DEVELOPMENT OF NOVEL FUNCTIONAL FOODS FROM TURKISH KABULI TYPE CHICKPEAS

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ABSTRACT

DEVELOPMENT OF NOVEL FUNCTIONAL FOODS FROM TURKISH KABULI TYPE CHICKPEAS

The aim of this thesis is to obtain novel functional chickpea and chickpea products by incorporating bioactive green and black tea phenolics into Turkish Kabuli type chickpeas during controlled rehydration. The rehydration of chickpeas in green or black tea infusions and green tea extracts increased the total phenolic content and bioactive properties of chickpeas. Chickpeas rehydrated in green tea extract or green tea infusions for 2 hours showed almost 2.2 − 2.5 fold higher total phenolic content, flavonoid content and free radical scavenging based antioxidant capacity, and rehydrated in green tea extract, green tea or black tea infusions almost 2.2-2.6 fold higher antidiabetic activity than control chickpeas rehydrated in water. The increase of rehydration period from 2h to 10h could increase the total phenolic content and antioxidant activity of chickpeas, but the increased incubation period did not have any considerable positive effects on antidiabetic activity of chickpeas. No significant reductions were observed in total phenolic content, flavonoid content and antioxidant activity of phenolic enriched chickpeas kept frozen at − 18°C while some limited reductions were observed in phenolic content and antioxidant activity of phenolic enriched chickpeas dried and stored at +4°C for 3 months. The extraction and then characterization of the phenolic content, and antioxidant and antidiabetic activity of protein from phenolic enriched chickpeas clearly showed the binding of the incorporated phenolic compounds onto chickpea protein. Thus, it appeared that the protein extracted from phenolic enriched chickpeas could also be used as a functional food ingredient. This work clearly showed the possibility of obtaining functional chickpeas and chickpea protein by use of controlled rehydration in presence solutions containing green and black tea phenolics.

ÖZET

KABULİ TÜRÜ TÜRK NOHUTLARINDAN YENİLİKÇİ FONKSİYONEL GIDALAR GELİŞTİRİLMESİ

Bu tezin başlıca amacı biyoaktif yeşil ve siyah çay fenoliklerinin kontrollü rehidrasyonla Türk Kabuli nohutlarına ilave edilmesi ve bu yolla yenilikçi fonksiyonel nohut ve nohut ürünleri geliştirilmesidir. Elde edilen sonuçlar nohutların yeşil veya siyah çay infüzyonları içerisinde rehidrasyonunun onların toplam fenolik madde miktarını ve biyokatif özelliklerini artırdığını göstermektedir. Nitekim nohutların 2 saat yeşil çay veya yeşil çay ekstraktı içerisinde rehidre edilmesi toplam fenolik ve flavonoid madde miktarı ile buna bağlı serbest radikal bağlama kapasitesine bağlı antioksidant aktivitelerini 2.2-2.5 kat, yeşil çay veya siyah çay içerisinde veya yeşil çay ekstraktı içerisinde antidiyabetik aktivitelerini ise 2.2-2.6 kat artırmıştır. Ancak, rehidrasyon süresinin 2 saatten 10 saate kadar artırılması toplam fenolik madde miktarı ve antioksidant aktiviteyi artırırken antidiyabetik aktivite üzerinde kayda değer bir artış sağlamamaktadır. Elde edilen fenolik maddelerce zenginleştirilmiş nohutlar fenolik madde miktarı ve antioksidant aktivitelerinde kayda değer bir değişiklik olmadan −18°C'de 6 ay depolanabilmektedirler. Ancak elde edilen ürünlerin yeniden kurutulması ve kuru halde +4°C'de 3 ay depolanması sırasında ürünlerin fenolik madde miktarı ve antioksidant aktivitesinde sınırlı bir kayıp görülmüştür. Fenolik madelerce zenginleştirilmiş nohutlardan ekstrakte edilen proteinlerin kontrol nohutlara göre oldukça yüksek fenolik madde miktarı ile antioksidant ve antidiyabetik aktivite göstermesi, çay fenoliklerinin nohut proteinlerine bağlandığını göstermiştir. Buna göre fenolik maddelerce zenginleştirilmiş nohutlardan ekstrakte edilecek proteinlerin fonksiyonel gıda katkı maddesi olarak da kullanılabileceği açıktır. Bu tez çalışması yeşil ve siyah çay fenolik maddeleri ve kontrollü rehidrasyon tekniği kullanılarak fonksiyonel nohut ve nohut proteini üretilebileceğini göstermiştir.

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CHAPTER 1

INTRODUCTION

Legumes including beans, peas, lentils and chickpeas have an important role in people's diet for thousands of years. These ancient crops are excellent source of antioxidant phenolic compounds, a good source of protein, dietary fibers, complex carbohydrates, folate and thiamine. Moreover, the legume proteins are rich in lysine essential amino acids. Thus, these reduce the risk of some health concerns such as cancer, obesity, hearth disease, diabetes, hypertension and cardiovascular disease (Asif et al. 2013 ; Pekşen and Artık 2005).

In Turkey, legumes are widely consumed and many different kind of legumes are grown. Chickpeas are the leading of these crops. After India and Pakistan, Turkey is among the primary chickpea-producing countries worldwide and takes place on the top in the Mediterranean region in chickpea growing area and total chickpea production (Özer et al. 2010). Although chickpeas contain high protein, high available carbohydrate and crude fiber contents and chickpea protein quality is higher than most legumes (Arcan and Yemenicioğlu 2007 ; Asif et al. 2013), the phenolic contents in chickpeas are lower than most of the other legumes (Han and Baik 2008).

Phenolic compounds are common components of foods of plant origin and occur naturally in many fruits, vegetables and natural product supplements. The results suggest that polyphenols especially the flavonoids are major antioxidants on human diet because they act as radical scavenger, hydrogen or electron donor and chelators of metal ions that cause oxidation (Damodaran 1996). Due to potential positive impacts of polyphenols on human health, they are widely used in the development of functional foods. The European Commission's Concerted Action on Functional Food Science (FUFOSE) defined the functinal foods as "A food can be considered as functional if it is evidenced that it has beneficial effects on one or more target functions in the body together with the basic nutritional effects in a sense which is either improving the general and physical conditions or/ and decreasing the risk of the evolution of diseases" (Diplock et al. 1999).

In this study, we increased antioxidant potential of chickpeas grown in Turkey by controlled rehydration in phenolic rich infusions and extracts and developed novel uses for antioxidant chickpeas as functinal food and food ingredients. Natural phenolic rich tea infusions and tea extracts with high bioactivity were used as the source of phenolic compounds for antioxidant chickpeas. Although tea is one of the well known excellent source of antioxidant, the consumption of it, especially green tea is not prefered by most of people because of its own bitter taste. Accordingly, this study shows that tea phenolics may be intake during the consumption of chickpeas which are the another important food source in the human diet due to this method. They can be easily used as frozen intermediate moisture antioxidant chickpeas or dried antioxidant chickpeas in the industry. Production of antioxidant chickpea flour and antioxidant chickpea protein extract is also possible with this method.

CHAPTER 2

PHENOLIC COMPOUNDS

2.1 Phenolic Compounds

Phenolic compounds, such as p-hydroxybenzoic acid, catechol, caffeic acid, gossypol, and quercein, are found in all plant tissues (Damodaran 1996). They are large group of molecules and have very important role on plant growth and defense. Since they include pigments and flavors, they make effect the color, taste and aroma of food (Vermerris and Nicholson 2006). Natural polyphenols can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. Flavonoids and tannins are an integral part of human and animal diets in the cause of representing one of the most numerous groups of plant metabolites (Bravo 1998). Exceeding 8000 polyphenols which contains over 4000 flavonoids have been identified and the number increases day by day (Ignat et al. 2011). There are lots of phenolic copmounds but phenolic acids, flavonoids and tannins only occur in foods. Tannins exist highly reactive combine with SH and amino groups of proteins (Bravo 1998 ; Damodaran 1996). The polyphenolic content of different foods and beverages is shown in Table 2.1. Most of the polyphenols listed in Table 2.1 are phenolic acids and flavonoids lesser are tannins (Bravo 1998).

The simplest phenolic compound is formed of one benzene ring with one hydroxyl group namely ‘phenol’. All other phenolic compounds are derived from this substance (Cemeroğlu et al. 2009). The compounds having more than one phenolic hydroxyl group in its structure are termed as polyphenols. They are formed of one or more aromatic rings (benzene) with one or more hydroxyl groups in it and also some side chains. Polyphenols commonly exist in seeds, fruits and other plant tissues either in free state or in conjugation with sugars as glycosides and esters (Baruah 2011).

Table 2.1 Phenolic content of different foods and beverages

(Source: Bravo 1998)

Food/Beverage[a]	Total Polyphenols	Food/Beverage[a]	Total Polyphenols
Cereals (mg/100 g dm)		Fruits (mg/100 g fm)	
Barley	1200–1500	Blackcurrant	140–1200
Corn	30.9	Blueberry	135–280
Millet	590–1060	Cherry	60–90
Oats	8.7	Cowberry	128
Rice	8.6	Cranberry	77–247
Sorghum	170–10,260	Gooseberry	22–75
Wheat	22–40	Grape	50–490
		Grapefruit	50
Legumes (mg/100 g dm)		Orange	50–100
Black gram	540–1200	Peach	10–150
Chickpeas	78–230	Pear	2–25
Cowpeas	175–590	Plum	4–225
Common beans	34–280	Raspberry	37–429
Green gram	440–800	Red currant	17–20
Pigeon peas	380–1710	Strawberry	38–218
		Tomato	85–130
Nuts (% dm)			
Betel nuts	26–33	Fruit juices (mg/L)	
Cashew nuts	33.7	Apple juice	2–16
Peanuts	0.04	Orange juice[b]	370–7100
Pecan nuts	8–14		660–1000
		Beverages	
Vegetables (mg/100 g fm)		Tea leaves (% dm)	
Brussels sprouts	6–15	Green	20–35
Cabbage	25	Black	22–33
Leek	20–40	Tea, cup (mg/200 mL)	150–210
Onion	100–2025	Coffee beans (% dm)	0.2–10
Parsley	55–180	Coffee, cup (mg/150 mL)	200–550
Celery	94	Cacao beans (% dm)	12–18
		Wine (mg/L)	
Fruits (mg/100 g fm)		White	200–300
Apple	27–298	Red	1000–4000 (6500)
Apricot	30–43	Beer (mg/L)	60–100

[a]dm=dry matter; fm=fresh matter.

[b]Values for different orange varieties.

They can be classified based on their basic skeleton and the number of benzene rings (Table 2.2) (Baruah 2011).

The phenolic compounds which are available in vegetal materials divide into two main groups named flavonoids and phenolic acids. The subgroups of flavonoids are comprise a large group of organic substances called flavones, flavonols, flavonones, flavanols or flavan-3-ols, anthocyanidins and isoflavones. Phenolic acids are classified as hydroxybenzoic acids and hydroxycinnamates (Cemeroğlu et al. 2009).

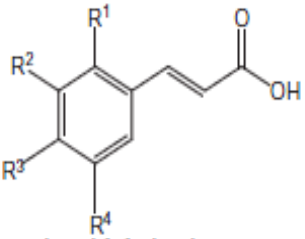
Table 2.2 Phenolic compounds in plants based on building blocks

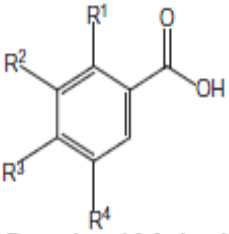
(Source: Baruah 2011)

Basic skeleton	Classes	Examples
C_6	Phenols	Catechol, hydroquinone
C_6-C_1	Phenolic acids	Gallic acid, salicylic acid
C_6-C_2	Acetophenones, tyrosine derivatives, phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde, tyrosol, p-hydroxy-phenylacetic acid
C_6-C_3	Hydroxycinnamic acid, phenyl propenes, coumarins, Isocoumarins.	Caffeic, ferulic, myristicin, eugenol, umbelliferone, aesculetin.
C_6-C_4	Naphthoquinones	Juglone, plumbagin
C_6-C_1-C_6	Xanthones	Mangiferin
C_6-C_2-C_6	Stilbenes, anthraquinone	Resveratrol, emodin
C_6-C_3-C_6	Flavonoids, isoflavonoids	Quercetin, cyanidin, genistein
$(C_6$-$C_3)_2$	Lignans, neolignans	Pinoresinol, eusiderin
$(C_6$-C_3-$C_6)_2$	Biflavonoids	Amentoflavone
$(C_6$-$C_3)_n$;$(C_6)_n$; $(C_6$-C_3-$C_6)_n$	Lignins; melanins; flavolans	Phenolic polymers

2.2 Phenolic Acids

Phenolic acids are present in almost all plant-derived foods, play an significant part of the human diet. Phenolic acids do not exist as free in the live plant tissues but they ocur with hydrolysis when proccessed (Cemeroğlu et al. 2009). Phenolic acids represent a various group of compounds including the overwhelmingly hydroxybenzoic acid and hydroxycinnamic acids. Hydroxybenzoic acids have characteristic C_6-C_1 and hydroxycinnamic acids have C_6-C_3 carbon skeleton (Figure 2.1). There are three main reactions contained in the formation of phenolic acids are deamination, hydroxylation and methylation (Heleno et al. 2015).They differ from each other because of different hydroxylations and methoxylations of their aromatic rings (Baruah 2011).





