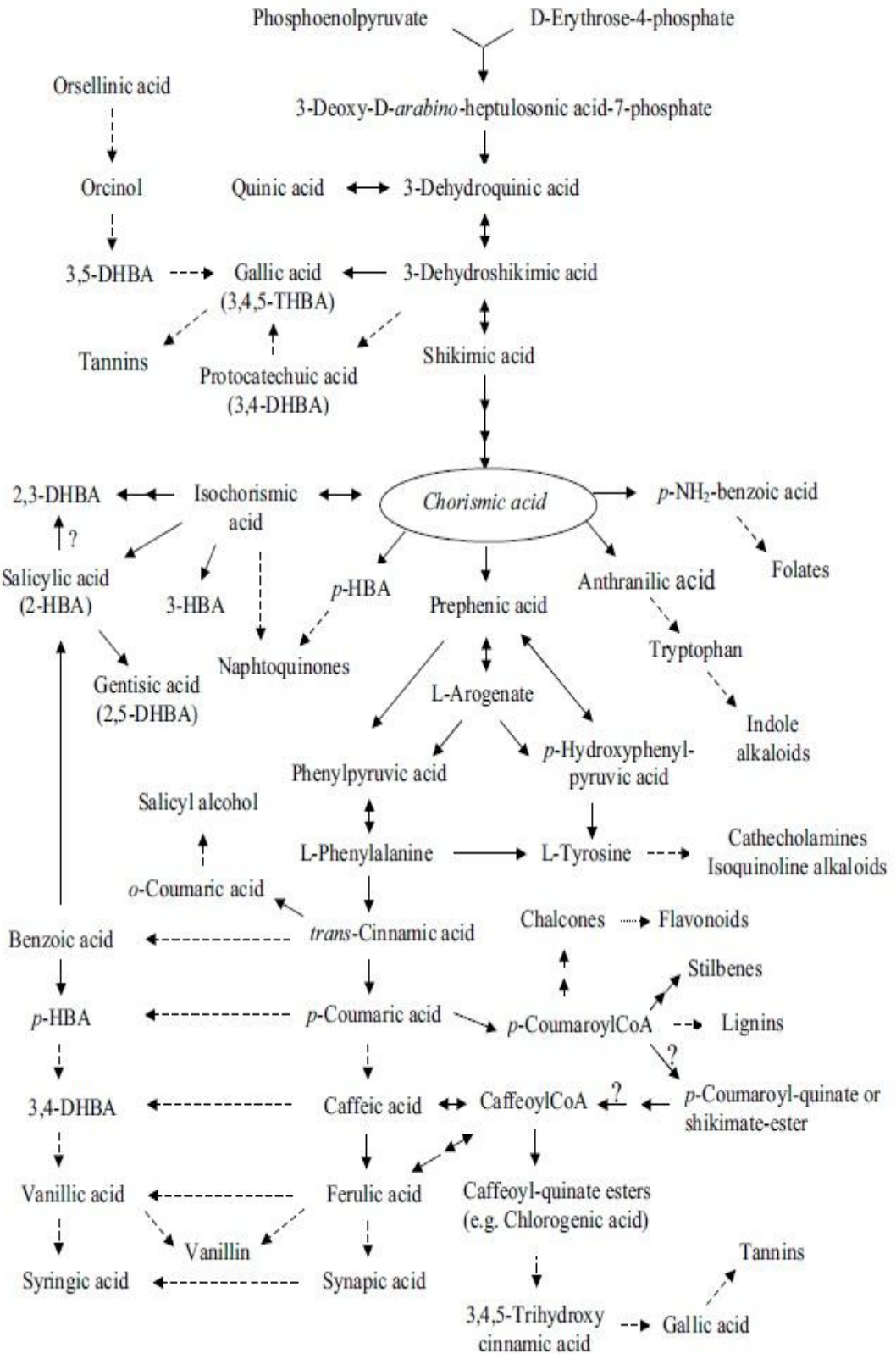


Figure 2.17. The pathway of some phenolic compounds biosynthesis. A small-dashed line represents multi-steps reactions. (Source: Mustafa and Verpoorte 2007)

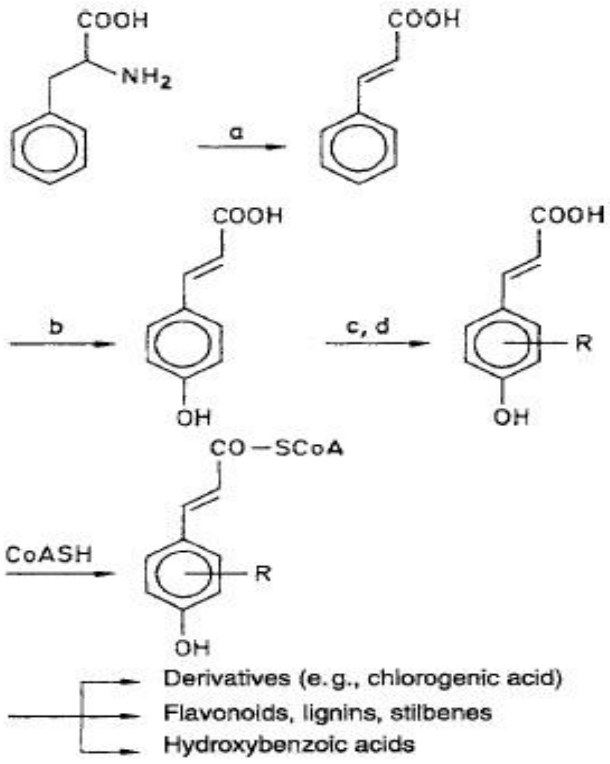


Figure 2.18. The basic pathway of hydroxycinnamic acid biosynthesis from phenylalanine. a) phenylalanineammonia lyase; b) cinnamic acid 4-hydroxylase; c) phenolases; d) methyl transferases, R: OH and OCH3 in various positions. (Source: Belitz, Grosch et al. 2009)

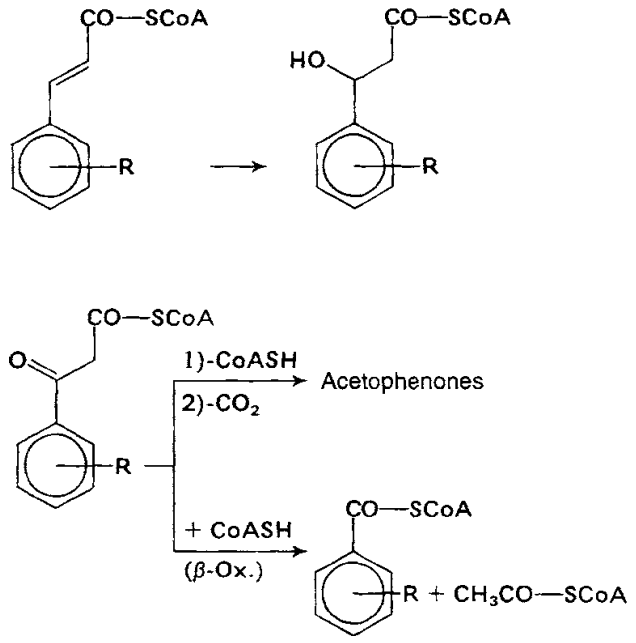
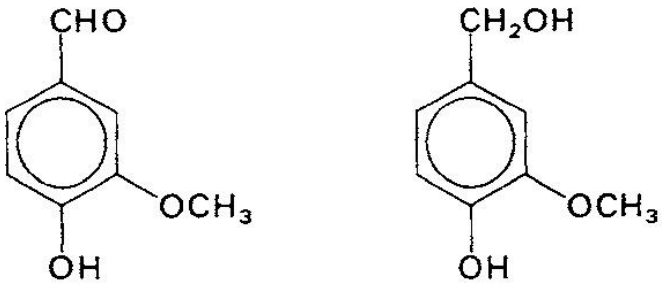


Figure 2.19. Hydroxybenzoic acids biosynthesis from hydroxycinnamic acid. (Source: Belitz, Grosch et al. 2009)



Vanillin Vanillyl alcohol

Figure 2.20. The structures of vanillin and vanillyl alcohol.
(Source: Belitz, Grosch et al. 2009)

Fragmentation of plant tissue provides releasing of free coumaric acids from glucosides of cis-o-coumaric acids, then the part of carboxylic acid of free coumaric acids close spontaneously to the ring and forms coumarins (Figure 2.21) (Belitz, Grosch et al. 2009).

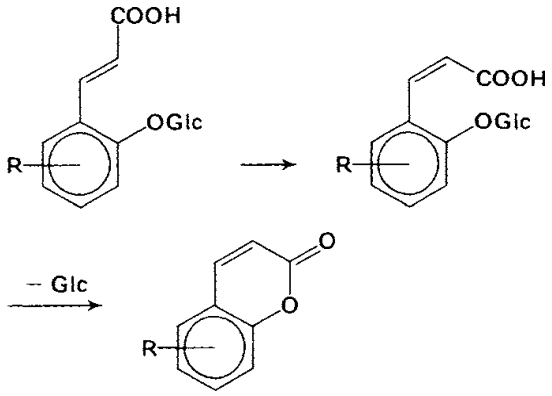


Figure 2.21. The synthesis of coumarin from cis-o-coumaric acid glucosides. (R: OH and OCH3 in various positions). (Source: Belitz, Grosch et al. 2009)

Lignin is formed through phenylpropanoid pathway. In this pathway, the coniferyl, sinapyl, and p-coumaryl alcohol form polymerization with dehydrogenation, and lignin is produced. Peroxidase and H_2O_2 play important role throughout the pathway (Belitz, Grosch et al. 2009).

Biosynthesis of flavonoids has several steps. It starts condensation of hydroxycinnamic acid with three malonic acid molecules and chalcone is produced. Stilbene can also be produced by 2,7-cyclization of hydroxycinnamic acid condensed with three malonic acid molecules.

Chalcone is converted to flavanone. There are two ways after flavanone step. Flavanones are converted to flavones in first way, or flavanones are converted to flavanonols in second way. After these steps, produced flavanonols can be converted to flavandiols, flavanols, flavonols, endiols, and enols, as well as anthocyanidins via enols (Figure 2.22) (Belitz, Grosch et al. 2009).

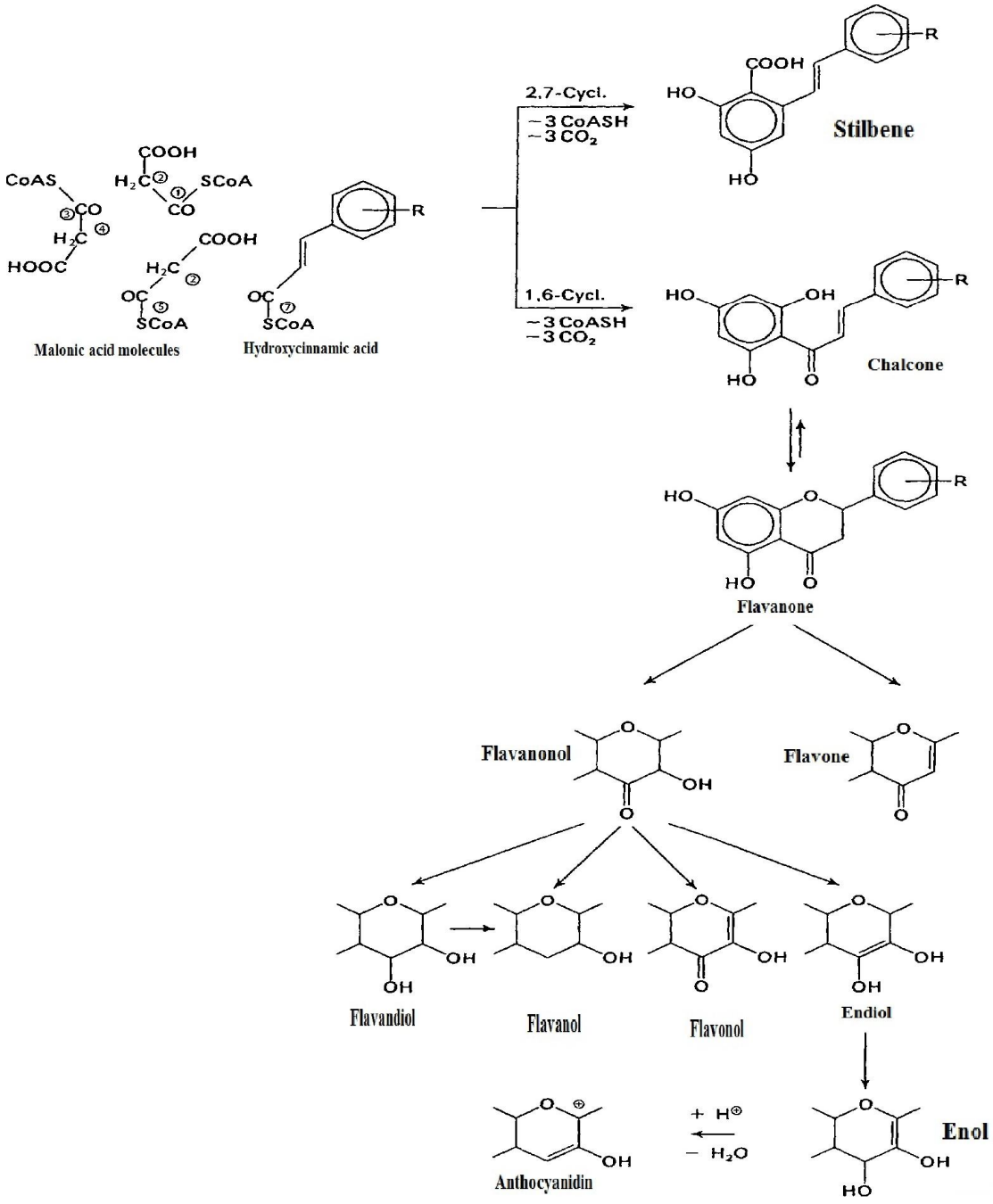


Figure 2.22. Flavonoid biosynthesis pathway.
(Source: Belitz, Grosch et al. 2009)

2.4. Antioxidant Mechanisms of Phenolic Compounds

Naturally occurring phenolic compounds (such as some flavonoids and nonflavonoid phenolic compounds) or synthetic phenolic compounds (such as butylated hydroxyanisole-BHA and butylated hydroxytoluene-BHT) show significantly antioxidant activity. They retard or inhibit oxidation of foods by several mechanisms which are free radical scavenging, metal chelating, and singlet oxygen quenching.
Thus, they increase shelf life of food products and improve food quality and consumer acceptability. They also contribute to the human health in a good way (Choe and Min 2009).