Substitution	Cinamic acid derivatives	Benzoic acid derivatives
R^1=OH	*o*-Coumaric acid	-
R^3=OH	*p*- Coumaric acid	*p*- Hydroxybenzoic acid
R^3=R^4=OH	Caffeic acid	Protocatechuic acid
R^2=OCH_3,R^3=OH	Ferulic acid	Vanillic acid
R^2=R^3=OCH_3	-	Veratric acid
R^2=R^3=R^4=OH	-	Gallic acid
R^1=R^4=OH	-	Gentisic acid
R^2=R^4=OCH_3, R^3=OH	Sinapic acid	Syringic acid
R^1=OH, R^4=HSO_3	-	5- Sulphosalicylic acid
R^2=R^3=OH	3,4 or 5-*O*-caffeoylquinic acid *	-

* The carboxylic group is esterified with quinic acid.

Figure 2.1 Chemical structures of primary benzoic and cinnamic acid derivatives (Source: Heleno et al. 2015)

2.2.1 Hydroxycinnamic Acids

Hydroxycinnamic acids known as hydroxycinnamates are an important group of low-molecular-weight phenolics. Hydroxycinnamic acid compounds are formed predominantly as simple esters with hydroxy carboxylic acids or glucose. Caffeic acid, p-coumaric acid, ferulic acid and sinapic acid are major hydroxycinnamic acids found in foods of plant origin (Baruah 2011).

2.2.2 Hydroxybenzoic Acids

Hydroxybenzoic acids are represented with a carboxyl group substituted on a phenol. Hydroxybenzoic acids also exist in bound form as component of hydrolysable tannins and lignins. Gallic acid, p-hydroxybenzoic acid, protocathechuic acid, salicylic acid and vanillic acid are most important benzoic acid derivatives (Vermeirssen et al. 2002). Ellagic acid especially ellagitannins (ETs) which release gallic acid is known as “hydrolysable tannis” and it is one of the most important non-flavonoid polyphenol group because of its potential benefits on cardiovascular health and high antioxidant

activities. In addition to this, many other bioactive properties such as anticarcinogenic, antiviral, antimutagenic and anti-angiogenic effects of ETs have been reported in a number of studies (Larrosa et al. 2010). Raspberry, strawberry, blackberry, pomegranate and walnut can be given as example of ellagitannis-rich foods (Cemeroğlu et al. 2009 ; Larrosa et al. 2010).

2.3 Flavonoids

Flavonoids are the most important group and represent the overwhelming majority of plant phenolics. They have characteristic $C_6C_3C_6$ carbon skeleton (diphenylpropanes skeleton) and have one or more aromatic ring which contains at least one hydroxyl group. Two aromatic rings are linked together by a group of three carbons that usually form an oxygenated heterocycle (Bravo 1998 ; Cemeroğlu et al. 2009 ; Damodaran 1996). They occur as glycosides with a single or multiple sugar components linked through an OH group (O-glycosides) or through carbon–carbon bonds (C-glycosides) (Acosta-Estrada et al. 2014). Figure 2.2 shows the basic structure containing A-, B- and C-ring and carbon numbering system of the flavonoids.

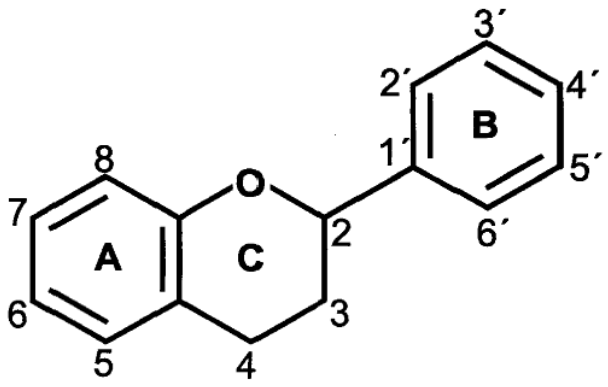


Figure 2.2 Basic structure of the flavonoids

(Source: Bravo 1998)

Flavonoids divide into six main subgroups which are flavones, flavonols, flavanones, isoflavones, anthocyanidins and flavanols (catechins) or flavan-3-ols (Figure 2.3). The substitutions such as oxygenation, alkylation, glycosylation, acylation to rings A and B cause different compounds within each group of flavonoids (Ignat et al. 2011).

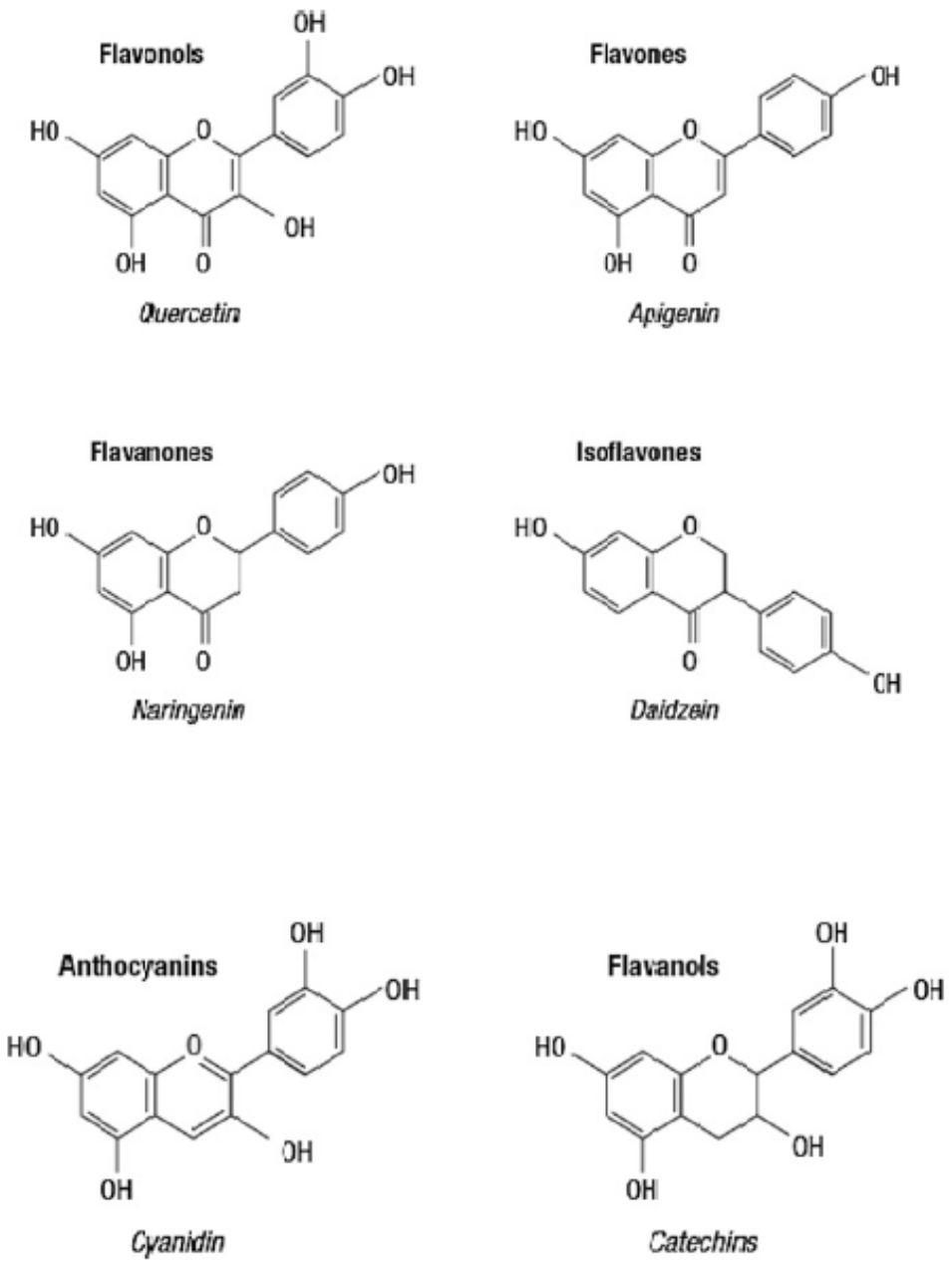


Figure 2.3 Chemical structures of main classes of flavonoids

(Source: Ignat et al. 2011)

2.3.1 Flavones

Flavones and flavonols are the most widely occurring and structurally diverse of the flavonoids. Citrus fruits, spinach, parsley and celery are the most common sources of flavones (Ignat et al. 2011). Apigenin and luteolin are known as flavones (Peluso et al. 2015).

2.3.2 Flavonols

Flavonols (e.g., quercetin, myricetin, kaempferol) are one of the most common phenolic compounds in fruits and the highest concentrations occur in the outer parts of plants. Therefore environmental factors such as light, growing conditions, storage, cooking conditions effect the content of these composites (Bravo 1998 ; Ignat et al. 2011). The variability of this group of flavonoids is considerable with about 200 different quercetin glycosides which is one of the potent natural antioxidant (Bravo 1998). Quercetin, kaempferol and their glycosides are found in foods such as broccoli, grapefruit, cranberry, apple and beverages which are black and green tea and red wines (Table 2.3) (Rice-Evans et al. 1996).

Table 2.3 Some sources of flavonoids

(Source: Rice-Evans et. al 1996)

Flavanol	
Epicatechin	
Catechin	
Epigallocatechin	green and black teas
Epicatechin gallate	red wine
Epigallocatechin gallate	
Flavanone	
Naringin	peel of citrus fruits
Taxifolin	citrus fruits
Flavonol	
Kaempferol	endive, leek, broccoli, radish, grapefruit, black tea
Quercetin	onion, lettuce, broccoli, cranberry, apple skin, berries, olive, tea, red wine
Myricetin	cranberry, grapes, red wine
Flavone	
Chrysin	fruit skin
Apigenin	celery, parsley
Anthocyanidins	
Malvidin	red grapes, red wine
Cyanidin	cherry, raspberry, strawberry, grapes
Apigenidin	coloured fruit and peels
Phenyl propanoids	
Ferulic acid	wheat, corn, rice, tomatoes, spinach, cabbage, asparagus
Caffeic acid	white grapes, white wine, olives olive oil, spinach, cabbage asparagus, coffee
p-Coumaric acid	white grapes, white wine, tomatoes spinach, cabbage, asparagus
Chlorogenic acid	apples, pears, cherries, plums peaches, apricots, blueberries tomatoes, anis

Flavonols have keto group at 4 position and a double bond at position 2,3 of the C ring in the basic flavonoid nucleus (Baruah 2011). They present as glycoside linked with sugars like anthocyanidins (Cemeroğlu et al. 2009).

2.3.3 Flavanones

Naringenin which is responsible of bitter taste in grapefruits and hesperidin are best known flavanones. Citrus foods and prunes are rich source of them (Bravo 1998 ; Rice-Evans et al. 1996). Chickpeas, cumin, licorice, peppermint contain a few of flavanones (Peterson and Dwyer 1998). The carbon-carbon bonds of them are saturated and this attribute makes the different from flavones (Figure 2.4).