2.4.1. Free Radical Scavenging

Free radicals are produced as a result of daily cellular metabolism. They have harmful effect on human health (such as carcinogenic, diabetic, atherosclerotic effect, etc.). Phenolic compounds as antioxidants donate their hydrogen (H) or electron to scavenge free radicals and they terminate free chain reaction in initiation and propagation steps (Figure 2.23). Antioxidant radicals being highly stable and having low reduction potential are also produced in the result of donating hydrogen or electron by antioxidants. Antioxidant radicals can react with free radicals to retard the free chain reaction other than donating their hydrogen or electron (Figure 2.24). Also, Antioxidant radicals can react with each other to produce hydroquinone and phenolic dimer. The higher stability of antioxidants owes their resonance delocalization throughout the phenolic ring. Some examples of phenolic compounds functioning as free radical scavenger are tocopherols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG), lignans, flavonoids, and phenolic acids. The bond dissociation energy between oxygen and hydrogen, pH of the medium, reduction potential, and delocalization of radical phenolic ring structure play an important role on effectiveness of radical scavenging of phenolic compounds (Choe and Min 2009).

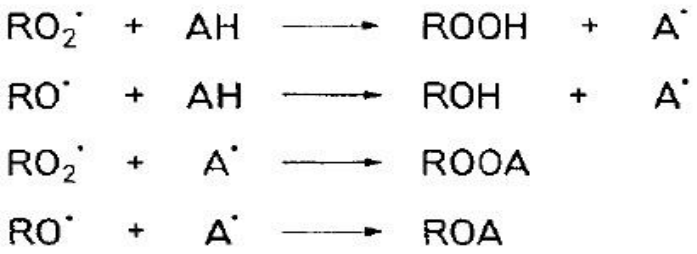


Figure 2.23. Antioxidant activity as a radical scavenger; RO• : Alkoxy radical, ROO• : Alkyl peroxy radical, AH: Antioxidant, A• : Antioxidant radical, nonradical products (ROOH, ROH, ROOA, ROA). (Source: Belitz, Grosch et al. 2009)

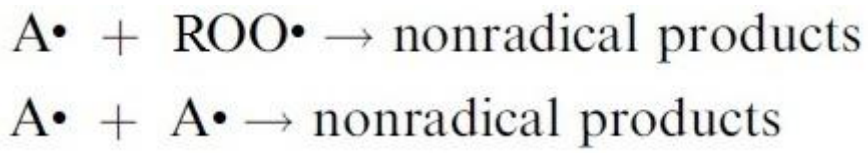


Figure 2.24. Antioxidant radicals react with each other and alkyl peroxy radicals. (Source: Jaganath and Crozier 2010)

In phenolic compounds, the lower dissociation energy of the bond between hydrogen and oxygen, the higher antioxidant property would be. Also, antioxidant effect of phenolic compounds depends on the number and position of their OH groups (Choe and Min 2009).

2.4.2. Metal Chelating

Metals such as iron or copper minimize the threshold value of oxidation in initiation step and it provides the acceleration of lipid oxidation. They abstract the hydrogen of food component to form food radicals such as alkyl peroxy radicals and alkoxy radicals. They also provide decomposition of hydrogen peroxide or hydroperoxides to hydroxy radicals (Figure 2.25) (Choe and Min 2009).

Polyphenols and flavonoids also act as metal chelators, and they form insoluble metal complexes, provide steric hindrance between metals and food components. They also inhibit oxidation of food components by blocking metal redox cycling.

Metal chelating activity of flavonoids attributed to their 3´, 4´-dihydroxy group in the B ring, the 4-carbonyl and 3-hydroxy group in the C ring, or the 4-carbonyl group in the C ring together with the 5-hydroxy group in the A ring (Choe and Min 2009).

$$H_2O_2 + \text{Ligand} - M^{(n)+} \rightarrow \bullet OH + -OH + \text{Ligand} - M^{(n+1)+}$$

$$ROOH + \text{Ligand} - M^{(n+1)+} \rightarrow ROO^{\bullet} + H^{+} + \text{Ligand} - M^{(n)+}$$

$$ROOH + \text{Ligand} - M^{n+} \rightarrow RO^{\bullet} + OH^{-} + \text{Ligand} - M^{(n+1)+}$$

Figure 2.25. Radicals formation in the presence of metal.
(Source: Jaganath and Crozier 2010)

2.4.3. Singlet Oxygen Quenching

Singlet oxygen has higher reaction rate with lipids than ground state triplet oxygen. Tocopherols, some phenolics, and ascorbic acid can be given as an example of singlet oxygen quencher. Singlet oxygen quenching mechanism has two ways as physical quenching mechanism and chemical quenching mechanism. Singlet oxygen quenching depends on the conjugated double bonds of quencher. The quencher has more conjugated double bonds, the better it shows quenching activity. Also, the presence of oxo and conjugated keto groups, or cyclopentane ring increase quenching activity (Choe and Min 2009).

In physical quenching, The energy level of singlet oxygen generally is near or higher than the energy level of quencher. The quencher gives an electron or energy to singlet oxygen and forms singlet state charge transfer complex and then it transforms to triplet state charge transfer complex by inter crossing system. The produced triplet state charge transfer complex is transformed to triplet oxygen and a quencher. There is no oxygen consumption and there is no production in this mechanism. It only occurs physically and singlet oxygen is quenched at this way (Figure 2.26) (Choe and Min 2009).

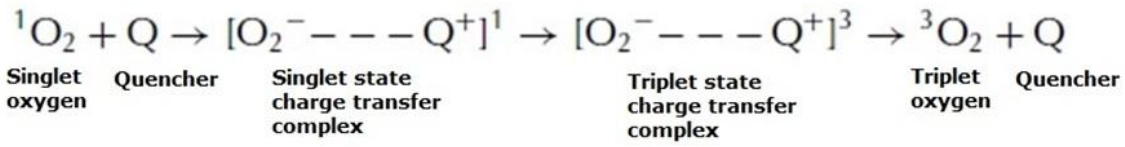


Figure 2.26. Fundamentally the physical quenching mechanism.
(Source: Choe and Min 2009)

In chemical quenching, singlet oxygen chemically reacts with the quencher. The quencher is oxidized, and unstable hydroperoxide of quencher is produced. β-Carotene, tocopherols, ascorbic acid, and some phenolics can be given as an example of chemical singlet oxygen quencher (Choe and Min 2009).

2.4.4. Photosensitizer Inactivation

The natural phenolic quenchers (such as tocopherols and flavonoids) prefer to inactivate the photosensitizer instead to inactivate the singlet oxygen. The photosensitizers, chlorophylls or riboflavins, are naturally found in the foods. Photosensitizers are excited and activated by light. The energy of photosensitizer is transferred to quencher. The the energy of excited photosensitizer is quenched by the quencher. It prevents formation of singlet oxygen and photosensitizer is inactivated in this way (Gordon 2001, Choe and Min 2009).

2.4.5. Lipoxygenase Inactivation

Flavonoids and phenolic acids have ability to inactivate the lipoxygenase enzyme by chelating ferrous form iron (Fe^{2+}) of lipoxygenase or reduction of hydroperoxides which is essential activators of lipoxygenase. Tea polyphenols, namely the catechins EGCG, ECG, EGC, EC, theaflavin monogallate B and theaflavin digallate have also inhibiting activity of lipoxygenase (Jisaka and Chedea 2011).

In chelating mechanism includes two ways, the first way is that flavonoids and phenolic acids bind covalently to ferrous state iron of lipoxygenase and they block the access of the substrate to iron.

The second way is that active ferric state iron (Fe^{3+}) is reduced by isoflavones donating an electron to (Fe^{3+}) ferric state iron. It is reduced to silent ferrous state iron (Fe^{2+}) and lipoxygenase is inhibited (Jisaka and Chedea 2011).

In reduction of hydroperoxide mechanism, this mechanism occurs enzymatically and includes conversion of silent ferrous species to the active ferric form (Jisaka and Chedea 2011).

CHAPTER 3

HEALTH BENEFITS OF PHENOLIC COMPOUNDS

3.1. Introduction

People are exposed to so many factors in daily life such as pollution, cigarette smoke, drugs, pesticides, insecticides, radiation, illness, and stress. Addition to these factors, unhealthy life style, excessive exercise, alcohol, malnutrition, and insufficient consumption of fruits and vegetables provide the formation of free radicals including reactive oxygen species (ROS) and reactive nitric oxide species (RNS) in the cells. Free radicals are molecules being electrically charged, and they possess an unpaired electron. Free radical formation includes a series of chain reactions. Free radicals generally need to neutralize themselves due to their nature. Initial step of this chain reaction starts with a free radical by capturing an electron from other substance, and it goes on until the last free radical is deactivated. Thousands of free radicals are produced between initial and termination step. Free radicals, including reactive oxygen and nitric oxide species, have the ability to attack to membrane lipids, nucleic acids, proteins, enzymes, and other small molecules in the cells of body. They lead to cellular damage, loss of their structure and function by causing to oxidative stress. Free radicals cause more than 50 severe diseases depending on the cellular damage such as atherosclerosis, multiple sclerosis, cancer, pancreatitis, pulmonary dysfunction, inflammatory bowel disease and colitis, cataracts, parkinson's disease, arthritis and inflammatory diseases, neonatal lipoprotein oxidation, diabetes, shock, trauma, and ischemia, skin lesions, renal disease and hemodialysis, chronic fatigue immunodeficiency syndrome (CFIDS), and aging.

Fortunately, free radical formations are controlled by naturally occurring phenolic compounds functioning as antioxidants. Antioxidants also have the ability to prevent attacks of free radicals to the healthy cells of body. Phenolic compounds and flavonoids scavenge directly free radicals. Free radicals are stabilized or deactivated at this way. A sufficient amount of intake of phenolic compounds and flavonoids from fruits and vegetables protect us against degenerative diseases in daily life. They provide to be healthier to us (Figure 3.1).

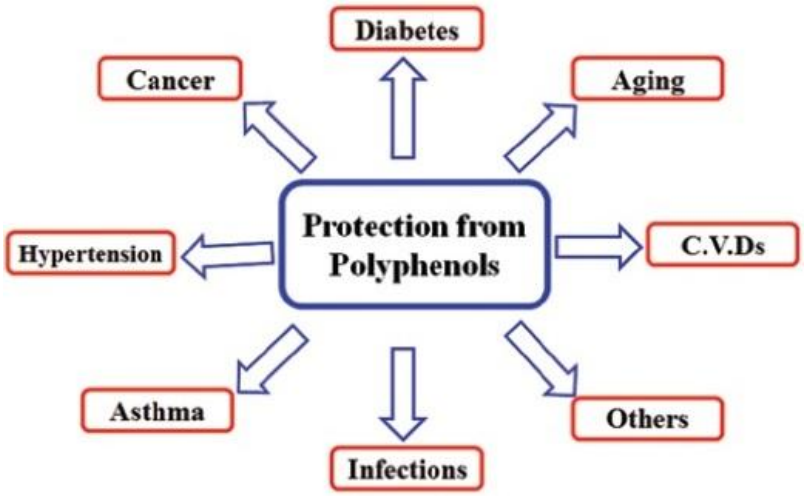


Figure 3.1. Phenolic compounds as a antioxidant provide protection from more than 50 severe diseases. (Source: Pandey and Rizvi 2009)

3.1.1. Antioxidant Effects of Phenolic Compounds

Antioxidant generally used by people is a term that to describe health benefits of phenolic and polyphenolic compounds. These beneficial compounds possess the antioxidative effects on human health as free radical scavenging, pro-oxidative metal ions chelating or reducing agents (Shahidi 2012). Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) are produced as a result of daily cellular metabolic reactions in mitochondrial respiratory chain through biological reduction of molecular oxygen. Under low level of oxygen conditions, nitric oxide (NO) produced by mitochondrial respiratory chain and reactive nitrogen species (RNS) are formed. They play important role in the stimulation of signalling pathways. Other reactive species are produced by ROS and RNS caused by lipid peroxidation. Oxidative stress caused by ROS/RNS and other free radicals is in a balance with antioxidants as protective mechanism. When our defence mechanism is weakened, especially in disease conditions, the balance of oxidant/antioxidant shift toward the direction of oxidant and the oxidant level increases (Figure 3.2). Increasing oxidative agents can cause damage cellular components such as lipids, proteins, carbohydrates, and even DNA. These damages construct the molecular basis of some important diseases such as cardiovascular diseases, cancer, diabetes, neurodegenerative disorders, autoimmune disorders, and aging (Blaženković, Štefan et al. 2012).