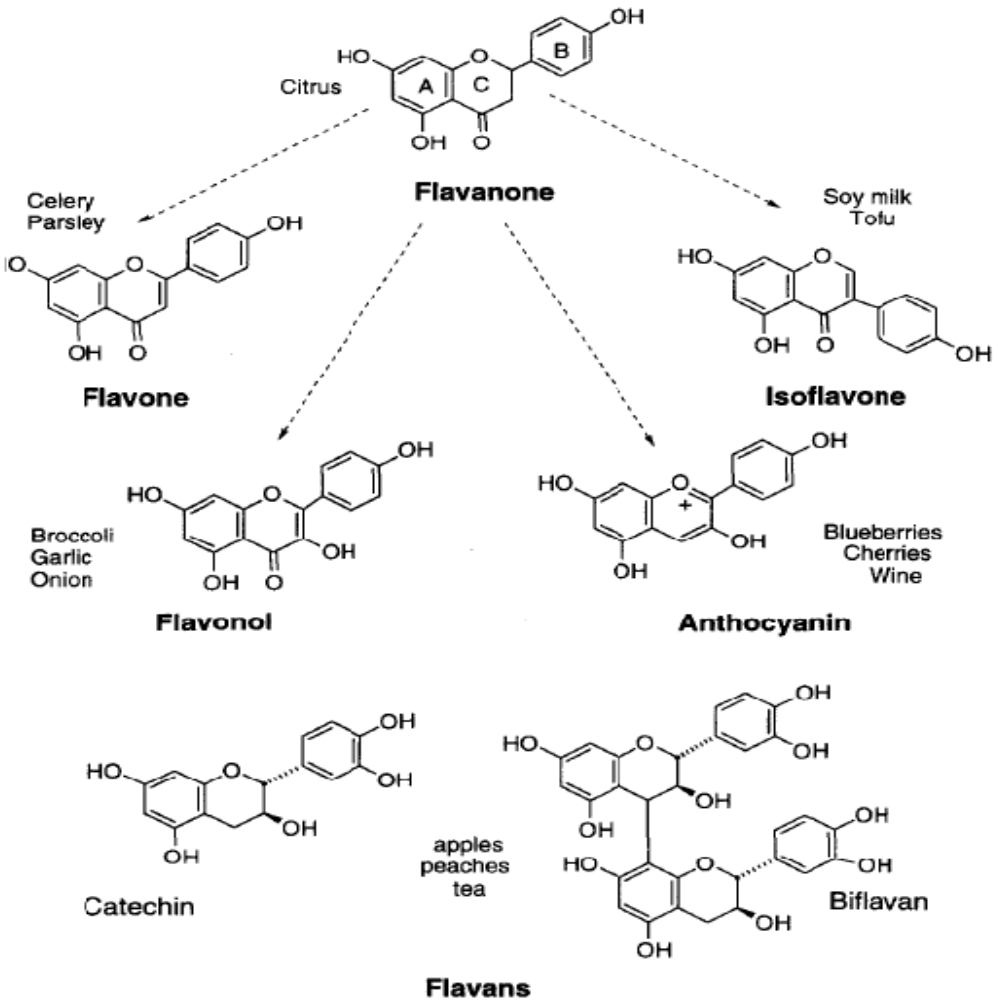


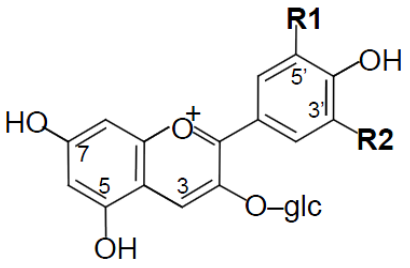
Figure 2.4 Chemical structure differences and some dietary sources of flavanoids (Source: Peterson and Dwyer 1998)

2.3.4 Isoflavones

Isoflavones are known with the similarities to estrogens having hydroxyl groups in the C7 and C4, positions, like estradiol molecule and they are structurally different from common flavonoids in B ring orientation (Ignat et al. 2011 ; Peterson and Dwyer 1998). Daidzein and genistein are best known isoflavones (Bravo 1998 ; Peluso et al. 2015). Isoflavones are compounds existing in great amounts in legumes. Soybeans are the major sources of these compounds espcially daidzein and genistein. Black beans, green split beans, clover sprouts, miso are other source of isoflavones (Medina et al. 2013 ; Peterson and Dwyer 1998). The dark varieties of legumes such as red kidney beans, black beans and black gram contains higher polyphenolic contents than other varieties (Bravo 1998).

2.3.5 Anthocyanidins

The anthocyanidins (or aglycons) consist of an heterocylic ring C containing a charged oxygen and it is bonded to aromatic ring A and also has a carbon–carbon bond with the aromatic ring B (Ignat et al. 2011). The anthocyanidins are basic structures of anthocyanins which are water soluble glycosides of them and the most common glycoside is the 3-O-glycoside. They are not found as free aglycons in nature, bonded to a sugar moiety such as glucose, galactose, rhamnose and arabinose. Anthocyanins include several anthocyanidins in nature, but only six anthocyanidins occur most frequently in plants: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Figure 2.5) (Damodaran 1996 ; Ignat et al. 2011).



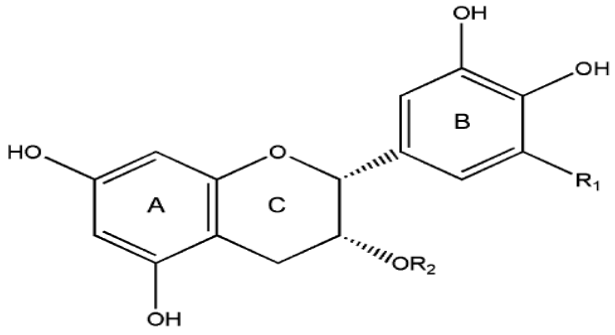
R1	R2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OH	OCH_3	Petunidin
OCH_3	H	Peonidin
OCH_3	OCH_3	Malvidin

Figure 2.5 Structures of most common anthocyanidins in plant-derived foods (Source: de Pascual-Teresa et al. 2010)

Anthocyanins are natural pigments and responsible for red, purple, or blue depending on pH occuring in many leaves, stems, roots, flowers, and fruits. Several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions affect their stability. Free aglycone form of anthocyanins (Anthocyanidins) has very low stability and solubility (Ignat et al. 2011).

2.3.6 Flavanols (Flavan-3-ols)

The flavanols is the most common flavonoid subgroup in plant tissues and very complex group of polyphenols. They have effect astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation of food (Aron and Kennedy 2008). They contain the C ring which is a saturated heterocycle with a hydroxyl group in position 4 and they are mostly found as free form in nature unlike anthocyanidins which are commonly found as their glycosylated form (Cemeroğlu et al. 2009). On the other hand, flavanols are commonly found in foods in their polymerized forms as oligomer or polymers. Their monomeric compounds (Figure 2.6) including catechin and epicatechin, oligomeric and polymeric compounds such as procyanidins (Figure 2.7 and 2.8) known as condensed tannins are typcially constituents of tea, cocoa, grape and wine. They are however almost unavailable in vegetables and legumes (de Pascual-Teresa et al. 2010).

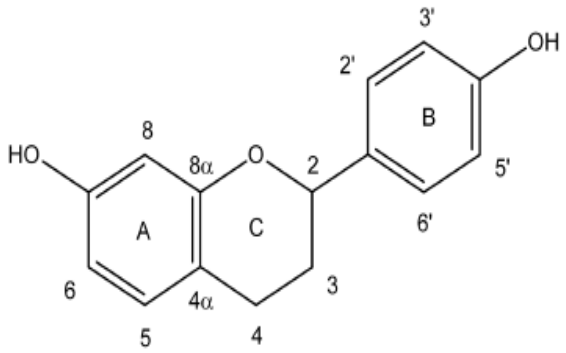


Name	R1	R2
(-)-Epicatechin (EC)	H	H
Epigallocatechin (EGC)	OH	H
Epicatechin gallate (ECG)	H	gallate
Epigallocatechin gallate	OH	gallate

Figure 2.6 Flavanols monomer examples in foods

(Source: Hackman et al. 2008)

Most flavanols present in nature are stereoisomers in cis or trans configuration of which (−)-epicatechin (cis) and (+)-catechin (trans) to carbons 2 and 3 in ring C. (−)epicatechin derivatives are the most common oligomers existing in edible plants (Fraga and Oteiza 2011).



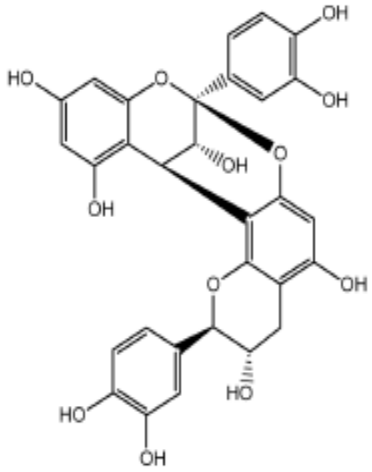
Proanthocyanidin type	3	5	8	3′	5′
Procassinidin	H	H	H	H	H
Probutinidin	H	H	H	OH	H
Proapigeninidin	H	OH	H	H	H
Proluteolinidin	H	OH	H	OH	H
Protricetinidin	H	OH	H	OH	OH
Propelargonidin	OH	OH	H	H	H
Procyanidin	OH	OH	H	OH	H
Prodelphinidin	OH	OH	H	OH	OH
Proguibourtinidin	OH	H	H	H	H
Profisetinidin	OH	H	H	OH	H
Prorobinetinidin	OH	H	H	OH	OH
Proteracacinidin	OH	H	OH	H	H
Promelacacinidin	OH	H	OH	OH	H

Figure 2.7 Proanthocyanidin types

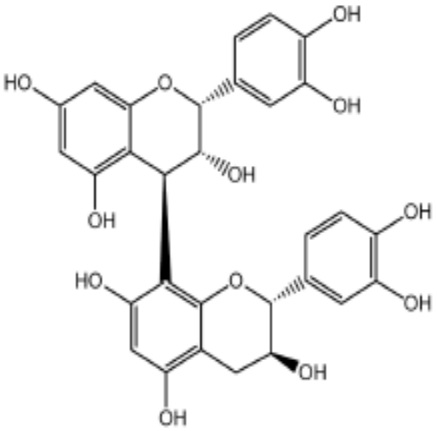
(Source: Aron and Kennedy 2008)

Most procyanidins are degraded into monomer or dimer units before absorption (Hackman et al. 2008). Figure 2.9 shows the flavanol dimers (A and B type) and trimer (C type) structures.

A-type Dimer (A1)



B-type Dimer (B-1)



C-type Proanthocyanidin

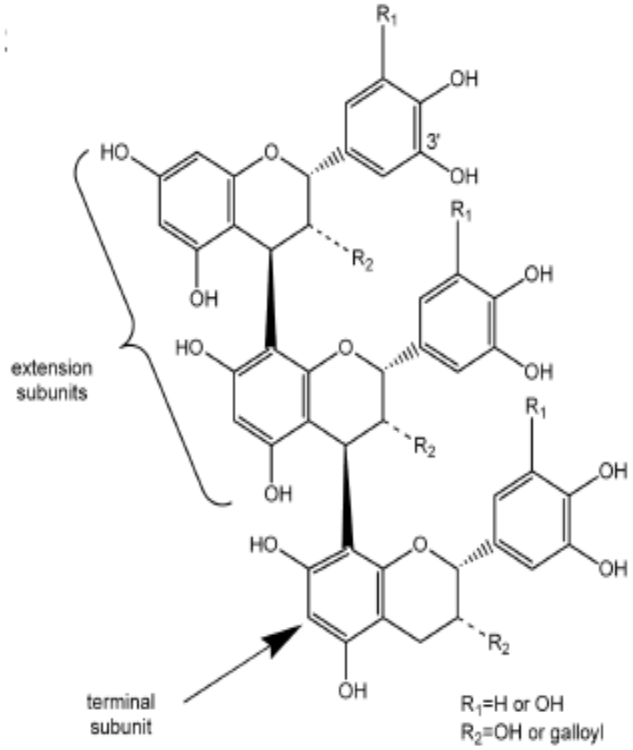


Figure 2.8 Flavanol dimers and trimer

(Source: Aron and Kennedy 2008)

2.4 Antioxidant Mechanisms of Phenolic Compounds

Antioxidants are substances which can be natural or synthesized retarding start or slow the rate of oxidation of oxidisable materials. They can inhibit the formation of free radicals in the initation step or discontinue propagation of the free radical chain by several mechanisms such as free radical scavenging, metal chelating or singlet oxygen quenching (Damodaran 1996). Activity of antioxidants against differing substrates is different from each other for example some are potent free radical scavengers whilst others are stronger metal chelaters. Free radicals including reactive oxygen (ROS) and nitrogen species are very reactive constituents which form in all living organisms during oxidation reactions and increase with environmental stress, wounding, and pathogen attack. Several human diseases involve free radicals. They damage to biomolecules such as lipids, nucleic acids, proteins in a cell and cause cellular membranes peroxidation (Baruah 2011 ; Leopoldini et al. 2011 ; Santhakumar et al. 2014).

Phenolic compounds are very effective natural antioxidants. Many flavonoids and esters of phenolic acids offer several health benefits due to functional and some biological activities in plant foods. They are the most consumed group of antioxidants in the diet (Wootton-Beard and Ryan 2011). Plant polyphenols can act as hydrogen or electron donating antioxidants (primary antioxidants) and metal chelaters (secondary antioxidants) (Figure 2.9). In hyrdogen atom transfer (HAT) mechanism, the antioxidant (designated as ArOH) transfers to free radical ($R^{\cdot}$) a hyrdogen atom. RH species and antioxidant radical form, which is stil antioxidant but less reactive ($ArO^{\cdot}$), are produced. In single electron transfer mechanism (SET), an electron is donated to the free radical. The anion R^{-} and cation antioxidant radical species ($ArOH^{+}$) are formed at the end of reaction. In another antioxidant mechanism, metals ions can be chelated by polyphenols and they provide to slow down the oxidation in food by this way (Leopoldini et al. 2011).

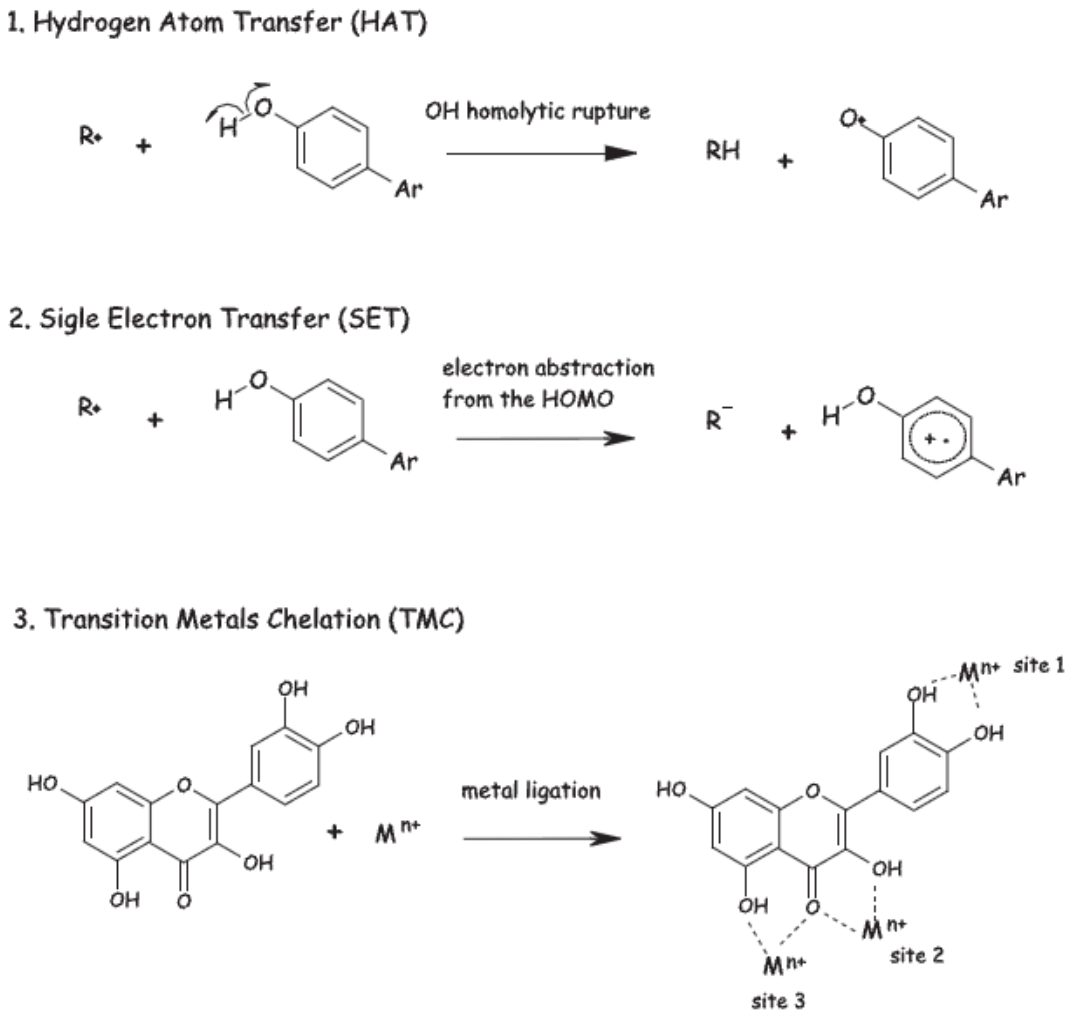


Figure 2.9 Antioxidant activity mechanisms of plant polyphenols
(Source: Leopoldini et al.2011)

2.4.1 Antioxidant Activity of Phenolic Acids

Phenolic acids have been greatly studied because of their bioactivities such as antioxidant, antimicrobial, anticancerogenic effects and others. Hydroxycinnamic acids show higher and more effective antioxidant activity than hydroxybenzoic acids due to their proton donating ability. The presence of −CH=CH−COOH groups in cinnamic acids have greater proton donating ability.

The antioxidant activity of phenolic acids and their esters is based on to the number and position of hydroxyl groups in the molecule in relation to the single carboxyl functional group. While the monohydroxybenzoic acids have no antioxidant activity in the *ortho* and *para* positions, *m*-hydroxy benzoic acids have high antioxidant

activity in the way of hydrogen-donating capacity against radicals. Besides, gallic acid (3,4,5-trihydroxy benzoic acid) has very high and powerful antioxidant activity since it has three available hydroxyl groups (Rice-Evans et al. 1996).

2.4.2 Antioxidant Activity of Flavonoids

Flavonoids are very high antioxidants in nature and many flavonoids have much stronger antioxidant potential than vitamin C and E. Antioxidant capacity of flavanoids depends on the configuration and total number of hydroxyl group in the B ring which is the most effective factor of free radical scavenging by hydrogen atom donation. The properties of high free radical scavenging capacity of flavanoids can be specified as an ortho-dihydroxy structure in the B ring, the presence of hydroxyl groups at positions 3 and 5 and 2,3-double bond in conjugation with a 4-oxo group in the C ring (Figure 2.10) (Prochazkova et al. 2011).

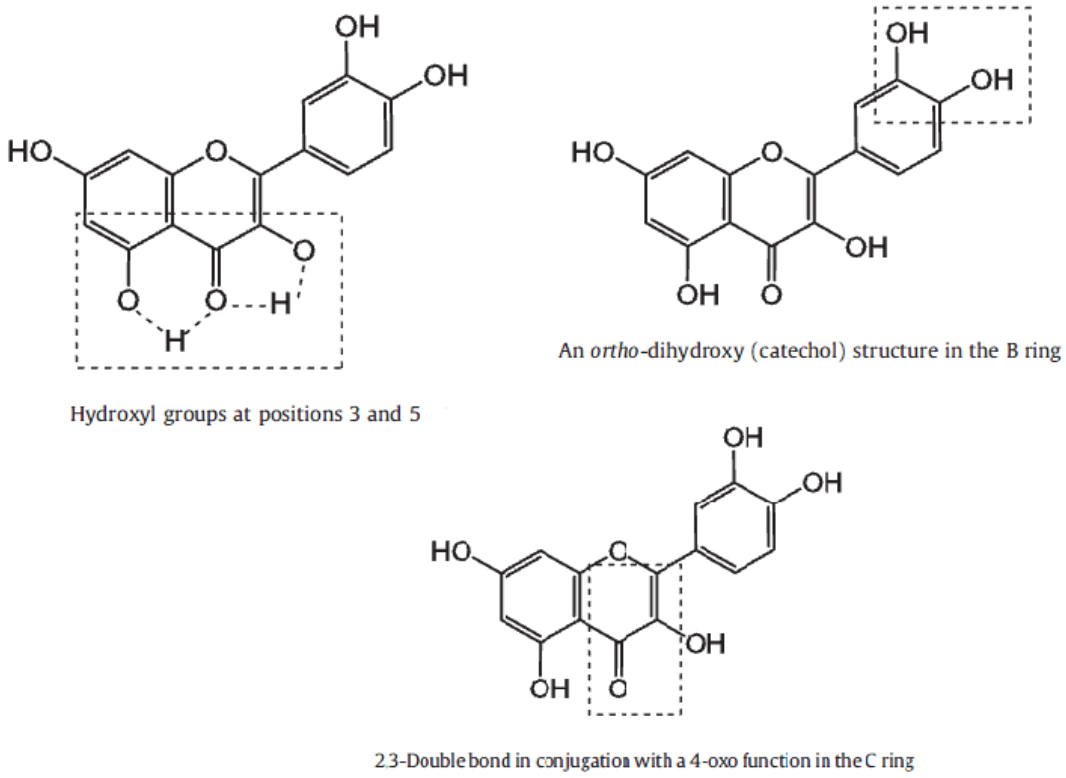


Figure 2.10 The main structural properties of flavonoids required for radical scavenging (Source: Prochazkova et al. 2011)

Another mechanism preventing damages by free radicals of flavanoids is metal chelation. An orto-dihydroxy structure in the B ring is the major responsible site of metal chelation with the free radical scavenging feature. Polymerization cause also increasement of antioxidant activity of flavanoid monomers such as the polymers of catechins which are perfect antioxidants due to the high number of hydroxyl groups in the molecules. On the other hand, glycosylation of flavanoids has reducing effect on their antioxidant activities when compared to their aglycones (Prochazkova et al. 2011).

CHAPTER 3

TEA PHENOLICS

3.1 General Information of Tea Phenolics

Tea, produced from the leaves of *Camellia sinensis* by different manufacturing processes with a worldwide yearly production of approximately 20% green, 2% oolong and the 78% black tea leaves, is an ancient and one of the most widely consumed beverage in the world (da Silva Pinto 2013). People's Republic of China, India, Kenya, Sri Lanka, and Turkey are the top producers of tea (Bansal et al. 2013). Traditionally, people consume to improve blood flow, eliminate toxins, and to gain resistance to illnesses (Dufresne and Farnworth 2001).

Tea is divided into three main types depending on the level of oxidation. They are green tea (non-fermented), oolong tea (semi-fermented) and black tea (fermented). Tea leaves are freshly harvested and they must be processed to inactive the polyphenol oxidase enzyme for green tea production, or to control the oxidation by the leaf enzymes for the production of oolong and black teas. Tea contains a large amount of polyphenol compounds called catechins which are very active flavonoids (flavan-3-ols). Catechins are formed unchanged in green tea but they are oxidized and polymerized to the theaflavins and thearubigins which are main pigments in black tea during the process of fermentation (Figure 3.1) (Chen et al. 2009 ; Sang et al. 2011). Oolong tea has small amount of both catechin and theaflavins. The fresh tea leaves have significant amount of caffeine (3-6%) which is unaffected by the processes, lignin (6.5%), organic acids (%1.5), theanine (4%) and free amino acids (1-5.5%). Tea is also known to contain other polyphenols such as gallic acid, quercetin, kaempferol, myricetin, and their glycosides (Namal Senanayake 2013 ; Zaveri 2006). The only flavone is apigenin defined in tea but represent a very small fraction of the tea polyphenols (Sang et al. 2011).

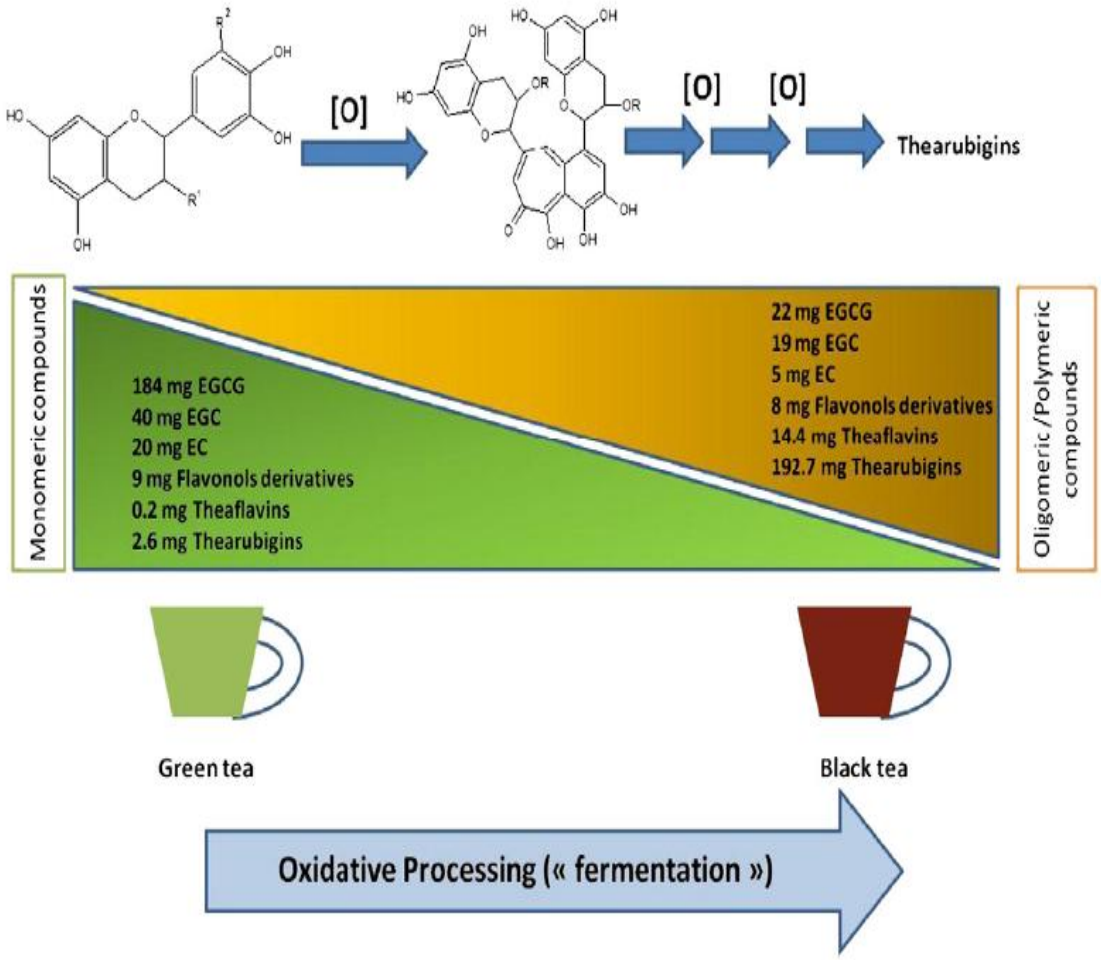
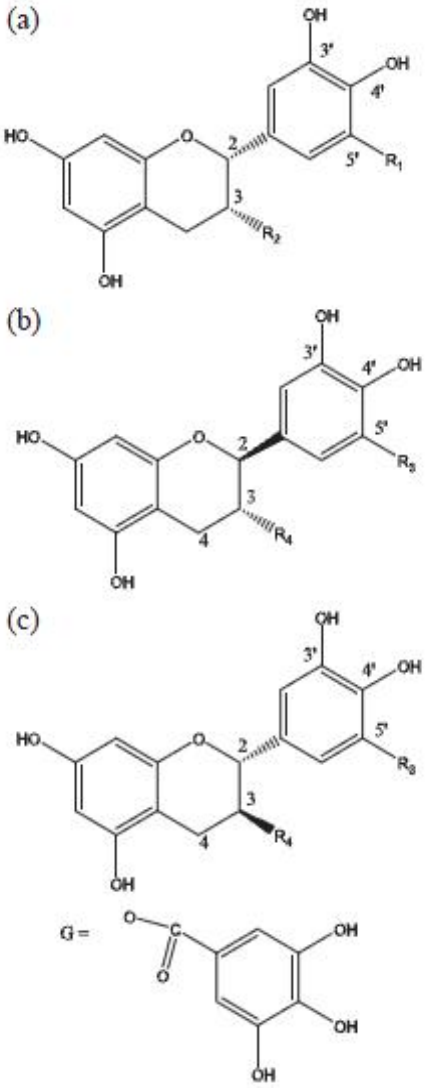