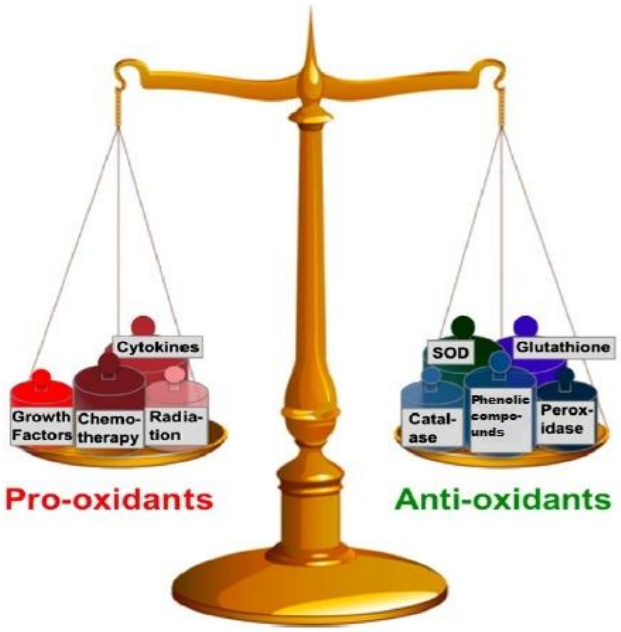


Figure 3.2. A representative model of balance of pro-oxidants / antioxidants (This figure was modified from original source). (Source: Reuter, Gupta et al. 2010)

In order to be protected the destructive effects of oxidative stress either endogenous antioxidant enzyme defences (such as super oxide dismutase, glutathione peroxidase, glutathione reductase and catalase) or non-enzymatic defences (such as glutathione, vitamins) should be increased through consumption of antioxidant rich fruits and vegetables (Figure 3.3) (Blaževković, Štefan et al. 2012).

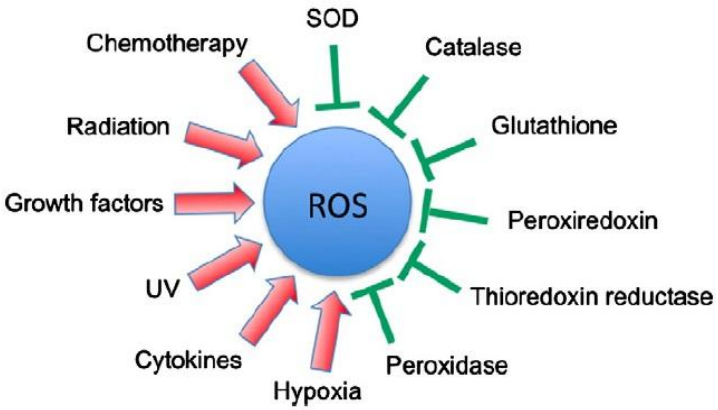


Figure 3.3. The factors and substances that provide ROS generation and prevention. (Source: Reuter, Gupta et al. 2010)

Fruits and vegetables, as plant derived foods, are excellent sources of phenolic compounds. Especially, skin and seeds of fruits, and leaves are rich in terms of phenolic compounds (Shahidi 2012). Polyphenols with regard to antioxidative effects have free radical scavenging activity that stop the free radical chain reaction, inhibition of free radical formation by regulation of enzyme activity, and chelation of metal ions activity (Blažeković, Štefan et al. 2012). Glycosylated phenolic compounds reduce diabetes, cataract, and neuropathy risks. Table 3.1 summarizes different health benefit mechanisms of phenolic compounds as antioxidants (Shahidi 2012).

• Direct Removal of ROS/RNS or potentiation of cellular antioxidant capacity
• Affecting cell differentiation
• Increasing the activity of carcinogen detoxifying enzymes
• Blocking the formation of *N*-nitrosamines
• Altering the estrogen metabolism and/or colonic milieu
• Increasing apoptosis of cancerous cell and/or decreasing cell proliferation
• Affecting DNA methylation and/or maintaining DNA repair
• Preserving the integrity of intracellular matrices
• Other mechanisms

Table 3.1. Action mechanisms of phenolic compounds.
(Source: Shahidi 2012)

3.1.2. Bioavailability of Polyphenols

Bioavailability is digestion, absorption, and metabolism process of consumed a food product. Each of polyphenols has a different bioavailability feature. Foods contain polyphenols in the form of esters, glycosides, and polymers. These compounds are hydrolysed by intestinal enzymes or colonic microflora, and then absorption occurs. Also, aglycone form of phenolic compounds are easily absorbed in the small intestine (Pandey and Rizvi 2009). Deconjugation and reconjugation reactions plays an important role in phenolic compound methabolism. A phenolic compound firstly hydrolyzed to their free aglycones by intestinal enzymes or colonic microflora, and then these aglycones are conjugated in the intestinal cells. After that, the absorption occurs and these conjugates undergo methylation, sulfation, glucuronidation, or a combination in the liver. The methyl substituents are added to C3 or C4 positions of a phenolic compound in methylation process.

The glucuronidation occurs in C3 position of a phenolic compound by the addition of glucuronic acid. The sulfation is catalyzed by tyrosylprotein sulfotransferase (TPST) to transfer sulfate, and this process takes place in golgi apparatus of liver cells.

The methylation, sulfation, and glucuronidation provide to increase of hydrophilicity of absorbed phenolic compounds and ease their excretion and elimination by means of urine and bile (Figure 3.4) (Karakaya 2004, Pandey and Rizvi 2009).

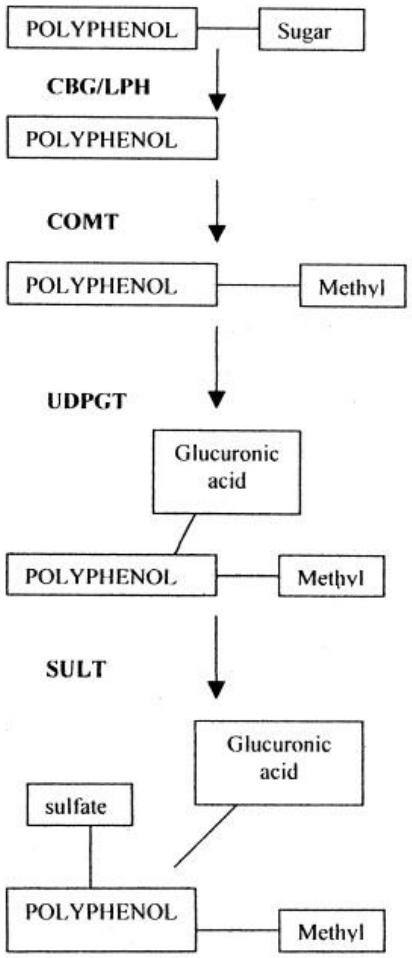


Figure 3.4. Possible metabolism process of polyphenols; CBG: cytosolic β-glucosidase, LPH: lactase phlorizin hydrolase, COMT: catechol-O-methyltransferase, UDPGT: glucuronosyl transferase, SULT: phenol sulfotransferases. (Source: Karakaya 2004, Calabrese, Butterfield et al. 2008)

The produced polyphenol forms as a result of metabolism may present in blood and tissues. Each of phenolic compounds has a different absorption place in human body. For example, some of them are absorbed in the gastrointestinal system while others are absorbed in other part of digestive system.

Most of flavonoids present as glycosides in foods and they have resistance to acidic conditions in stomach so some glycosides and merely aglycones are absorbed in the intestine. Albumin is an important molecule for circulation of polyphenol metabolites and for bioavailability of polyphenols, because polyphenol metabolites present in the blood as bound to albumins. They provide the delivery of polyphenol metabolites to cells and tissues. In excretion and elimination of polyphenol metabolites, the high molecular structure metabolite conjugates are eliminated in bile, while low molecular structure metabolite conjugates, such as monosulfates, are excreted in urine (Figure 3.5) (Pandey and Rizvi 2009).

Urinary excretion rate decreases from isoflavones to flavonols in terms of flavonoids. The relationship between bioavailability and intake of polyphenols shows their health effects on the human body (Pandey and Rizvi 2009).

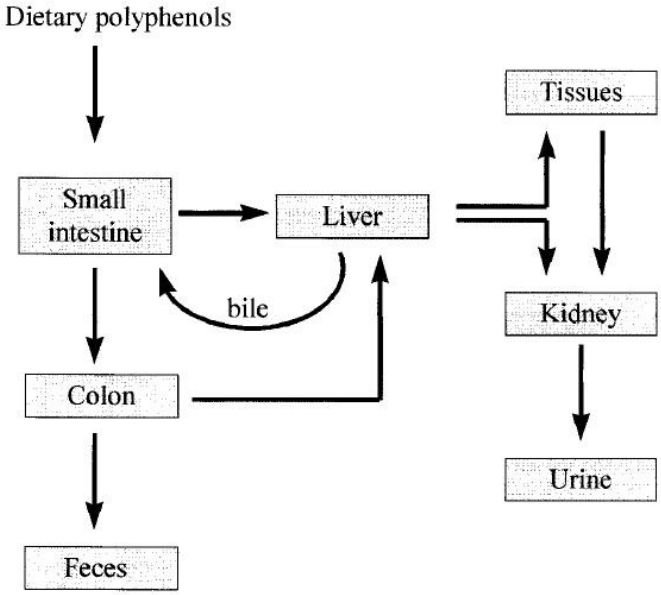


Figure 3.5. Possible bioavailability ways of consumed dietary polyphenols in humans. (Source: Scalbert and Williamson 2000)

3.1.3. Cardio-Protective Effect of Phenolic Compounds

Atherosclerosis is a multifactorial illness. Chronic inflammation plays important role in all steps of atherosclerosis from initiation to progression and each risk factor (e.g. smoke, cholesterol, hypertension, and diabetes mellitus) causes to increase of oxidation of important cellular components and contributes to pathogenesis accelerating the underlying inflammatory process. Besides these risk factors environment-gene

interaction plays important role. Numerous studies have proven antiatherosclerotic effect of phenolic compounds on cardiovascular diseases. Before the mention about protective effect of phenolic compounds in cardiovascular diseases, we fundamentally need to know the formation mechanism of atherosclerosis. Oxidation of polyunsaturated fatty acids of low density lipoprotein (LDL) by free radicals, metal ions or lipoxygenase enzyme causes a common reaction, and starts a general inflammatory response. Oxidized LDL (ox-LDL) possesses also cytotoxic effect. Oxidized LDL (ox-LDL) firstly affects the endothelial cells which found in vascular structure as monolayer. Normally, endothelial cells have slick, shiny, and antithrombotic properties. Endothelial cells lose these properties with effect of ox-LDL and they become sticky and prothrombotic. Adhesion molecules (VCAM-1, ICAM-1), growth factors (PDGF, βFGF, TGF-β, IL-1, TNFα), and cytokines (M-CSF, GM-CSF) begin to secrete by affected endothelial cells. Monocytes and T lymphocytes are attached to the endothelial cells by adhesion molecule VCAM-1. While monocytes pass the subendothelial zone called as intima by monocyte chemoattractant protein-1 (MCP-1), T lymphocytes pass the intima by different chemokines (G-IP-10, MIG). Monocytes and T lymphocytes, passed the the subendothelial zone, begin to accumulate in the intima. Accumulated T lymphocytes cause to secretion of proinflammatory cytokines. Accumulated monocytes are transformed to the macrophage cells. Scavenger receptors of Macrophage cells are activated. They ingest LDLs and form “foam cells”. Macrophage colony stimulating factor (M-CSF) secreted from active endothelial cells increase the accumulation of macrophage in the intima. Also, M-CSF stimulates the immune system. Ox-LDL increases the inflammation by stimulating the toll-like receptors which are found in coronary atherothrombotic plaques, intima, and adventisya. Thus, the atheroma plaque grows gradually and causes vascular occlusion and heart attack (Tokgözoğlu 2009).

Fortunately, there are many beneficial natural compounds in diet for human health like phenolic compounds as phenolic acids and flavonoids. Flavonoids may downregulate or inhibit cyclooxygenase and lipoxygenase enzyme activity playing an important role in platelet aggregation and in the formation of macrophages, prostaglandins, and leukotrienes. Downregulation of cyclooxygenase and lipoxygenase enzymes by phenolic compounds provide reduction of hydroperoxides, downregulation of the arachidonic acid cascade, and reduction of platelet aggregation and thrombotic tendencies.

These effects also provide reduction of thrombosis and chronic inflammatory reactions

involved in immune suppressions (Frankel 1999).

Also, nitric oxide (NO) molecule has ability to regulate the vascular function inhibiting platelets aggregation, inducing vasorelaxation and repressing the expression of inflammatory proteins and adhesion molecules (ICAM-1 and VCAM-1) (Larrosa, Garcia-Conesa et al. 2010).

Phenolic compounds show three possible protective mechanisms against cardiovascular diseases. They firstly inhibit LDL oxidation, protect cholesterol from forming cytotoxic compounds and prevent atherosclerosis. They secondly interfere with the immune response by reducing monocytes from vessel walls. They thirdly interfere with platelet aggregation involved in the clotting process and preventing thrombosis (Frankel 1999). Recent studies performed with phenolic compounds of pomegranate juice and walnut extract showed that protection of lipoproteins against oxidation, anti-atherogenic effects, effects on cellular cholesterol metabolism and uptake, modulation of proatherogenic effects, protection against the accumulation of harmful atherogenic oxidized lipids, anti-inflammatory and cardioprotective effect at the endothelium level, in vitro inhibitor effect to LDL oxidation by inhibiting induced-HDL oxidation, increasing the association of paraoxonase1 (PON1) with HDL, increasing PON1 expression and activity, inducing secretion of active PON1, reducing platelets induced aggregation and thromboxane A(2) production, reducing the cellular total peroxides, inhibiting the native and ox-LDL uptake, stimulating HDL efflux, decreasing the cell cholesterol, increasing expression of endogenous nitric oxide synthase (eNOS), reducing the activation of oxidation-responsive elements ETS like gene 1 (ELK-1) and cAMP responsive element binding protein (p-CREB), showing cytoprotection against oxidative (H2O2, ox-LDL) cell damage, upregulating paraoxonase 2 (PON2) expression and activity, reducing oxidative stress and cell mediated- LDL oxidation, decreasing expression of adhesion molecules, ICAM-1 and VCAM-1, increasing the lag time in mediated-LDL oxidation compared with control, respectively (Larrosa, Garcia-Conesa et al. 2010). Also, green tea is known as one of the significant catechin sources. There are numerous studies about protective effect of green tea in cardiovascular diseases. A Study performed with green tea catechins demonstrate that 0.08 to 5 ppm of the green tea extracts inhibited the 3.9–98% of LDL oxidation and provided nutritional benefits on cardiovascular diseases (Thielecke and Boschmann 2009).

3.1.4. Anti-Cancer Effect of Phenolic Compounds

Cancer often seeming a single disease but it is a complex diseases group affecting cells and tissues due to excessive and uncontrolled proliferation of a cell group in the body (Klug and Cummings 2012). Cancer, being many derivatives which are originated according to the type of cells, is one of the most important diseases having high rate of incidence worldwide. There are lots of cancer types which originated according to the type of cells such as lung, breast, stomach, prostate, liver, and etc. According to datas of "International agency for research on cancer and cancer research UK", 6.6 million cases in men, and 6.0 million cases in women, in total an estimated 12.7 million cancer cases were seen around the world in 2008. They also reported that this number could reach to 21 million by 2030. Figure 3.6 shows the incidence of different types of cancers seen in year of 2008, and it also shows lung, female breast, colorectal and stomach cancers being the most commonly diagnosed cancers and they form more than 40% of all cases. Worldwide in 2008, the numbers of people who died from cancer were approximately 7.6 million. The numbers of people who died from lung cancer, stomach, liver, colorectal and female breast cancers were more than other types of cancer (Figure 3.7) (IARC and UK 2012).

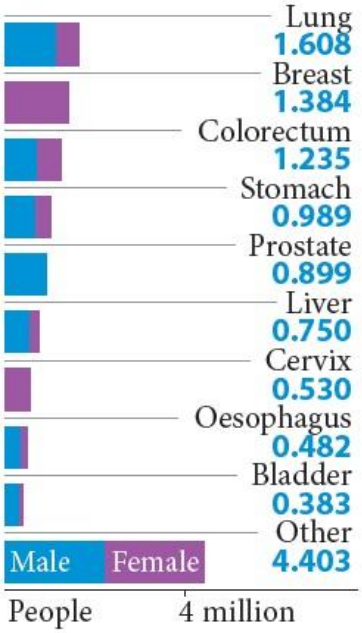


Figure 3.6. The incidence of different types of cancers that seen in year of 2008. (Source: IARC and UK 2012)

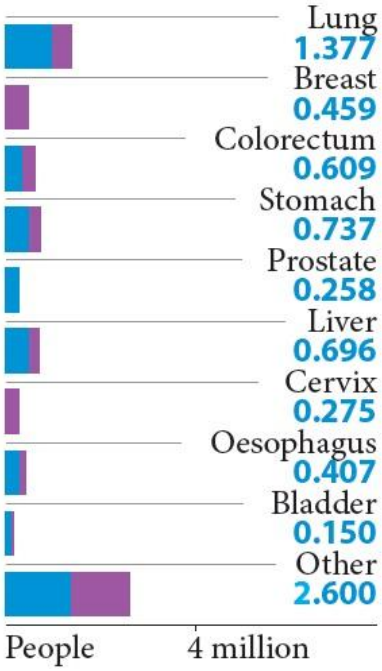


Figure 3.7. The worldwide mortality rates in different cancer types in 2008 (blue colors represent males and purple colors represent females). (Source: IARC and UK 2012)

Genetic and environmental conditions play an important role in the formation of cancer. Expose to stress, microbial and viral infections, allergens, expose to radiation, and toxic chemicals, autoimmune and chronic diseases, obesity, consumption of alcohol, tobacco use, a high-calorie diet, irregular lifestyle, and unbalanced nutrition comprise the environmental conditions. Sustained exposure to these factors causes to increase of reactive species including reactive oxygen species (ROS) and reactive nitrogen species (RNS), and they cause oxidative stress in cells of the body. ROS and RNS are also produced in the mitochondria through normal cellular metabolism. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$), and organic peroxides. Hydroxyl radical ($OH^\bullet$) attack on DNA produces 8-hydroxydeoxyguanosine (8-OHdG) known as one of the major products of DNA oxidation by oxidative stress. 8-OHdG play a crucial role in carcinogenesis progression. Nitric oxide (NO) as RNS is produced from I-arginine in hypoxic conditions and can cause to formation of other reactive species (for example, reactive aldehydes, malondialdehyde, and 4-hydroxynonenal) by inducing excessive lipid peroxidation. Small amounts of nitric oxide acts as neurotransmitter and vasodilator, but nitric oxide that produced much larger amount in conditions of the oxidative stress leads to inflammation and cancer (Reuter, Gupta et al. 2010).

Cancer is usually described at least three phases as initiation, promotion, and progression. ROS level increases in the cells exposed sustained oxidative stress, and can damage by attacking to the cellular components as proteins, lipid membranes, carbohydrates, DNA. ROS induced DNA damage produces gene mutations and structural alterations into the DNA. This stage is called as initiation stage of cancer. ROS induced promotion stage includes the aberrant gene expression and cell-to-cell communication, change in second-messenger systems. These abnormalities cause to an increase in cell proliferation and to a decrease in apoptosis. In the progression stage, ROS causes lots of DNA mutations contributing angiogenesis, tumor development and metastasis (Reuter, Gupta et al. 2010).

Phenolic compounds as a part of the nutrient content have beneficial and protective effect against cancer. They can block initiation, reverse the promotion, and also stop the progression stage of cancer, thorough iron chelating, reactive species scavenging (ROS and RNS), cyclooxygenase and lipoxygenase enzyme inhibiting activities (Devasagayam, Tilak et al. 2004, Vermerris and Nicholson 2008).

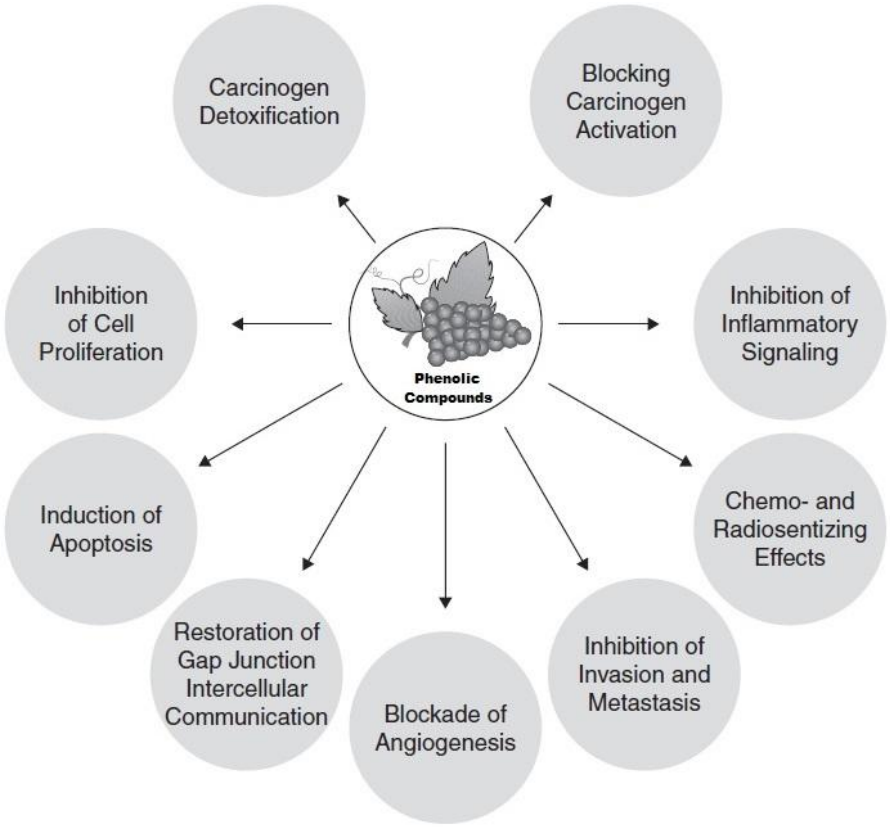


Figure 3.8. Anticancer effects of phenolic compounds (This figure was modified from original source). (Source: Kundu and Surh 2010)

Phenolic compounds prevent oxidation of cellular macromolecules, eliminate carcinogens, stimulate carcinogen detoxification enzymes, increase anti-inflammatory activity, repress the proliferation, induce cell cycle arrest and apoptosis in cancer cells, block angiogenesis and metastasis, regulate cellular signaling and estrogenic activity, and increase sensitivity of cancer cells to chemotherapy and radiotherapy (Figure 3.8) (Pandey and Rizvi 2009, Kundu and Surh 2010). A study performed with digalloyl-resveratrol as a phenolic compound showed that di-GA exhibits three distinct anticancer activities: induction of apoptosis, the inhibition of cell division and cell-cycle arrest, and disruption of cancer cell-induced lymphendothelial disintegration (Madlener, Saiko et al. 2010). Wang, Wang et al. (2012) studied the anti-angiogenesis effects and mechanisms of ellagic acid on human breast cancer utilizing in-vitro and in-vivo methodologies. They clearly showed that ellagic acid significantly inhibited a series of VEGF-induced angiogenesis processes including proliferation, migration, and tube formation of endothelial cells. Also, it directly inhibited VEGFR-2 tyrosine kinase activity, its downstream signaling pathways including MAPK and PI3K/Akt in endothelial cells, MDA-MB-231 cancer growth and P-VEGFR2 expression.

3.1.5. Anti-Aging Effect of Phenolic Compounds

Generally, all mammals, including humans, are born, grow, grow old, and dies. Aging is a quite complex, multifactorial depending on oxidative stress including accumulation of molecular damage of DNA, proteins, and lipids, and unhindered process which resulting with reduction of body functions through time (Reuter, Gupta et al. 2010, Poljsak and Milisav 2013). The cells, tissues and organs in our bodies are exposed to various irreversible changes (e.g., insufficient DNA damage repair, genetic instability, noninfectious chronic inflammation, alterations in fatty acid metabolism, accumulation of end products such as advanced glycation end products, amyloid, and proteins, alterations in neuroendocrine systems, loss of post-mitotic cells and decreasing number of neurons and muscle cells) through aging process. Until now, so many theories trying to explain all aspects of aging mechanism has been put forward. These theories are exemplified by, respectively, the telomere shortening hypothesis, the reproductive-cell cycle theory, the programmed theories (e.g. aging clock theory), mitohormesis theory, the disposable soma theory, evolutionary theory, and finally free radical or oxidative stress theory (Poljsak and Milisav

2013). The common point of all these theories was the oxidative stress and oxidative stress related telomere shortening (Figure 3.9). Oxidative stress is an important indicator of aging in different species. Because free radical and ROS levels that cause to oxidative stress, increases in senescent organisms. In our daily life, 1%-4% of the consumed oxygen is converted into the superoxide ion by mitochondria. After that, the produced superoxide ion may be transformed to hydrogen peroxide, hydroxyl radical and other reactive species (such as other peroxide types and singlet oxygen). These reactive species have ability to harm on cell constituents (e.g., structural proteins, lipids, and mitochondrial/nuclear DNA).

They also lead to accumulation of oxidative damages in senescent cells. Due to internal and external factors increased mitochondrial activity causes high ROS generation, which is causative to mutations in the mitochondrial DNA. The reduction of ATP production, ROS and proton leakage to mitochondrial inner membrane and other cellular components are seen in damaged mitochondria and cells including damaged mitochondria. Also, the number of mitochondria decreases in cells which exposed to oxidative damage. ROS and proton leakage from damaged mitochondria cause detriment to cellular macromolecules including enzymes, nucleic acids and membrane lipids. They also cause mutational loading of mtDNA, and a defective respiratory chain which produce more ROS and damage. These events generate a vicious cycle related with aging (Figure 3.10) (Poljsak and Milisav 2013).