Figure 3.1 The process of fermentation and average values of phenolic compounds per cup (8 oz) for green and black tea (Source: da Silva Pinto 2013)

Catechins are usually present for 30-42% of dry weight of the solids in brewed green tea (Sang et al. 2011). Four major tea catechins are identified as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG). These epicatechins can change to their epimers that are non-epicatechins; (-)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (+)-catechin (C) and (-)-catechin gallate (CG), respectively (Figure 3.2) (Ananingsih et al. 2013 ; Sharma and Zhou 2011). EGCG is the most copious catechin in green tea existing 65% of the total catechin content (Zaveri 2006). The major theaflavins (TF) formed during the production of black tea are theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3′-gallate (TF2B) and theaflavin-3,3′-digallate (TF3) (Figure 3.3) (Lun Su et al. 2003).



Component	Abbrev.	R_1	R_2	R_3	R_4
(-)-Epigallocatechin gallate	(-)-EGCG	OH	G	-	-
(-)-Epicatechin gallate	(-)-ECG	H	G	-	-
(-)-Epigallocatechin	(-)-EGC	OH	OH	-	-
(-)-Epicatechin	(-)-EC	H	OH	-	-
(-)-Gallocatechin gallate	(-)-GCG	-	-	OH	G
(-)-Gallocatechin	(-)-GC	-	-	OH	OH
(-)-Catechin gallate	(-)-CG	-	-	H	G
(+)-Catechin	(+)-C	-	-	H	OH

Figure 3.2 Chemical structures of (a) epicatechins, (b) non-epicatechins and (c) (+)-catechin (Source: Ananingsih et al. 2013)

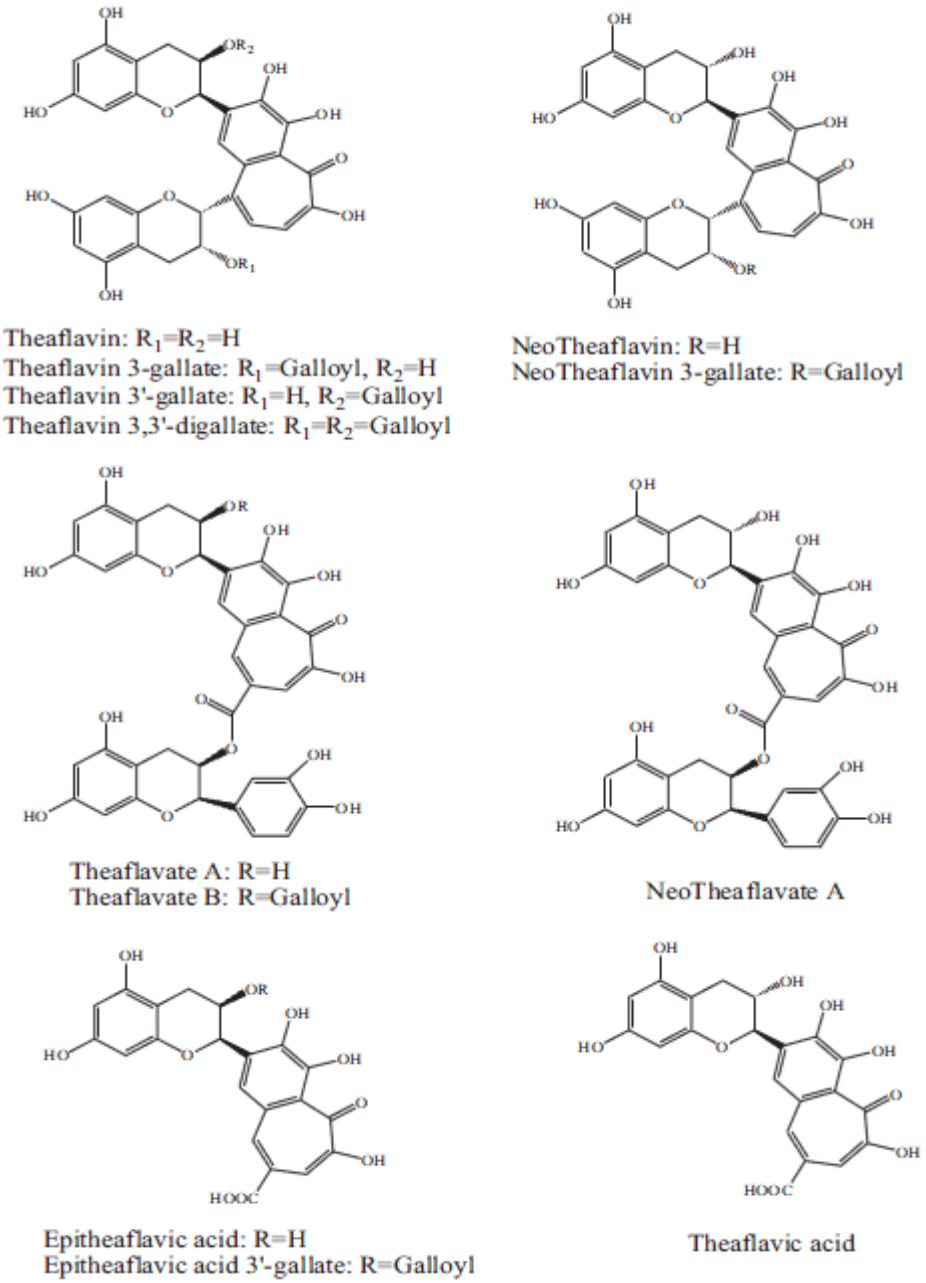


Figure 3.3 Chemical structures of major theaflavins in black tea

(Source: Sang et al. 2011)

Stereoisomers of theaflavins and theaflavin derivatives such as theaflavic acids and theaflavates also occur in black tea. The theaflavins make effects to the properties of black tea including mouthfeel, color and crem formation. While theaflavins are bright orange or orange-red and responsible for the astringency, thearubigins exhibit red-brown or dark-brown in color (Sang et al. 2011).

3.2 Health Benefits of Tea Phenolics

The bioactive properties of tea and its benefits on human health have been studied greatly with epidemiological studies, in vitro studies and animal studies all around the world in recent years. Tea contains a large amount of very active flavonoids called flavan-3-ols which present several health benefits by having antioxidant, anticancer, anticariogenic, antihypertensive, cardiopreventive, antimicrobial, anti-viral and antidiabetic effects (Aron and Kennedy 2008 ; Dufresne and Farnworth 2001). Consumption of tea effect to reduce cholesterol and prevent conversion of LDL to harmful oxidized chemical structures owing to being rich source of flavan-3-ols (Aron and Kennedy 2008). It has been reported that tea consumpion providing long term intake (approx.30years) is related with reduced levels of fasting blood glucose and lower prevalence of type 2 diabetes among people living in Mediterranean islands (da Silva Pinto 2013). Green and black tea can inhibit the oxidation of lipoproteins induced by Cu^{2+} in vitro that facilitate to the prevention of atherosclerosis and other cardiovascular diseases (Khan and Mukhtar 2007).

Animal studies shows that green tea catechins make beneficial effects in Parkinson's disease and Alzhemier's disease, prevention of cancer such as lung, skin, esophagus, liver and stomach, prevention of ischemic damage, hypercholesterolemia, anti-inflammatory and others due to its high antioxidant and metal chelating properties. In addition to these properties, green tea and EGCG have been shown prevention of tumor blood vessel growth and anti-mutagenic effects (Rapaka and Coates 2006 ; Zaveri 2006). Many possible health effects of green tea on obesity, type-2 diabetes and cardiovascular risk factors are also associated with its EGCG content (Thielecke and Boschmann 2009).

Another study about green tea consumption (at least a cup of tea /day) demonstrates to be significantly related with decrease potential for caries. The main anticaries compounds are EGC which is in green tea and theaflavins and thearubigins which is oxidized complex polyphenols in black tea (Gazzani et al. 2012). There are also many studies about the relation between human health and black tea consumption such as prevention of some cancers, diabetic and cardiovascular diseases. Black tea phenolics may also show antimutagenic and anticlastogenic effects like green tea phenolics (Gupta et al. 2002 ; Kumar and Rizvi 2014).

Table 3.1 The possible positive health effects of tea phenolics

Type of Tea	The Possible Positive Health Effects of Tea Phenolics	Source
Green Tea	Anticarcinogenic effect Antihypertensive effect Antidiabetic effect Protection against paralysis Protection against metabolic syndrome Protection against cardiovascular disease Effects on oral and dental health Antiviral effect Protection against Parkinson and Alzheimer disease Antiaging effect	(Actis-Goretta et al. 2006) (Dufresne and Farnworth 2001) (Gazzani et al. 2013) (Koh et al. 2010) (Thielcike and Boschmann 2009) (Zaveri 2006)
Black Tea	Anticarcinogenic effect Antihypertensive effect Antidiabetic effect Antiinflammatory Protection against cardiovascular disease Effects on oral and dental health	(Actis-Goretta et al. 2006) (Dufresne and Farnworth 2001) (Gazzani et al. 2013) (Koh et al. 2010)

3.2.1 Antioxidant Properties of Tea

Phenolic compounds of tea are well-known for their high antioxidant activities. Bioactive properties especially antioxdiant activities of tea and its products have been researched on several occasions. Although comparison of antioxidant activities of green, oolong and black tea may show differences between the studies, generally antioxidant activity of green tea is stronger than black tea in literature. However, it is not approved to make general comparison as cultivar type, growth conditions and different technologies of the tea production may affect tea components (Carloni et al. 2013).

Tea protect against oxidative damage in humans and reduce the harm of reactive oxygen species including superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide and peroxynitrite. Green tea catechins have metal-

chelating properties and it contributes to their antioxidant acctivities. Effectiveness of antioxidant activity of catechins is associated with the chemical structure containing the close dihydroxy or trihydroxy structure which can chelate metal ions and provide high reactivity to quench free radicals. EGCG is most effective and high ability in reacting with most reactive oxygen species (Khan and Mukhtar 2007). EGCG has also protective effects in neurodegenerative diseases due to iron-chelating properties (Zaveri 2006). The effectiveness of the antioxidant potentials of the tea catechins as radical scavengers in the aqueous phase decrease respectively as epicatechin gallatte ≈ epigallocacethin gallate > eppigallocatechin > gallic acid > epicatechin ≈ catechin (Rice-Evans et al. 1996).

The effects of antioxidant properties of the black tea are generally associated with its flavonoid components; theaflavins, bisflavanols and theaflavic acids (Kumar and Rizvi 2014). Amount and position of hydroxyl groups of theaflavins structure existed in black tea have acted on the antioxidative properties and decrease respectively as $TF_3 > TF_2 > TF_1$. Besides, theaflavin gallates show stronger antioxidative properties compared with free theaflavins due to the amount of gallic acid residues (Luczaj and Skrzydlewska 2005).

3.2.2 Antidiabetic Properties of Tea

Polysaccharides particularly starch play an important role as energy supply in human diet. Starch is formed of two types of molecules; amylose which is bound to each other through α (1→4) glycosidic linkages and amylopectin which is polymer of α-1,4 linked-glucose molecules in a linear way and α-1,6 bound-glucose molecules at the branching sites. α-Amylase and α-glucosidase are the main enzymes in starch digestion. α-Amylase hydrolyzes the α-1,4 linkage of starches, but its impact is sterically blocked in the adjacency of α-1,6 branching sites. Maltose, maltotriose and α-dextrins are the products of α-amylase digestion. On the other hand, α-glucosidase catalyzes the hydrolysis of α-1,4 linkages in oligosaccharides and exhibits to hydrolyze α-1,6 bonds in α-dextrins (Figure 3.4). The major product of α-glucosidase digestion is glucose which is released into the bloodstream and increasing blood sugar levels for this reason. Some carbohydrate-containing foods cause rapidly digestion of carbohydrates when consumed. These foods called high glycemix index foods constitutes the risk of several

chronic diseases such as type 2 diabetes and obesity for people (Koh et al. 2010). Type 2 diabetes is in charge for more than 90% of all diabetes diseases (Tang et al. 2013). In addition to diabetic drugs such as acarbose, the consumption of some plant-based foods or supplements can retard starch digestion and reduce the glycemic index through acting as natural glucosidase inhibitors (Koh et al. 2010 ; Yilmazer-Musa et al. 2012). Many scientific studies have reported that tea is one of the source having great potential in preventing type 2 diabetes due to its flavonoid contents. Theaflavins and tea catechins especially EGCG play a role on inhibiting salivary α-amylase and α-glucosidase (Kao et al. 2006 ; Tang et al. 2013).