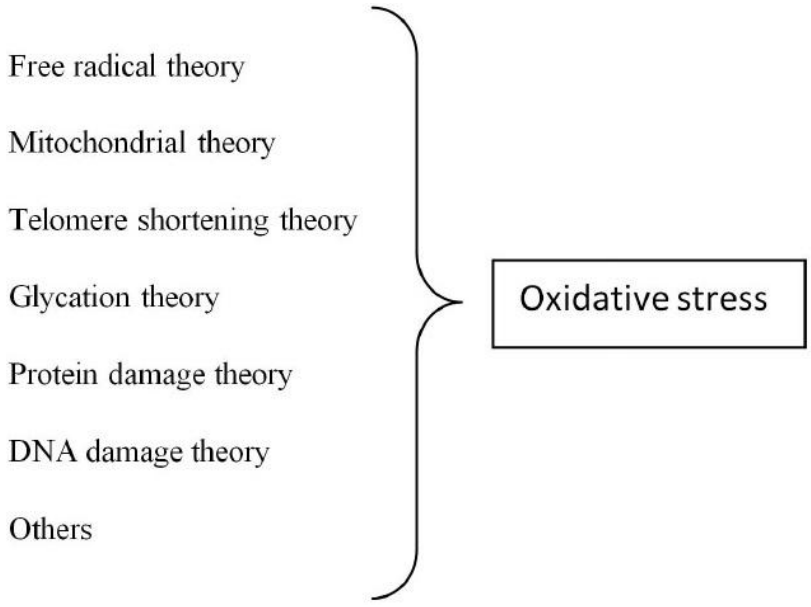


Figure 3.9. The common point of all these theories is the oxidative stress.
(Source: Poljsak and Milisav 2013)

The intake of polyphenols (phenolic acids and flavonoids) as a antioxidant supplement from fruit and vegetables provide increase in antioxidant defence system of cells, decrease in oxidative stress and cellular damages, prevent early aging of individuals (Poljsak and Milisav 2013).

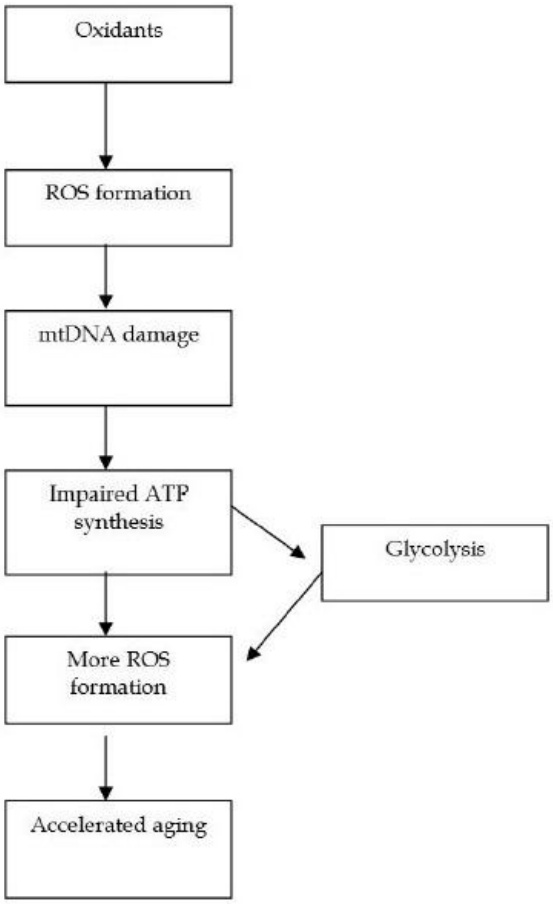


Figure 3.10. The vicious respiratory cycle playing role in aging process.
(Source: Poljsak and Milisav 2013)

3.1.6. Others

The some important health benefits of phenolic compounds have been shown up to this section. Additionally, phenolic compouds have much more beneficial health effects than explained above. Phenolic compounds exert anti-diabetic effect when they consumed regularly. Diabetus mellitus associated with impairment of glucose methabolism causes imbalance with hyperglycemia. It has two main categories as type 1 and type 2. Diabetes leads to some complements such as retinopathy resulting with blindness, nephropathy resulting with the risks of amputations, foot ulcers, and sexual dysfunctions. A lot of studies

have been performed to exert anti-diabetic effects of phenolic compounds (Pandey and Rizvi 2009). A recent study performed with phenolic extracts of *Terminalia chebula, Terminalia belerica, Emblica officinalis* and their combination demonstrated that block lipid peroxide formation and scavenge hydroxyl and superoxide radicals in vitro. When the extracts consumed regularly, they observed that the blood sugar level in normal and in alloxan diabetic rats decreased significantly and having a sustained effect (Sabu and Kuttan 2002).

Asthma is a chronic inflammatory disease of the respiratory tracts caused by combination of genetic and environmental factors, and the illness is characterized by reversible respiratory tract obstruction and bronchial spasm. Asthma symptoms can be generally indicated with wheezing, coughing, chest tightness, and shortness of breath. The studies on the disease have shown that polyphenols possess positively regulatory effect on the lung function, and protective effects against asthma (Pandey and Rizvi 2009). A study performed with polyphenols of edible red alga (Laurencia undulata) on mice having ovalbumin-induced murine allergic airway reactions showed that inhibited significantly all asthmatic reactions and possessed therapeutic potential against bronchial asthma (Jung, Choi et al. 2009).

Oxidative stress affecting nerve cells in the central nervous system causes neurodegenerative diseases including Alzheimer’s disease. Alzheimer’s disease is the most common type of brain disorders that affect a person's ability and quality of life. The illness has no cure it continues progressively and causes to death.

CHAPTER 4

MATERIALS AND METHODS

4.1. Materials

The dried fruits and walnuts were purchased from the Işık Tarım Ürünleri Sanayi ve Ticaret A.Ş in İzmir (Turkey) and green tea was provided by the Beta Tea Organic Green Tea (Turkey) (Table 4.1). Ethanol, hydrochloric acid (32%), di-sodium hydrogen phosphate, sodium carbonate, sodium hydroxide pellets, sodium chloride, sodium dihydrogen phosphate monohydrate, potassium peroxodisulfate were purchased from Merck KGaA (Darmstadt). ABTS (2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) and sodium nitrite were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Aluminum chloride phosphate and Folin-Ciocalteu's phenol reagent were purchased from Fluka (Switzerland).

Table 4.1. Dried Fruits and Catechin sources

Products - Materials	**The Average Initial Moisture Content % (w/w)**	**Source**
Raisins	13.17	Işık Tarım Ürünleri Sanayi ve Ticaret A.Ş.
Dried Figs	16.41	Işık Tarım Ürünleri Sanayi ve Ticaret A.Ş.
Prunes	14.83	Işık Tarım Ürünleri Sanayi ve Ticaret A.Ş.
Dried Apricots	16.61	Işık Tarım Ürünleri Sanayi ve Ticaret A.Ş.
Walnut Shell	-	Işık Tarım Ürünleri Sanayi ve Ticaret A.Ş.
Green tea	-	Beta Tea Organic Green Tea

4.2. Methods

4.2.1. Preparation of Phenolic Extracts Used In Controlled Rehydration

4.2.1.1. Preparation of Green Tea Extracts

The green tea extraction was determined using the extraction with water method of (Perva-Uzunalić, Škerget et al. 2006). Green tea extracts were prepared at 1g/20ml proportion. According to this proportion, 1000 ml distilled water was heated to 85°C by heating mixer. 50 g green tea leaves were weighed and added to boiled water. Green tea was heated at 85°C for 20 minutes by heating mixer that was adjusted second stir step option. After the heating, Green tea was filtered to remove its leaves via using synthetic filter. Filtered liquid was centrifuged at 15000 rpm for 15 minute. Centrifuged extract was lypholized and stored at -18°C.

4.2.1.2. Preparation of Walnut Shell Extracts

The walnut shell extraction was determined using the extraction with water method of (Perva-Uzunalić, Škerget et al. 2006). Walnut Shell extracts were prepared at 1g/6ml proportion. According to this proportion, 3000 ml distilled water was heated to 85°C by heating mixer. 500 g Walnut Shell was weighed and added to boiled water. Walnut shell was heated at 85°C for 20 minutes by heating mixer that was adjusted second stir step option. After the heating, walnut shell was filtered to obtain its extraction liquid via using synthetic filter. Filtered liquid was centrifuged at 15000 rpm for 15 minute. Centrifuged extract was lypholized and stored at -18°C.

4.2.2. Controlled Rehydration of Dried Fruits

20 g dried fruit was taken into 150 ml distilled water or green tea / wallnut shell extract to rehyrate the organic dried fruits. Following that, the samples incubated for 3h at 27-30°C. After the incubation, rehyrated dried fruit was filretered to remove liquid

and dried fruit sample was measured to determine that how much water taken. Rehydrated moisture contents of dried fruits were detemined After determining the initial moisture content.

4.2.3. Extraction of Phenolic Compounds from Dried Fruits

The rehydrated samples were added to 150 ml dH_2O, and the suspension solution cut up with Waring blender for 2 minutes. The obtained paste was further homogenized with an IKA homogenizer-disperser at 18000 rpm for 2 min. The obtained homogenate was centrifuged for 30 min at 15000 g (+4°C) and pellet was left from liquid, pellet was collected and first extract is obtained. The step that is until the end of the centrifuge from homogenization is called as 'Washing step' and this washing step was performed two times. First and second extract were mixed, measured and assayed for its total phenolic and flavonoids content and antioxidant capacity. This extract is designated aqueous extract. The precipitate obtained during first centrifugation was combined with the precipitate obtained from second centrifugation. The total precipitate was then suspended in 150 ml ethanol and homogenized with an IKA homogenizer-disperser at 18000 rpm for 2 min. The homogenate was clarified by centrifugation at 15000 g (+4°C) for 30 min and then assayed for its total phenolic and flavonoids content, and antioxidant capacity. This extract was designated ethanolic extract.

4.2.3.1. Preparation of Dried Fruit Extracts Treated with Green Tea/Walnut Shell

Lypholised green tea and walnut shell were used as catechin sources. To prepare extracts of dried fruits treated with green tea or walnut shell, Different amount of green tea or walnut shell (0.75 g and 1.5 g) was added to 150 ml distilled water and 20g dried fruit was rehyrated with these mixtures for 3h at 27-30°C. The remaining steps was similar with in the section of 5.2.3 Extraction of Phenolic compounds from dried fruits.

4.2.4. Preparation of Insoluble Fractions of Dried Fruits

Preparation of insoluble fractions of foods were performed to using the method described by Serpen et al. Serpen, Capuano et al. (2007) with some modifications. The obtained precipitates from extraction washed with ethanol. The washing procedure was ended after 2 washing cycles with 10 gram of precipitate correspond to 40 mL of ethanol (1 g/4 ml). Each washing step was followed by a stirring in magnetic stirrer at 500 rpm for 30 min and centrifugation at 15000 *g* for 10 min. After final washing and centrifugation, the residual precipitate was lyophilized to obtain the insoluble fraction of dietary fibers and fruit samples, which was kept at 4 °C prior to antioxidant activity measurement.

4.2.5. Determination of Antioxidant Potential of Dried Fruits

4.2.5.1. Determination of Total Phenolic Content of Dried Fruits

The total phenolic content of dried foods water and ethanolic extracts were determined using the Folin-Ciocalteu method of Singleton and Rossi (1965). A 0.2 ml sample of appropriately diluted aqueous or ethanolic extract was mixed with 1 ml of 1/10 diluted Folin-Ciocalteu reagent. After 3 minutes incubation, 0.8 ml of a 7.5 % Na_2CO_3 solution was added to the mixture and shaken. The mixture was further incubated for 2 hours, and its absorbance at 765nm was measured with a spectrophotometer. Total phenolic contents of dried foods were expressed as milligrams of gallic acid equivalents per kg of dry dried foods. All measurements were conducted five times.

4.2.5.2. Determination of Total Flavonoids Content of Dried Fruits

The total flavonoids content of dried foods were determined using the method described by Zhishen (1999). Before analysis 250µl of dried food water and ethanolic extracts were diluted with 1 ml of distilled water. Then, 75 µl of 5 % $NaNO_3$ was added into the diluted sample and mixed. After 5 min incubation, 75 µl of 10 % $AlCl_3$ was added into the mixture and it was incubated for 1 min. At the end of

the incubation period, 0.5 ml of 1 M NaOH solution and 0.6 ml distilled water were added into the mixture and its absorbance was determined at 510nm. The total flavonoids content was expressed as milligrams of epicatechin equivalents per kg of dry dried foods. All measurements were conducted five times.

4.2.5.3. Determination of Free Radical Scavenging Capacity (TEAC) of Dried Fruits

The antioxidant activity of dried foods used in this work has been mainly based on free radical scavenging capacity. The tests were conducted using the ABTS radical by the method given in Re et al (1999). The ABTS free radical cation was obtained by treating 7 mM ABTS solution with 2.45 mM potassium persulfate. The ABTS radical solution was diluted with 5 mM pH 7.4 phosphate buffer containing 150 mM NaCl (PBS) until its absorbance reached 0.70 units at 734 nm. The reaction mixture was prepared by mixing 25, 50 and 75 µl of dried food water and ethanolic extracts with 2 ml of ABTS radical cation solution. The absorbance of each reaction mixture was then monitored and recorded after 1, 3, 6, 9, 12, and 15 min. To calculate the AUC, the percent inhibition/concentration values for the extracts and trolox were plotted separately against test periods. The division of the areas of curves for each dried food water and ethanolic extracts to that of trolox was used to calculate the AUC value. All measurements were conducted three times and antioxidant activity was expressed as trolox equivalents (mmol) per kg of dry dried foods.

4.2.5.4. Determination of Bound Antioxidant Activity

The method described by Serpen et al. Serpen, Capuano et al. (2007) with some modifications was used to determinate immobilize antioxidant activity. 20 mg lyophilized sample tranferred to an eppendorf was mixed with 19 ml ABTS radical solution and stirred for 12 min at 150 x rpm in a shaking incubator at a constant temperature (30°C). After centrifugation at 6000 x g for 2 min, the absorbance of the supernatant was measured at 734 nm exactly 15 min after mixing the insoluble matter with the ABTS radical solution. All measurements were performed five times.

4.2.5.5. Determination of The Moisture Content of Dried Organic Fruits

The initial moisture contents of dried organic fruits were determined by the standart vacuum oven method for dried fruits (AOAC, 1995) with some modifications. This experiment was performed at a temperature of 70 ^{o}C $\pm$ l °C and at a pressure 60 mm Hg for six cycle (each cycle took 1 hour).

4.2.6. Statistical Analysis

The statistical analysis were carried out by using ANOVA with analyzing data for the analysis of variance. Values are significantly different at $P < 0.05$ as determined by Fisher's protected least significant difference.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1. Phenolic Content and Antioxidant Potential of Major Organic Sun-Dried Fruits Grown in Turkey

The total phenolic content (TPC), total flavonoid content (TFC) and trolox equivalent antioxidant potential (TEAC) of organic sun-dried raisins, figs, prunes and apricots were given in Table 1 and 2. The total TPC, TFC and TEAC of sun dried fruits varied between 1762 and 4062 µg gallic acid/g (d.w.), 830 and 2559 µg catechin/g (d.w.), and 35.7 and 74.1 µmol trolox/g (d.w.), respectively. The total TEAC and TPC of prunes were 1.7 to 2.3 fold higher than those for apricots, raisins and figs which showed quite similar total TEAC and TPC values. On the other hand, the total TFC of prunes and figs were similar and 1.7 to 3 fold higher than those of raisins and apricots. These results obtained for total TEAC, TPC and TFC clearly suggest the high total antioxidant capacity and potential health benefits of prunes. The minimum total antioxidant capacity was observed for raisins but the slight differences in antioxidant capacities among raisins, apricots and figs should not be important considering the potential variations of the antioxidant phenolic contents of fruits depending of cultivars, growth conditions and climate.

Not only the total amount of phenolic compounds but also the solubility profiles of phenolic compounds in the products is also a very important factor effecting the potential health benefits of fruits. The amount of water soluble phenolic compounds is particularly important since these are the primary phenolic fractions released from the product within the gastrointestinal tract. The soluble TPF values of dried fruits clearly indicate the high solubility of anthocyanin rich phenolic compounds in prunes. However, it is worth to report that the prunes and figs have quite similar water soluble TFC values which are almost 2.6 and 4.5 fold higher than those of raisins and apricots. These results clearly showed that the prunes and figs are rich sources of flavonoids. Moreover, it is also clear that the apricots are the poorest source of flavonoids which might be association with critically important health benefits like carcinogenic activity.

The bound antioxidant activity determined in this work reflected the antioxidant activity originated from insoluble biopolymeric compounds including carbohydrates such as cellulose, hemicellulose and pectin, and proteins. These biopolymers could show antioxidant activity due to their reactive groups. For example, the proteins owe their antioxidant activity to their constituent amino acids such as aromatic, sulfur containing and basic amino acids which are capable to donate protons to free radicals (Hu, McClements et al. 2003, Je, Park et al. 2005, Rajapakse, Mendis et al. 2005). The basic and acidic amino acids also have ability of chelating metal ions that are responsible for initiation of lipid oxidation in foods (Je, Park et al. 2005, Rajapakse, Mendis et al. 2005). On the other hand, the antioxidant activity of carbohydrates in related mostly to their covalently or ionically bound phenolic content (Arcan and Yemenicioğlu 2007). The bound TEAC values determined in this work showed the 1.5-2 fold higher antioxidant capacity of bound antioxidants in prunes than those of apricots, raisins and figs which did not vary considerably in their bound antioxidant capacity values.

Table 5.1. The phenolic and flavonoid contents (given as water and alcohol soluble and total) of different controlled rehydrated organic dried fruits.

Products	Total Phenolic Content (µg gallic acid/g)			Total Flavonoid Content (µg catechin/g)		
	Water Soluble	**Alcohol Soluble**	**Total**	**Water Soluble**	**Alcohol Soluble**	**Total**
Raisins	**1634.97 ± 31.51 c**	**128.08 ± 7.36 b**	**1763.05**	**616.53 ± 19.28 b**	**554.82 ± 24.34 b**	**1171.35**
Dried figs	**1883.99 ± 37.96 b**	**157.54 ± 32.50 ab**	**2041.53**	**1628.43 ± 153.86 a**	**931.02 ± 118.09 a**	**2559.45**
Prunes	**3884.37 ± 52.37 a**	**178.62 ± 5.03 a**	**4062.99**	**1692.54 ± 61.39 a**	**329.28 ± 27.39 c**	**2021.82**
Dried Apricots	**1943.78 ± 40.73 b**	**142.95 ± 4.17 b**	**2086.73**	**371.70 ± 13.40 c**	**459.51 ± 7.91 b**	**831.21**

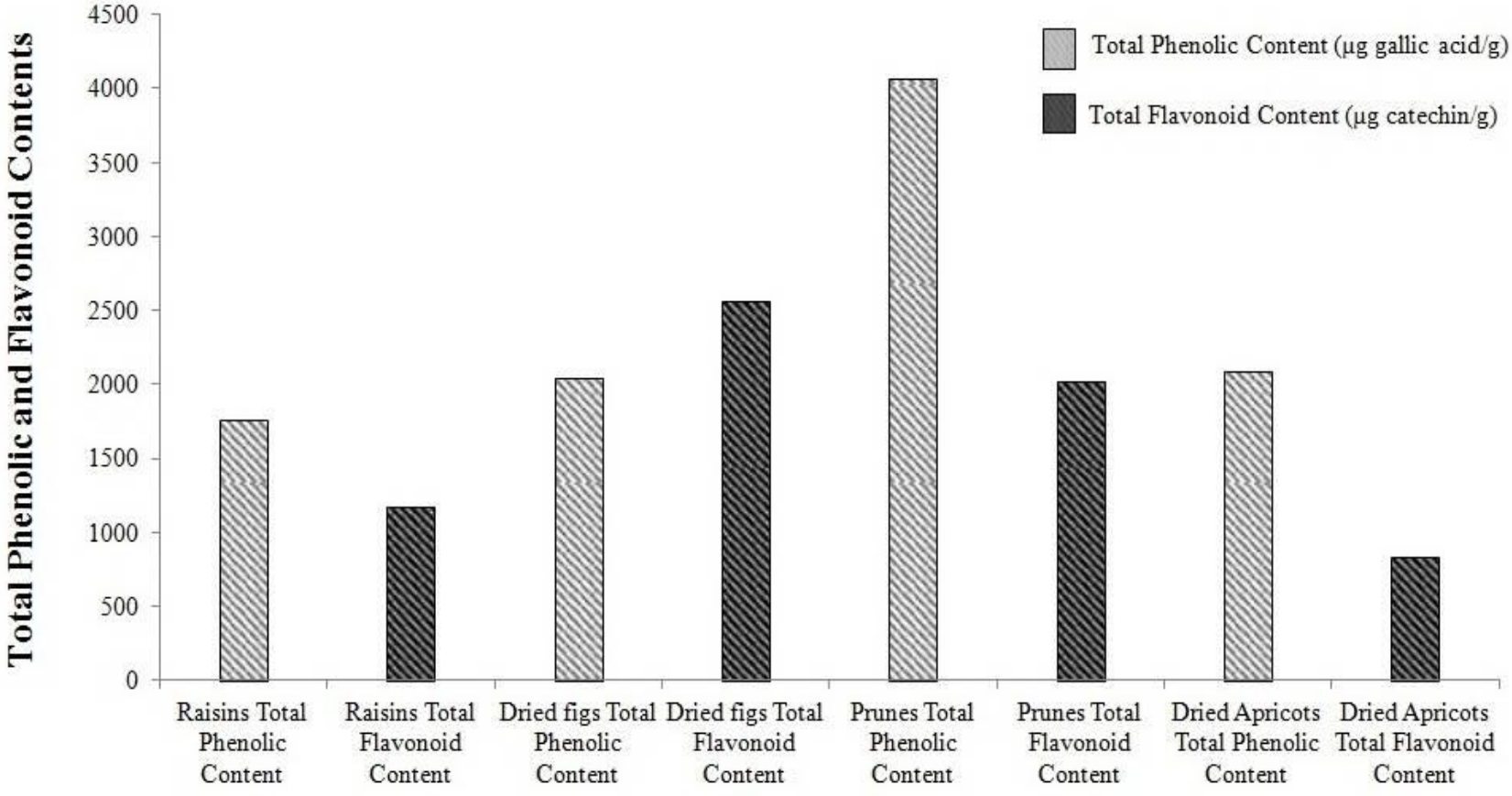
a-c: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Table 5.2. The TEAC values (given as water and alcohol soluble, bound, and total) of different controlled rehydrated organic dried fruits.

Products	TEAC (µmol/g)			
	Water Soluble	**Alcohol Soluble**	**Bound**	**Total**
Raisins	**12.05 ± 0.50 c**	**0.86 ± 0,01 b**	**22.76 ± 0.40 c**	**35.67**
Dried figs	**12.55 ± 0.16 c**	**0.91 ± 0.03 b**	**28.27 ± 3.35 b**	**41.73**
Prunes	**27.29 ± 0.69 a**	**1.16 ± 0.08 a**	**45.65 ± 2.44 a**	**74.10**
Dried Apricots	**15.83 ± 0.29 b**	**1.20 ± 0.16 a**	**27.64 ± 1.96 b**	**44.67**

a-c: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Figure 5.1. Total phenolic and flavonoid contents of different controlled rehydrated organic dried fruits.



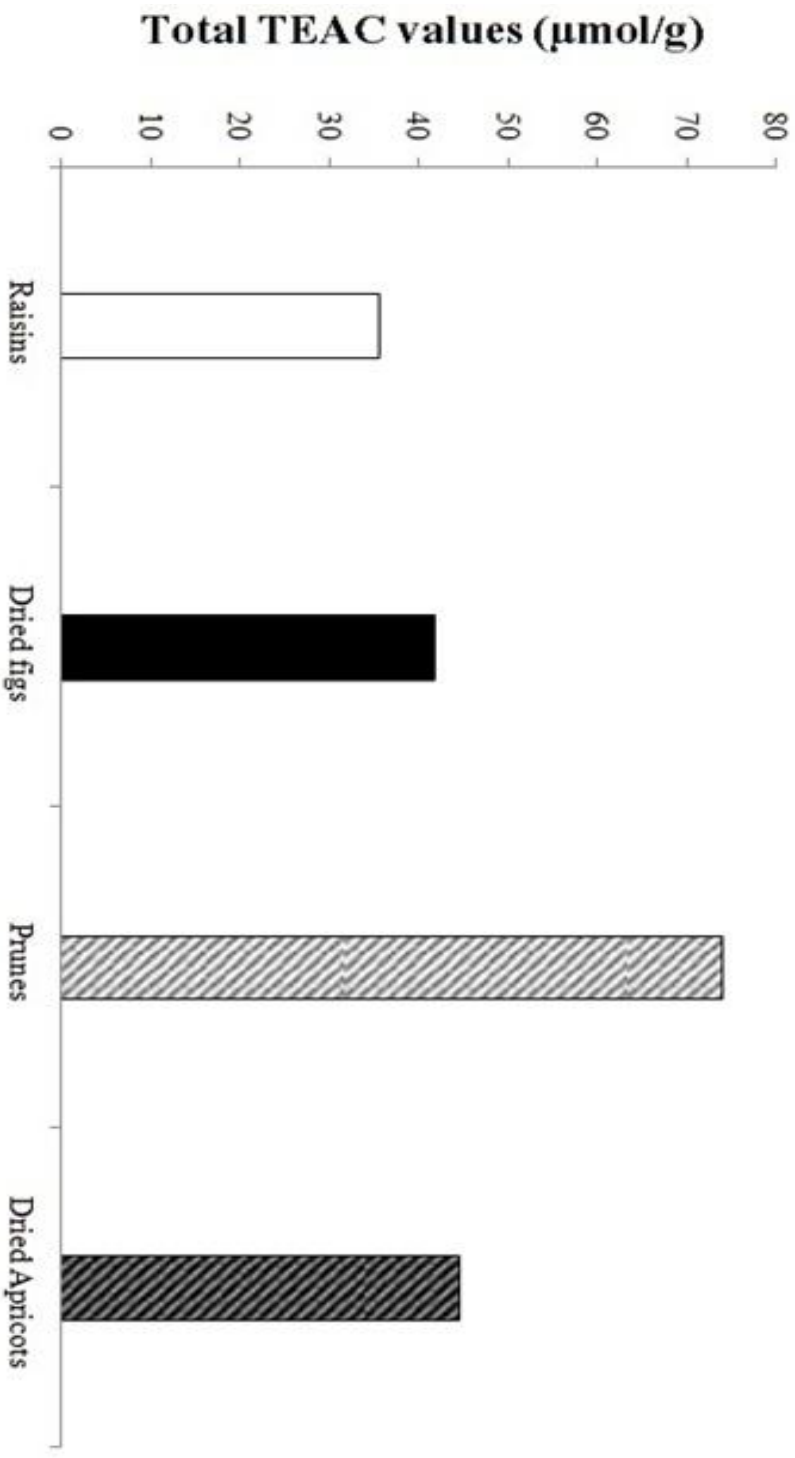


Figure 5.2. Total TEAC values of different controlled rehydrated organic dried fruits.