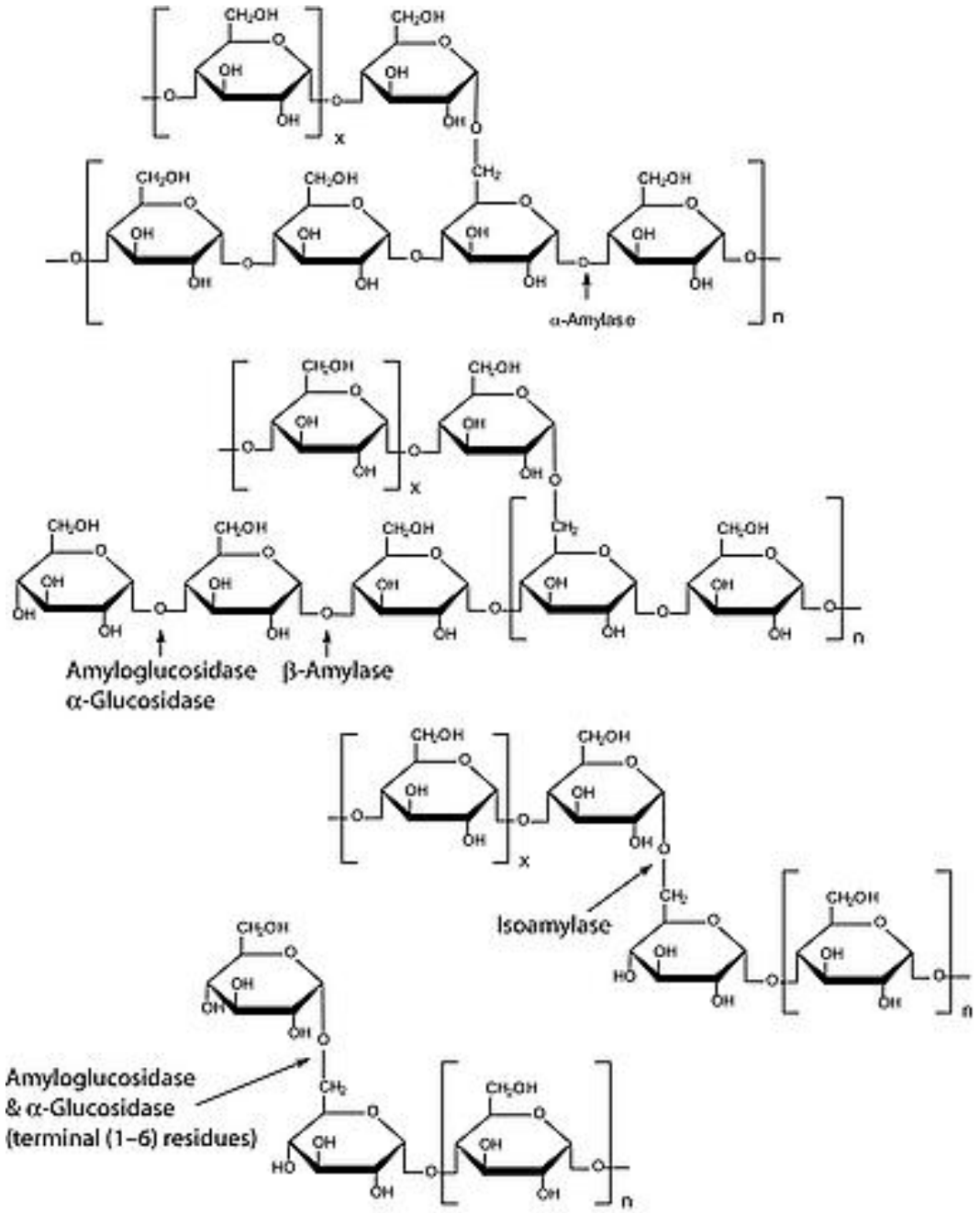


Figure 3.4 Polymers of α-(1-4)-D-glycopyranosyl units with α-(1-6)-D-glycopyranosyl branching (Source: (www.sigmaaldrich.com))

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

Kabuli Turkish type chickpeas were purchased from a Turkish market, green tea was purchased as 125g package (Çaykur, Zümrüt type), black tea was bought as 1kg package (Çaykur, Tiryaki type) from a Turkish market in İzmir. Green tea extract was provided by Rudolf Wild GmbH and Co KG (Berlin, Germany). Ethanol (Absolute), acetic acid (96%), di-sodium hydrogen phosphate, sodium carbonate, sodium hydroxide pellets, sodium chloride, sodium dihydrogen phosphate monohydrate and potassium peroxodisulfate were purchased from Merck KGaA (Darmstadt). ABTS (2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid), sodium nitrite, angiotensin-I-converting enzyme (ACE) from rabbit lung, N-[3-(2-Furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), 4 nitrophenyl R-D-glucopyranoside (PNPG), α-Glucosidase (AGH) from intestinal acetone powder from rat were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Aluminum chloride phosphate and Folin-Ciocalteu's phenol reagent were purchased from Fluka (Switzerland).

4.2 Methods

4.2.1 Determination of Phenolic Content, Antioxidant and Antidiabetic Potential of Tea Infusions and Solutions of Green Tea Extract

4.2.1.1 Preparation of Tea Infusions and Solutions of Green Tea Extract

Green tea was brewed at 80°C for 20 min in distilled water at the desired concentration (5% w/v) in waterbath. Identically, black tea was brewed at 90°C for 7.5 min in distilled water at the desired concentration (5% w/v) and then cooled to the room temperature. Green tea extract was dissolved at the desired concentration (1% w/v) at room temperature.

4.2.1.2 Determination of Total Phenolic Content of Tea Infusions and Solutions of Green Tea Extract

The phenolic content was determined using the method described by Singleton and Rossi (1965) by using Folin-Ciocalteu as reactive reagent. A 0.25 ml sample of in a fitting manner diluted aqueous was mixed with 1 ml of 1/10 diluted Folin-Ciocalteu reagent and incubated for 3 minutes. At the end of the incubation, 7.5% (w/v) Na_2CO_3 solution was added to the mixture and shaken. The absorbance of the sample was determined by a spectrophotometer at 765 nm after it was incubated for 2 hours at room temperature. Deionized water was used as control. The average of triplicate measurements was used to calculate the phenolic content as milligrams of catechin equivalents per 100 ml. Catechin was used for the preparation of standard curve.

4.2.1.3 Determination of Total Flavonoid Content of Tea Infusions and Solutions of Green Tea Extract

The flavonoid content was determined according to the method of Zhishen (1999). 0.20 ml sample of appropriately diluted aqueous was mixed with 0.075 ml of 5% $NaNO_2$ solution. After 5 minutes incubation, 0.075 ml of 10% $AlCl_3$ solution was added and incubated for 1 minute. 0.50 ml of 1M NaOH solution was added to the mixture for reaction termination and and the absorbance of the sample was determined by a spectrophotometer at 510 nm following dilution with 0.60 ml distilled water. The average of triplicate measurements was used to calculate the flavonoid content as milligrams of catechin equivalents per 100 mL. Catechin was used for the preparation of standard curve.

4.2.1.4 Determination of Free Radical Scavenging Capacity of Tea Infusions and Solutions of Green Tea Extract

The antioxidant activity of tea infusions and solution of extract was determined with spectro-photometric method (Shimadzu, Model 2450, Japan) according to the method of Re et al. (1999). The ABTS free radical cation solution was prepared by treating 7 mM ABTS solution with 2.45 mM potassium persulfate. The reaction mixture was formed by mixing 2 mL ABTS radical solution diluted with 75 mM phosphate buffered saline (PBS) containing 150 mM NaCl at pH 7.4 until its absorbance adjusted 0.68-0.720 units at 734 nm and 10, 20 and 30 μL of tea infusions and green tea extract samples. The decrease in absorbance was monitored for 6 min and recorded after 1, 2, 3, and 6 min. The results were calculated as area under the curve (AUC) values plotting the percent inhibition /concentration values for the samples and trolox separately against test periods. The slopes for each test period was used to create the curve and the division of the areas of curves for each samples to that of trolox was used to calculate the AUC value. The average of triplicate measurements was used to calculate antioxidant activity based on free radical scavenging and expressed as μmol trolox equivalents per 100 mL.

4.2.1.5 Determination of Antidiabetic Activity Including α-Glucosidase Inhibiton of Tea Infusions and Solutions of Green Tea Extract

The antidiabetic activity including α-glucosidase inhibition of tea infusions and extract was determined with spectrophotometric method (Shimadzu, UV-2600, Japan) according to the method of Koh et al. (2010) as acarbose equivalents. Mammalian α-glucosidase (AGH) mixture (16.5 mg/ml buffer) was centrifuged at 8760 x g at 4°C for 30 minutes after vortexing for 5 minutes and the top clear supernatant was utilized as live enzyme in the assay. A far amount of supernatant was treated at 100°C for 10 minutes to procure dead enzyme which is used both as control blank and sample blank instead of live enzyme. Buffer was used as control of the sample. 340 µl of sample was mixed with 20 µl of AGH and incubated at 37 °C for 10 minutes. Then 40 µl of PNPG solution was added to initiate the digestion to all vials. After 15 minutes, 200 µl of 1 M Na_2CO_3 was added for reaction termination. Determination of % inhibition of AGH was measured of absorbance at 400nm. The antidiabetic activity including α-glucosidase inhibition was expressed as mmol acarbose equivalents per 100 mL. All measurements were done as four times.

% α-Glucosidase inhibition = $[((A_{control} - A_{controlblank}) / (A_{sample} - As_{ampleblank})) / (A_{control} - A_{controlblank})] \times 100$

4.2.2 Controlled Rehydration of Chickpeas

25 g chickpeas were rehydrated in 100 ml distilled water to obtain control samples and rehydrated evenly in green tea infusion, black tea infusion and green tea extract solution to acquire functional chickpeas. Following this, they were incubated separately for each solution at 30°C for 2, 4, 6, 8 and 10 hours. At the end of the incubation, the weight of rehydrated chickpeas were measured to determine acquired liquid amount. The moisture content of rehydrated chickpeas were calculated with the acquired liquid amount since the initial moisture content of chickpeas was known.

4.2.3 Determination of Moisture Content of Chickpeas

To determine the moisture content of chickpeas, a method for the moisture and volatile matter content of oilseeds was used (ISO 2000). This test was applied at 103 ± 2°C in an oven at atmospheric pressure until practically constant mass is approached.

4.2.4 Determination of Phenolic Content, Antioxidant and Antidiabetic Potential of Chickpeas

4.2.4.1 Extraction of Phenolic Compounds from Chickpeas

To provide chickpeas extracts, 25 g rehydrated chickpeas were broken into pieces and homogenized with 150 ml 50% ethanol for 4 minutes with a Waring blender. The obtain paste was further homogenized with a homogenizator-disperser at 18000 rpm for 2 minutes and the the homogenate was filtered from synthetic cheesecloth. The obtained filtrate was centrifuged at 10 000 g (+4°C) for 15 minutes. The supernatant was collected and assayed for determination of phenolic content, antioxidant potential and other bioactive properties for soluble polyphenols.

4.2.4.2 Determination of Total Phenolic Content of Chickpeas

The total phenolic content of chickpeas was analysed by the method given in section 4.2.1.2 (Singleton and Rossi 1965). The extract of rehydrated chickpeas obtained by the method present in section 4.2.4.1 was used in the assay. The average of triplicate measurements was used to calculate the phenolic content as milligrams of catechin equivalents per 100 g dry matter of chickpeas.

4.2.4.3 Determination of Total Flavonoid Content of Chickpeas

The total flavonoid content of chickpeas was determined by the method given in section 4.2.1.3 (Zhishen et al. 1999). The extract of rehydrated chickpeas obtained by the method present in section 4.2.4.1 was used in the assay. The average of triplicate

measurements was used to calculate the flavonoid content as milligrams of catechin equivalents per 100 g dry matter of chickpeas.

4.2.4.4 Determination of Free Radical Scavenging Capacity of Chickpeas

The antioxidant activity of chickpeas was determined by the method given in section 4.2.1.4 (Re et al. 1999). The extract of rehydrated chickpeas obtained by the method present in section 4.2.4.1 was used in the assay. The average of triplicate measurements was used to calculate antioxidant activity based on free radical scavenging and expressed as μmol trolox equivalents per 100 g dry matter of chickpeas.