5.2. Development of a Novel Controlled Rehydration Method to Increase Phenolic Content and Antioxidant Capacity of Sun-Dried Fruits

5.2.1. Controlled Rehydration Studies with Raisins Using Green Tea Extracts (GTE) and Walnut Shell Extracts (WSE)

Since the raisins showed the minimum TEAC based antioxidant capacity, studies to improve phenolic content and antioxidant capacity of fruits were concentrated on this product. The novel method was based on controlled rehydration of dried fruits in phenolic extracts and it targets increasing of the antioxidant activity of fruits by incorporating phenolic compounds in extracts to fruit tissues. The rehydration studies with raisins conducted in different concentrations (0.5% or 1% (w/w)) of green tea extract (GTE) and walnut shell extract (WSE) at room temperature for 3h until reaching of final moisture content of 38.73% (w/w).

The results given in Table 3 and 4 clearly showed the possibility of increasing total TEAC, TPF and TPC of raisins with controlled rehydration. The rehydration of raisins in 0.5 % and 1 % GTE increased the total TEAC of raisins almost 1.5 and 1.8 fold, respectively. The total TPC of raisins rehydrated in 0.5 % and 1 % GTE also increased 1.5 and 1.8 fold, respectively. Although the rehydration in 1 % GTE caused a 1.6 fold increase in TFC of raisins, rehydration in 0.5% GTE did not cause a considerable change in TFC of samples. Thus, it is clear that the use of 1% solution of GTE is essential to obtain a considerable increase in all of the TEAC, TPC and TFC.

On the other hand, the total TEAC of raisins rehydrated in 0.5 % and 1 % WSE increased almost 1.3 and 1.6 fold, respectively. The rehydration in 0.5 % WSE did not cause a considerable increase in total TPF and total TFC of raisins, while only a 1.2 fold increase occurred in total TPF and total TFC by increasing WSE concentration to 1 %. A more careful study on the given results clearly showed that the increases in TPC and TFC of raisins rehydrated in WSE was originated mainly from the increase in alcohol soluble TPC of samples (1.7 to 2.0 fold). No considerable or very slight increases occurred in water soluble TPC and alcohol and water soluble TFC of raisins when WSC was used in controlled rehydration. This, results clearly showed the solubility and complex formation problems of WSC once it was incorporated into raisins.

The comparison of TEAC, TPC and TFC values of raisins obtained by rehydration in GTE and WSE clearly showed that the increase of phenolic extract concentration increased the antioxidant potential of final product. However, use of 1% of phenolic extracts is clearly more beneficial to obtain considerable increases in antioxidant parameters. Although a limited differences exist in TEACs of raisins rehydrated in WSE and GTE, use of 1% GTE instead of WSE at the same concentration gave almost 1.4 fold higher TPC and TFC in the final products. Thus, it appeared that the rehydration in GTE is more beneficial than rehydration in WSE to obtain reasonable increases in both total antioxidant activity and total phenolic contents of raisins.

Table 5.3. The phenolic and flavonoid contents (given as water and alcohol soluble, and total) of different controlled rehydrated raisins using green tea extracts (GTE) and walnut shell extracts (WSE).

Rehydrated Raisins	Total Phenolic Content (µg gallic acid/g)			Total Flavonoid Content (µg catechin/g)		
	Water Soluble	**Alcohol Soluble**	**Total**	**Water Soluble**	**Alcohol Soluble**	**Total**
Water (Control)	**1634.97 ± 31.51 e**	**128.08 ± 7.36 d**	**1763.05**	**616.53 ± 19.28 d**	**554.82 ± 24.34 b**	**1171.35**
0.5% GTE	**2441.55 ± 98.94 b**	**246.12 ± 27.28 b**	**2687.67**	**656.04 ± 17.14 c**	**561.21 ± 25.53 b**	**1217.25**
1% GTE	**2759.82 ± 42.12 a**	**345.67 ± 29.71 a**	**3105.49**	**1008.87 ± 34.12 a**	**835.14 ± 85.79 a**	**1844.01**
0.5% WSE	**1783.53 ± 41.69 d**	**213.67 ± 7.19 c**	**1997.20**	**613.57 ± 9.28 d**	**593.33 ± 83 b**	**1206.90**
1% WSE	**1887.25 ± 37.49 c**	**260.70 ± 7.66 b**	**2147.95**	**763.69 ± 28.82 b**	**585.79 ± 70.95 b**	**1349.48**

a-e: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Table 5.4. The TEAC values (given as water and alcohol soluble, bound, and total) of different controlled rehydrated raisins using green tea extracts (GTE) and walnut shell extracts (WSE).

Rehydrated Raisins	TEAC (µmol/g)			
	Water Soluble	**Alcohol Soluble**	**Bound**	**Total**
Water (Control)	**12.05 ± 0.50 e**	**0.86 ± 0,01 e**	**22.76 ± 0.40 b**	**35.67**
0.5% GTE	**26.57 ± 0.42 b**	**2.31 ± 0.17 b**	**24.20 ± 1.57 b**	**53.08**
1% GTE	**30.40 ± 0.45 a**	**2.84 ± 0.09 a**	**30.68 ± 1.64 a**	**63.92**
0.5% WSE	**18.16 ± 0.01 d**	**1.43 ± 0.08 d**	**25.84 ± 2.37 b**	**45.43**
1% WSE	**23.47 ± 0.02 c**	**1.84 ± 0.09 c**	**31.60 ± 0.93 a**	**56.91**

a-e: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Figure 5.3. Total phenolic and flavonoid contents of different controlled rehydrated raisins using green tea extracts (GTE) and walnut shell extracts (WSE).

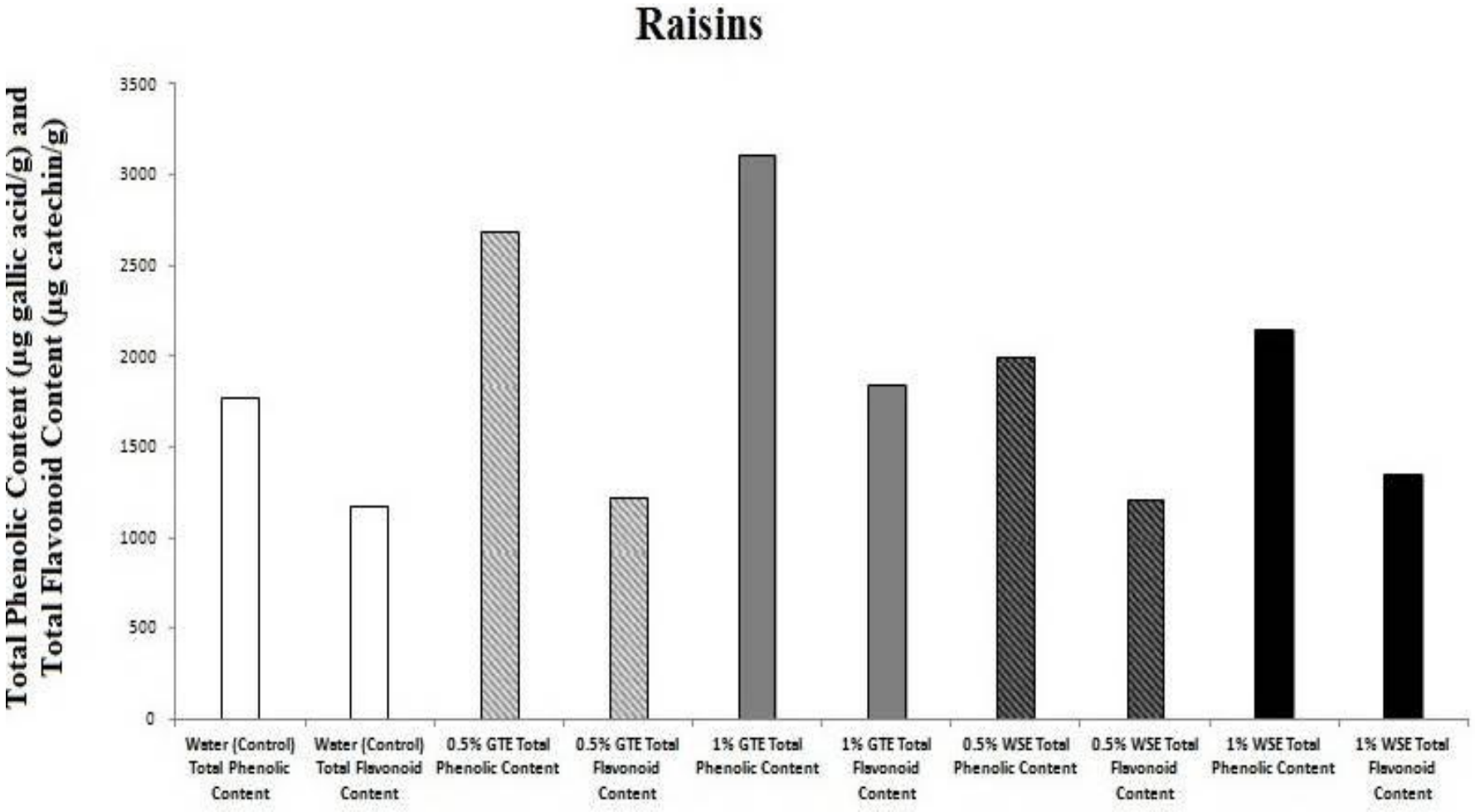
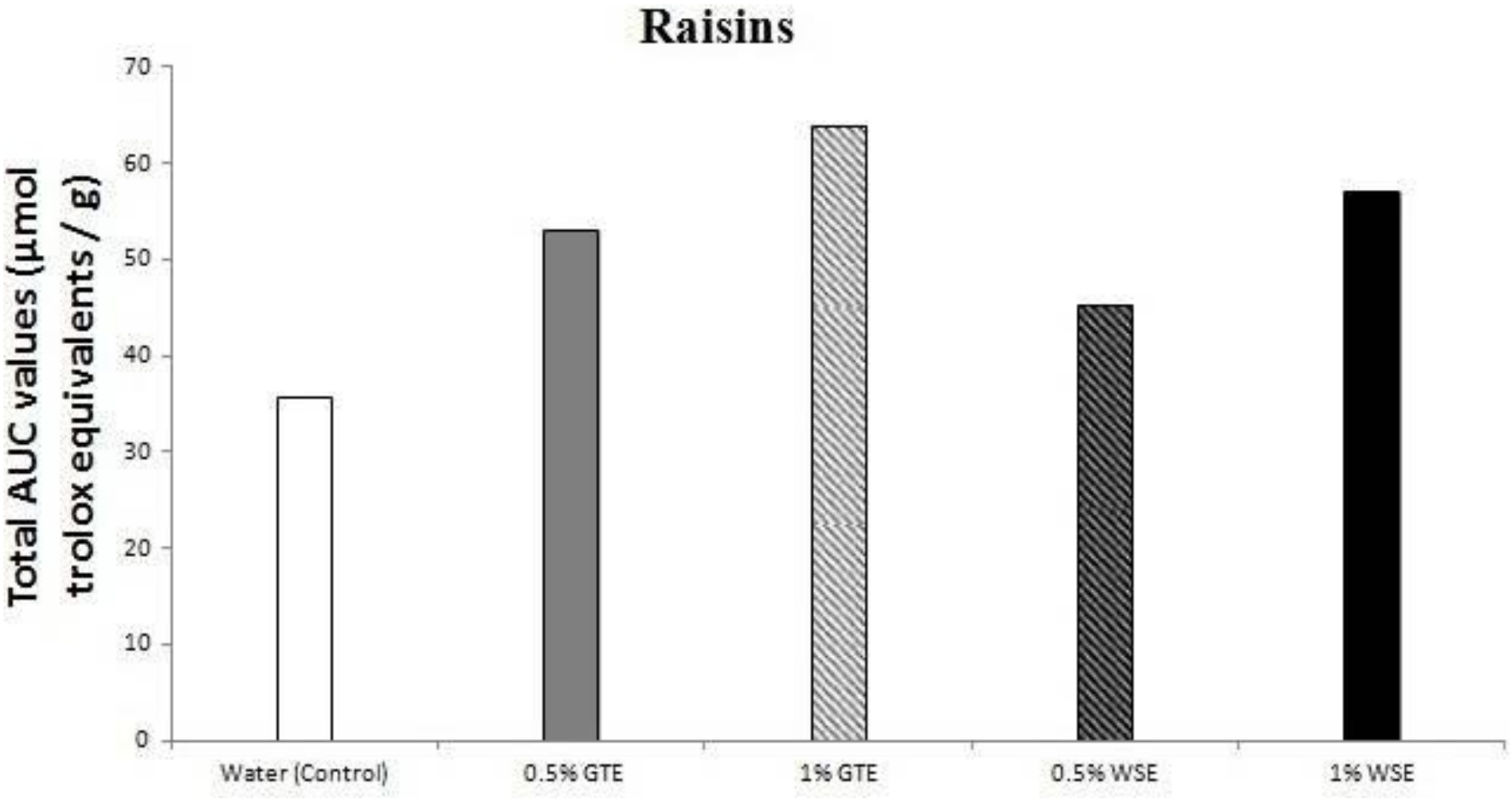


Figure 5.4. Total TEAC values of different controlled rehydrated raisins using green tea extracts (GTE) and walnut shell extracts (WSE).