4.2.4.5 Determination of Antidiabetic Activity Including α-Glucosidase Inhibiton of Chickpeas

The antidiabetic activity including α-glucosidase inhibition of chickpeas was determined according to the method given in section 4.2.1.6 (Koh et al. 2010). The extract of rehydrated chickpeas obtained by the method present in section 4.2.4.1 was used in the assay. The antidiabetic activity was expressed as mmol acarbose equivalents per 100 g dry matter of chickpeas. All measurements were performed as four times.

4.2.5 Determination of Phenolic Content, Antioxidant and Antidiabetic Potential of Chickpea Protein

4.2.5.1 Extraction of Phenolic - Protein Complexes from Chickpeas

50 g rehydrated chickpeas were homogenized with 200 ml distilled water for 2 minutes with a Waring blender. The pH of the obtained homogenate was adjusted to 9.5 by 0.1 N NaOH solution to maximize protein solubility while stirring with a magnetic stirrer for 30 minutes. The homogenate was filtered from synthetic cheesecloth and centrifuged at 15 000 g (+4°C) for 20 minutes. The supernatant was collected and then the pH of the supernatant was adjusted to 4.5 with 1 N acetic acid solution to provide

the classical isoelectric precipitation (IEP) of the chickpea proteins. The paste were then centrifuged at 15000 x g (+4°C) for 20min and the precipitate was collected. After the precipitate was dissolved in deionized water to ensure solving other compounds, the pH of the slurry was adjusted to 4.5 again. The proteins were then precipitated by centrifugation at 15000 x g (+4°C) for 20min. The obtained proteins are suspended in distilled water and lyophilized after setting of its pH to 7.0.

4.2.5.2 Determination of Total Phenolic Content of Protein Extracts

The total phenolic content of protein extracts was tested by the method of Singleton and Rossi (1965) given in section 4.2.1.2. The lyophilized protein extract was dissolved in distilled water to use in the assay. To prepare solution, 0.1 g lyophilized protein was dissolved in 9.9 ml distilled water by stirring with a magnetic stirrer for 30 min at 30°C. After stirring, the solution was centrifuged at 8760 g (+4°C) for 30 min for clarification. The average of triplicate measurements was used to calculate the phenolic content as milligrams of catechin equivalents per lyophilized protein in 100 g dry matter of chickpeas.

4.2.5.3 Determination of Total Flavonoid Content of Protein Extracts

The total flavonoid content of protein extracts was determined by the method of Zhishen (1999) given in section 4.2.1.3. The lyophilized protein extract was dissolved in distilled water to use in the assay. To prepare solution, 0.1 g lyophilized protein was dissolved in 9.9 ml distilled water by stirring with a magnetic stirrer for 30 min at 30°C. After stirring, the solution was centrifuged at 8760 g (+4°C) for 30 min for clarification. The average of triplicate measurements was used to calculate the flavonoid content as milligrams of catechin equivalents per lyophilized protein in 100 g dry matter of chickpeas.

4.2.5.4 Determination of Free Radical Scavenging Capacity of Protein Extracts

The antioxidant activity based on free radical scavenging capacity of protein extracts was determined by the method of Re et al. (1999) given in section 4.2.1.4. The lyophilized protein extract was dissolved in distilled water to use in the assay. To prepare solution, 0.1 g lyophilized protein was dissolved in 9.9 ml distilled water by stirring with a magnetic stirrer for 30 min at 30°C. After stirring, the solution was centrifuged at 8760 g (+4°C) for 30 min for clarification. The average of triplicate measurements was used to calculate antioxidant activity based on free radical scavenging and expressed as μmol trolox equivalents per lyophilized protein in 100 g dry matter of chickpeas.

4.2.5.5 Determination of Antidiabetic Activity Including α-Glucosidase Inhibiton of Protein Extracts

The antidiabetic activity including α-glucosidase inhibition of chickpeas was determined according to the method of Koh et al. (2010) given in section 4.2.1.6. The lyophilized protein extract was dissolved in distilled water to use in the assay. To prepare solution, 0.1 g lyophilized protein was dissolved in 9.9 ml distilled water by stirring with a magnetic stirrer for 30 min at 30°C. After stirring, the solution was centrifuged at 8760 g (+4°C) for 30 min for clarification. The antidiabetic activity was expressed as mmol acarbose equivalents per lyophilized protein in 100 g dry matter of chickpeas. All measurements were performed as four times.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 Preparation and Characterization of Tea Infusions

Preparation of tea infusions were optimized according to the total phenolic and flavonoid contents of 5% (w/v) green and black tea infusions brewed at two different temperatures (80 and 90°C).

5.1.1 Effect of Different Soaking Time and Temperature on Total Phenolic Content of Tea Infusions

The total phenolic content of tea infusions is given in Table 5.1. The samples were assayed for both green and black tea infusions incubated for 2.5, 5, 7.5, 10 and 15. minutes at 90°C and 7.5, 15, 22.5 and 30. minutes at 80°C. The total phenolic content of green and black tea infusions ranged between 231.1 and 452.0 mg catechin / 100mL and 94.3 and 231.8 mg catechin / 100mL respectively. The results clearly showed that the total phenolic content of black tea infusion considerably increased when the black tea infusion was brewed at 90°C. In contrast, the total phenolic content of green tea infusions prepared at 80 and 90 °C were similar (Figure 5.1).

Table 5.1 Total phenolic content of green tea infusion and black tea infusion

Soaking time (minutes)	**Total phenolic content of tea infusions (mg catechin / 100mL)**			
	Green Tea Infusion		**Black Tea Infusion**	
	80°C	**90°C**	**80°C**	**90°C**
2.5	−	231.1 ± 1.7 e	−	98.3 ± 0.4 i
5	−	266.4 ± 3.0 d	−	159.9 ± 1.2 g
7.5	283.1 ± 2.3 d	306.9 ± 3.5 c	94.3 ± 8.3 i	204.9 ± 20.9 f
10		312.4 ± 1.9 c	−	215.8 ± 18.2 ef
15	366.4 ± 4.0 b	379.9 ± 1.8 b	131.2 ± 16.4 h	231.8 ± 2.7 e

(cont. on next page)

Table 5.1 (cont.) Total phenolic content of green tea infusion and black tea infusion

22.5	452.0 ± 28.8 a	–	136.4 ± 4.0 h	–
30	449.3 ± 1.5 a	–	161.6 ± 6.7 g	–

a-i: Different letters at each column indicate significantly different results at P<0.05.

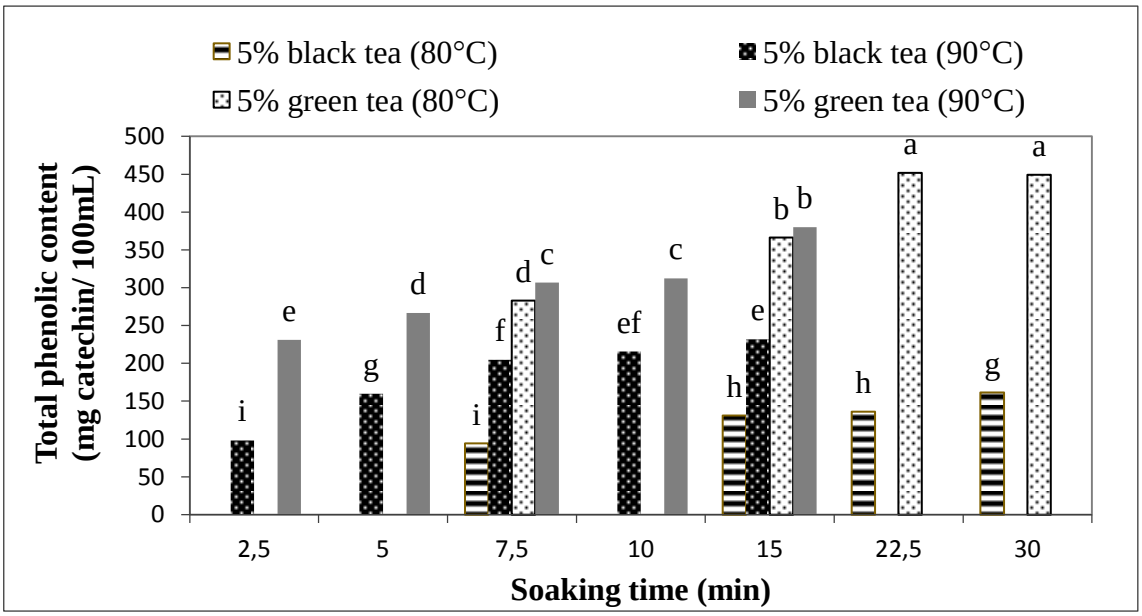


Figure 5.1 Total phenolic content of green tea infusion and black tea infusion. Different letters indicate significantly different results at P<0.05.

5.1.2 Effect of Different Soaking Time and Temperature on Total Flavonoid Content of Tea Infusions

The total flavonoid contents of tea infusions brewed for 2.5, 5, 7.5, 10 and 15. minutes at 90°C and 7.5, 15, 22.5 and 30. minutes at 80°C are given in Table 5.2. The total flavonoid content of green and black tea infusions ranged between 56.17 and 100.49 mg catechin / 100mL and 18.91 and 45.07 mg catechin / 100mL respectively. Similar to total phenolic content, the flavonoid contents of black tea infusions brewed at 90°C were significantly higher than those brewed at 80°C.

According to the total phenolic and flavonoid content of tea infusions, preparation conditions selected for the infusions were 90 °C for 7.5 minutes for the black tea infusion and 80°C for 20 minutes for the green tea infusion.

Table 5.2 Total flavonoid content of green tea infusion and black tea infusion

Soaking time (minutes)	Total flavonoid content of tea infusions (mg catechin / 100mL)			
	Green Tea Infusion		Black Tea Infusion	
	80°C	90°C	80°C	90°C
2.5	–	56.17 ± 3.45 e	–	23.07 ± 0.17 ij
5	–	64.75 ± 0.95 d	–	32.37 ± 1.30 h
7.5	68.15 ± 1.23 d	66.54 ± 5.14 d	18.91 ± 0.30 j	38.96 ± 0.93 g
10	–	73.09 ± 2.13 c	–	40.89 ± 0.84 fg
15	85.19 ± 7.20 b	87.65 ± 2.03 b	24.19 ± 0.39 i	45.07 ± 1.96 f
22.5	100.19 ± 0.77 a	–	25.85 ± 0.39 i	–
30	100.49 ± 4.45 a	–	31.04 ± 0.78 h	–

a-j: Different letters at each column indicate significantly different results at P<0.05.

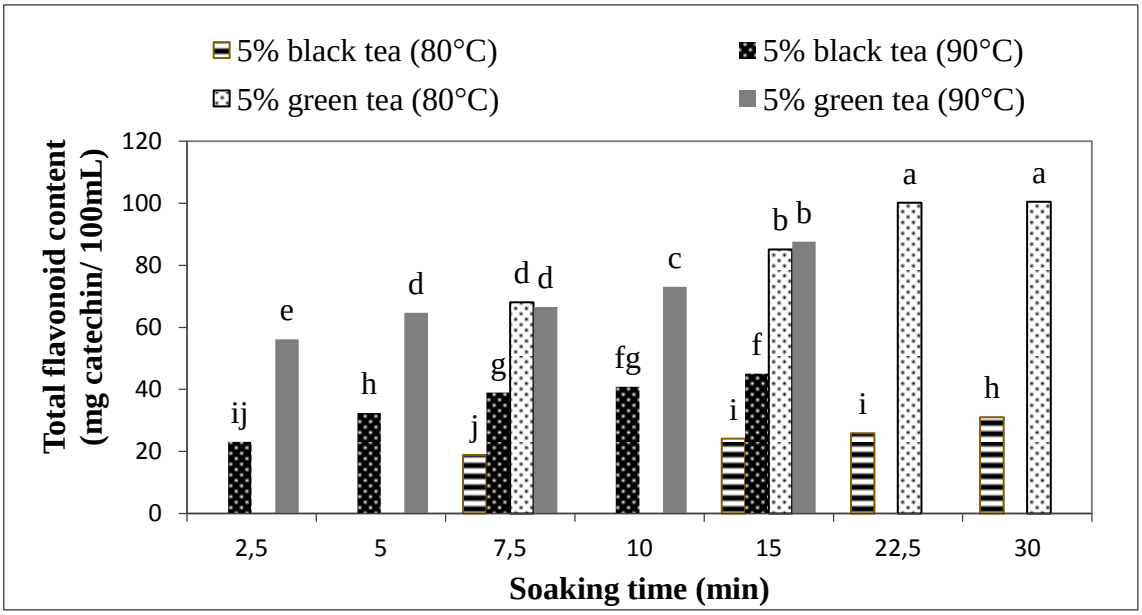


Figure 5.2 Total flavonoid content of green tea infusion and black tea infusion. Different letters indicate significantly different results at P<0.05.

5.2 Comparison of Phenolic Contents, Antioxidant Activities and Antidiabetic Activities of Prepared Tea Infusions with Commercial Green Tea Extracts

Total phenolic and flavonoids contents and free radical scavenging capacities of prepared infusions were compared with those of commercial green tea extracts.