5.2.2. Controlled Rehydration Studies with Prunes, Figs and Apricots Using Green Tea Extracts

The controlled rehydration studies with prunes, figs and apricots were applied with 1% solution of GTE since this extract at the specified concentration successfully increased the TEAC, TPC and TFC of raisins. The results given in Table 5 and 6 clearly show the contribution of controlled rehydration with GTE in bioactive properties of dry fruits. The rehydration of sun-dried figs, prunes and apricots in 1% of GTE for 3 h at room temperature caused almost 1.4, 1.1., 1.5 fold increase in total TPC; 1.3, 1.2, 1.6 fold increase in total TFC, and 1.6, 1.5, 1.3 fold increase in total TEAC, respectively. The most limited increases in total TPC and total TFC following rehydration occurred with prunes but the increase in total TEAC of all samples was considerable. The careful analysis of results revealed that the most significant increases in antioxidant parameters by GTE rehydration occurred in alcohol soluble TEAC and alcohol soluble TPC of dried fruits.

Table 5.5. The phenolic and flavonoid contents (given as water and alcohol soluble, and total) of different controlled rehydrated figs, prunes and apricots using green tea extracts.

Rehydrated Products	Total Phenolic Content (µg gallic acid/g)			Total Flavonoid Content (µg catechin/g)		
	Water Soluble	**Alcohol Soluble**	**Total**	**Water Soluble**	**Alcohol Soluble**	**Total**
Dried figs						
Water (Control)	**1883.99 ± 37.96 e**	**157.54 ± 32.5 de**	**2041.53**	**1628.43 ± 153.86 ab**	**931.02 ± 118.09 b**	**2559.45**
1% GTE	**2503.59 ± 98.83 d**	**464.22 ± 24.87 a**	**2967.81**	**1868 ± 1375 a**	**1526.28 ± 99.72 a**	**3394.28**
Prunes						
Water (Control)	**3884.37 ± 52.37 b**	**178.62 ± 5.03 d**	**4062.99**	**1692.54 ± 61.39 ab**	**329.28 ± 27.39 d**	**2021.82**
1% GTE	**4153.68 ± 27.44 a**	**281.30 ± 4.23 c**	**4434.98**	**2078.05 ± 77.86 a**	**351.21 ± 10.41 d**	**2429.26**
Dried Apricots						
Water (Control)	**1943.78 ± 40.73 e**	**142.95 ± 4.17 e**	**2086.73**	**371.70 ± 13.40 c**	**459.51 ± 7.91 dc**	**831.21**
1% GTE	**2810.22 ± 76.26 c**	**331.53 ± 7.26 b**	**3141.75**	**768.67 ± 31.72 bc**	**554.69 ± 32.16 c**	**1323.36**

a-e: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Table 5.6. The TEAC values (given as water and alcohol soluble, bound, and total) of different controlled rehydrated figs, prunes and apricots using green tea extracts.

Rehydrated Products	TEAC (µmol/g)			
	Water Soluble	**Alcohol Soluble**	**Bound**	**Total**
Dried figs				
Water (Control)	**12.55 ± 0.16 e**	**0.91 ± 0.03 d**	**28.27 ± 3.35 d**	**41.73**
1% GTE	**21.10 ± 0.53 c**	**4.05 ± 0.19 a**	**39.40 ± 3.29 c**	**64.55**
Prunes				
Water (Control)	**27.29 ± 0.69 b**	**1.16 ± 0.08 d**	**45.65 ± 2.44 b**	**74.10**
1% GTE	**43.75 ± 1.13 a**	**2.55 ± 0.17 c**	**68.42 ± 4.18 a**	**114.72**
Dried Apricots				
Water (Control)	**15.83 ± 0.29 d**	**1.20 ± 0.16 d**	**27.64 ± 1.96 d**	**44.67**
1% GTE	**21.60 ± 0.16 c**	**3.64 ± 0,18 b**	**32.98 ± 2.28 d**	**58.22**

a-e: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Figure 5.5. Total phenolic and flavonoid contents of different controlled rehydrated figs, prunes and apricots using green tea extracts.

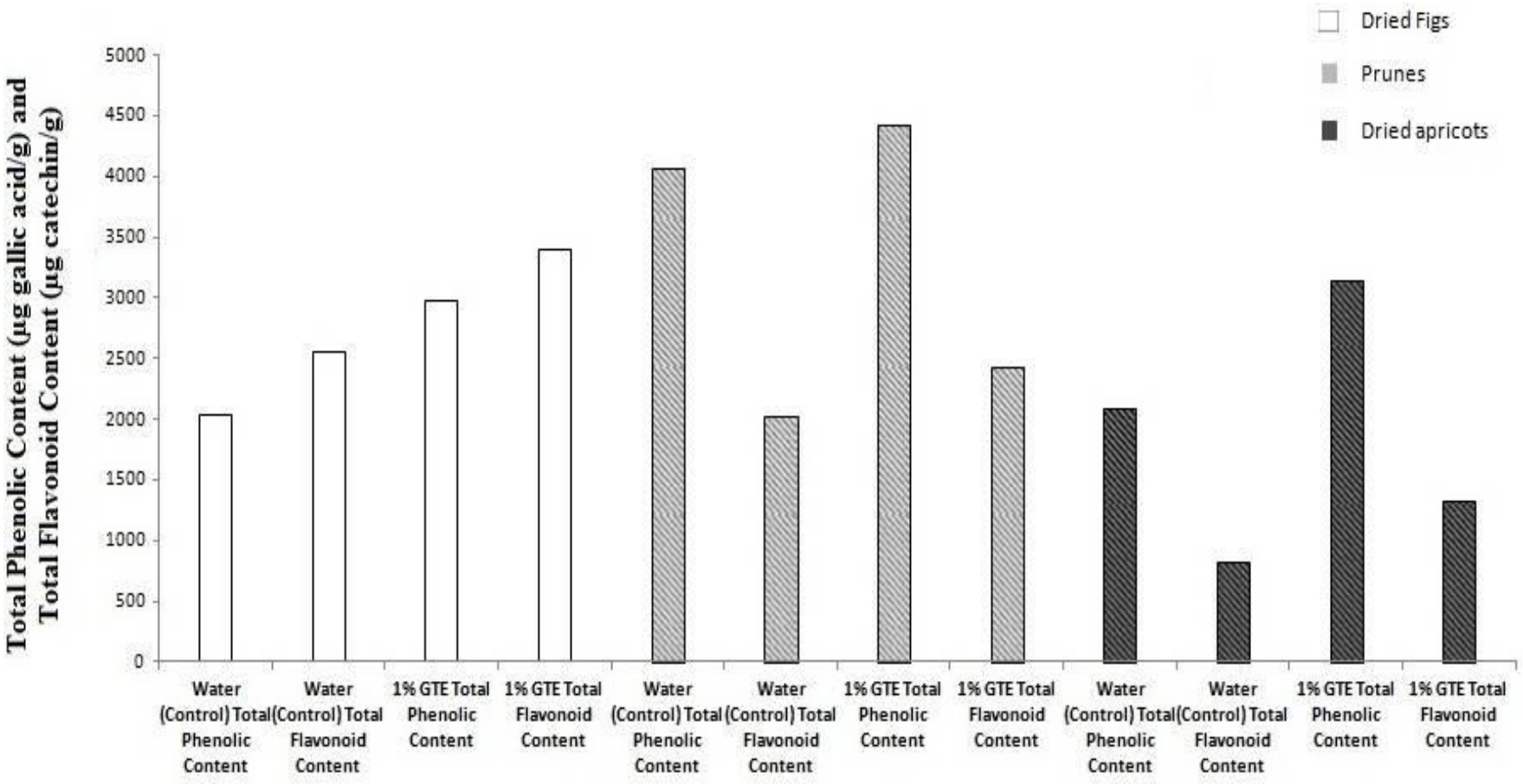
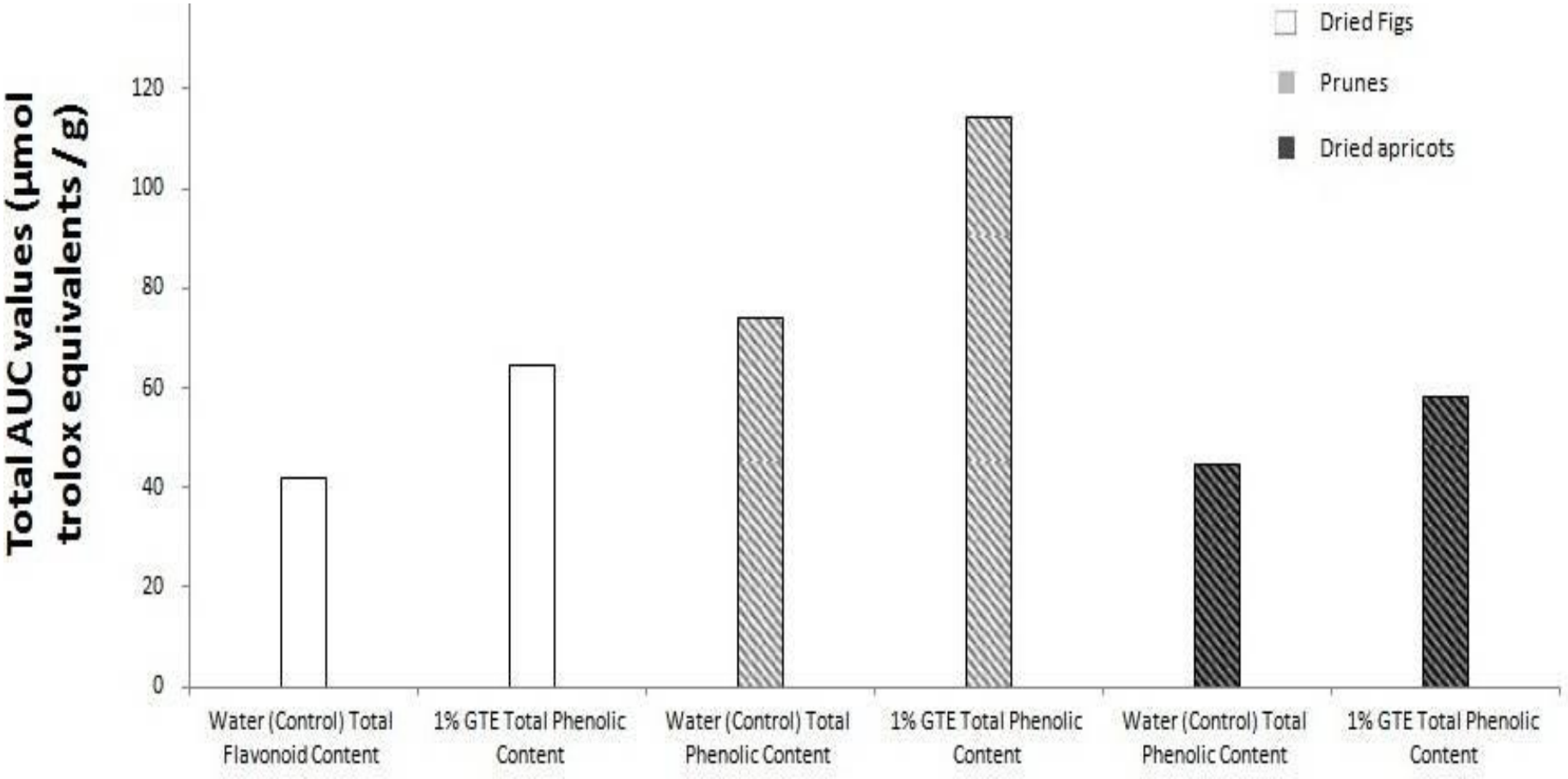


Figure 5.6. Total TEAC values of different controlled rehydrated figs, prunes and apricots using green tea extracts.



CHAPTER 6

CONCLUSIONS

- The results of this study clearly showed the considerably higher antioxidant activity and phenolic content, thus, greater potential health benefits of organic sun-dried prunes than organic sun-dried figs, raisins and apricots
- The organic sun-dried figs, raisins and apricots have quite similar antioxidant activity and phenolic contents.
- The antioxidant status of sun-dried figs, raisins and apricots could be improved by a novel process that involves controlled rehydration of fruits in green tea or walnut shell extracts which are rich sources of antioxidant phenolic compounds.
- The small size of raisins enables incorporation of high amounts of phenolic compounds into these fruits within short rehydration periods. Longer rehydration periods are needed for large sized figs, apricots and prunes than raisins to absorb the same amounts of antioxidant phenolic compounds.
- At the same rehydration conditions and extract concentrations the green tea extracts caused more considerable increases in antioxidant potential and phenolic content of dried fruits than the walnut extracts. This result suggest higher diffusivity (or lower molecular weighs) of green tea phenolics than walnut phenolics.
- At almost all rehydration conditions the antioxidant activity and phenolic content of dried fruits increased as concentration of green tea extracts and walnut extracts were increased.
- The final moisture contents of raisins (38.73%) , figs (39.83%), apricots (43.81%) and prunes (36.97%) within 3h rehydration at room temperature reached to the “Intermediate Moisture Product’s” level. Thus, preservative agents like sorbates and benzoates and cold storage are needed to obtain microbiologically stable functional fruit products.

- The controlled rehydration in phenolic extracts could also be employed for dried vegetables to improve functional properties of these food products.

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