5.2.1 Total Phenolic and Flavonoid Contents of Prepared Tea Infusions and Commercial Green Tea Extract

Total phenolic and flavonoid contents of tea infusions and 1% solutions of green tea extract were given in Table 5.3. The total phenolic and flavonoid contents of black tea infusion, green tea infusion and 1% solution of green tea extracts were determined as 262.6, 44.2 and 270.9 mg catechin / 100 ml and 58.3, 105.4 and 70.4 mg catechin / 100 ml respectively. These results clearly showed that both total phenolic and flavonoid contents of green tea infusion were quite higher than those of black tea infusion and 1% solution of green tea extract. The prepared black tea infusion and 1% solution of green tea extract had almost the same amount of total phenolic content (Figure 5.3), but total flavonoid content of green tea extract solution was almost 17% higher than that of black tea infusion (Figure 5.4).

Table 5.3 Total phenolic and flavonoid content of tea infusions and 1% solution of green tea extract

Solutions	**Total phenolic content (mg catechin /100 ml)**
Black tea infusion	262.6 ± 7.4 a
Solution of green tea extract	270.9 ± 1.6 a
Green tea infusion	440.2 ± 9.5 b
	Total flavonoid content (mg catechin / 100 ml)
Black tea infusion	58.3 ± 1.3 a
Solution of green tea extract	68.1 ± 3.9 b
Green tea infusion	105.4 ± 3.0 c

a-c: Different letters at each column indicate significantly different results at $P<0.05$.

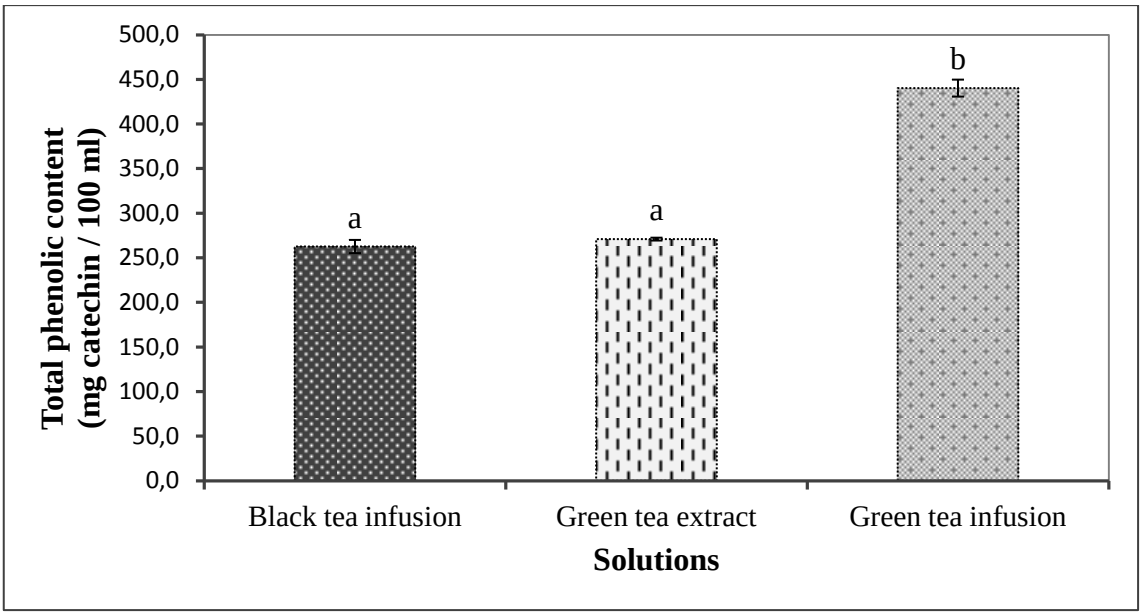


Figure 5.3 Total phenolic contents of tea infusions and 1% solution of green tea extract. Different letters indicate significantly different results at P<0.05.

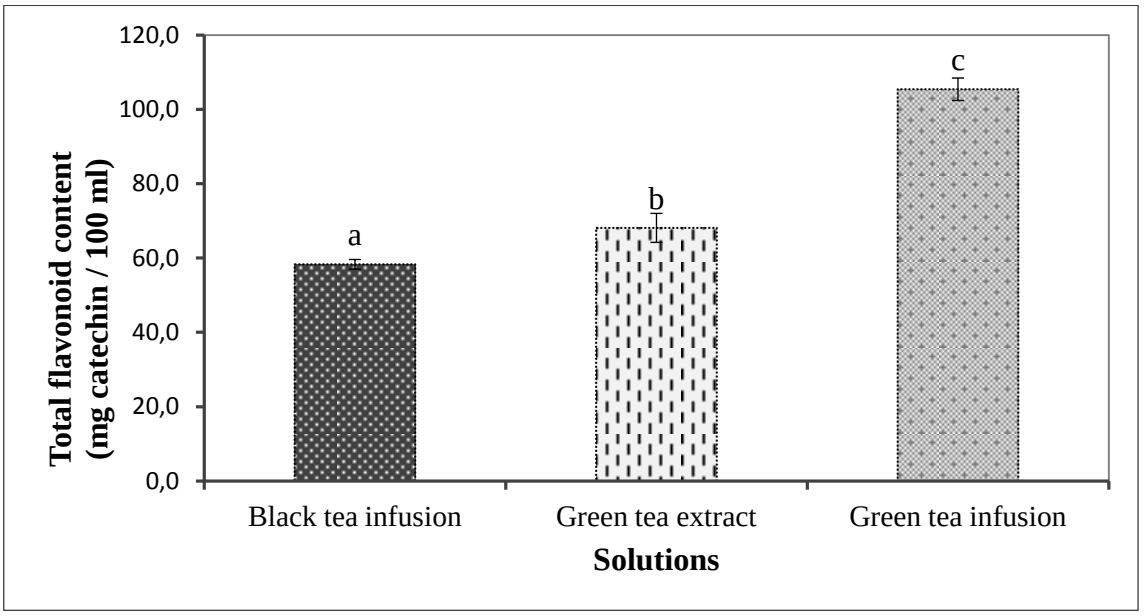


Figure 5.4 Total flavonoid contents of tea infusions and 1% solution of green tea extract. Different letters indicate significantly different results at P<0.05.

5.2.2 Free Radical Scavenging Based Antioxidant Capacity of Prepared Tea Infusions and Commercial Green Tea Extract

The antioxidant activities based on free radical scavenging capacity of tea infusions and 1% solution of commercial green tea extract were given in Table 5.4. The antioxidant activities of black tea infusion, green tea infusion and solution of green tea extract were determined as 3428, 6351 and 3727 μmol Trolox / 100 ml, respectively. As seen from the results, the highest free radical scavenging capacity was observed for green tea infusion. Black tea infusion and solution of green tea extract showed very similar free radical scavenging capacities. Thus, it appeared that there are high correlations among total phenolic content, total flavonoid content and free radical scavenging capacities of tea infusions and solution of green tea extract.

Table 5.4 Free radical scavenging based antioxidant capacities of tea infusions and 1% solution of green tea extract

Solutions	**Free radical scavenging capacity (μmol Trolox / 100 ml)**
Black tea infusion	3428 ± 112 a
Solution of green tea extract	3727 ± 107 b
Green tea infusion	6351 ± 28 c

a-c: Different letters at the column indicate significantly different results at P<0.05.

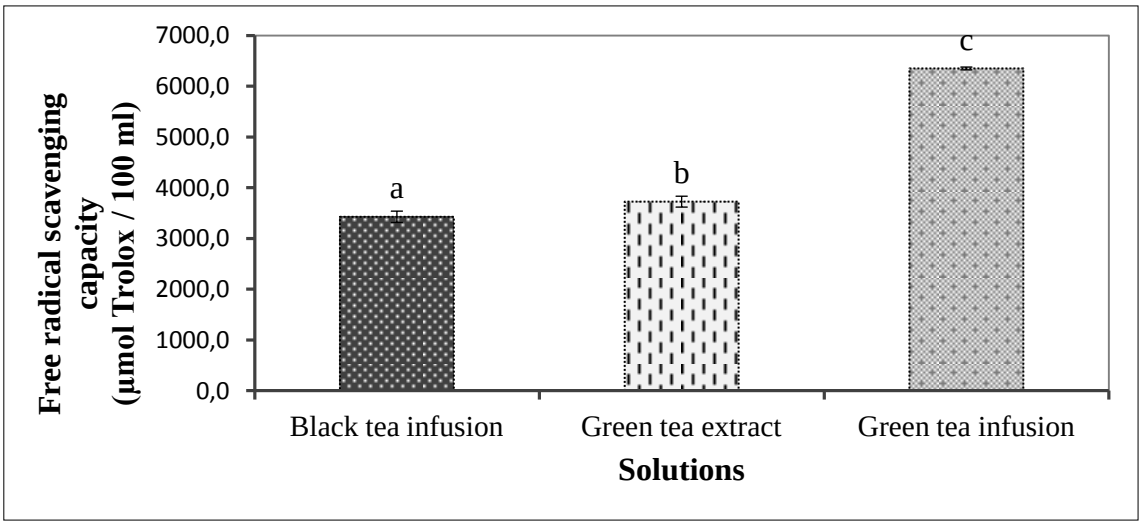


Figure 5.5 Free radical scavenging based antioxidant capacities of tea infusions and 1% solution of green tea extract. Different letters indicate significantly different results at P<0.05.

5.2.3 Antidiabetic Activity of Prepared Tea Infusions and Commercial Green Tea Extract

Antidiabetic activities of prepared tea infusions and 1% solution of commercial green tea extract were determined by assaying their capacity to inhibit α-glucosidase enzyme. The antidiabetic activities of 1% solution of green tea extract and prepared green tea infusion and black tea infusion were determined as 18.69, 42.56 and 75.31 mmol Acarbose / 100 ml, respectively. Interestingly the black tea infusion which had the lowest total phenolic content showed the highest antidiabetic activity. Thus, it seemed that not the total content of phenolics, but the composition of phenolics is important for the antidiabetic activity. In fact, this was expected since Koh et al. (2010) reported that theaflavins found in black tea are better inhibitors of α-glucosidase than catechins found in the green tea.

Table 5.5 Antidiabetic activities of tea infusions and 1% solution of green tea extract

Solutions	Antidiabetic Activity (mmol Acarbose / 100 ml)
Solution of green tea extract	18.69 ± 3.46 a
Green tea infusion	42.56 ± 5.87 b
Black tea infusion	75.31 ± 3.74 c

a-c: Different letters at the column indicate significantly different results at P<0.05.

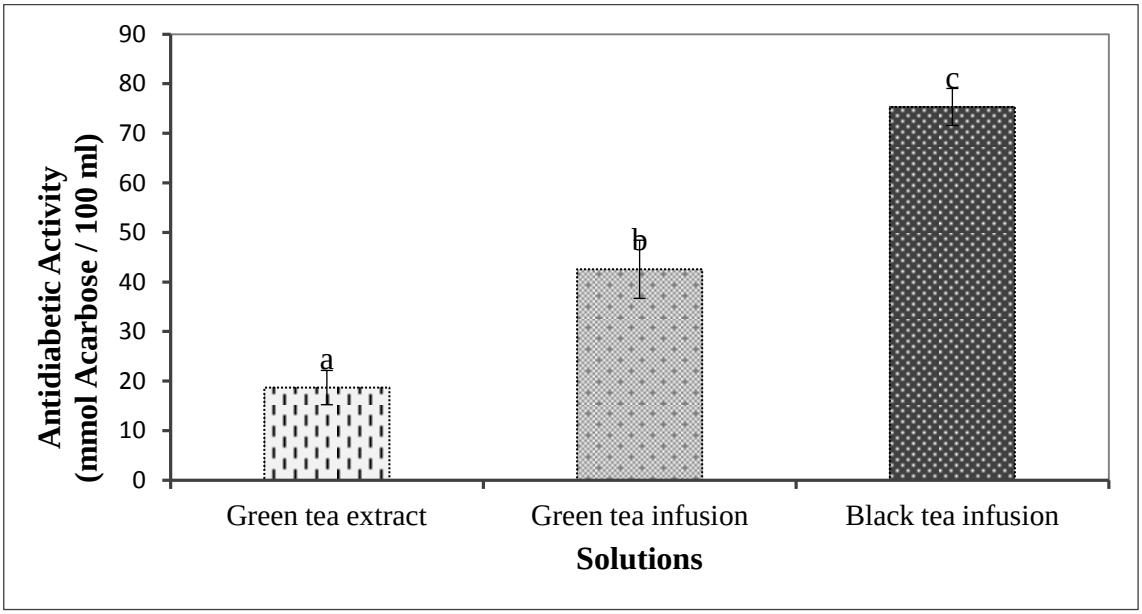


Figure 5.6 Antidiabetic activities of tea infusions and 1% solution of green tea extract. Different letters indicate significantly different results at P<0.05.

5.3 Rehydration Profiles of Chickpeas

In this study, the rehydration kinetics of chickpeas was also investigated in water, tea infusions and green tea extract at 30°C during 10h incubation period (Figure 5.7).

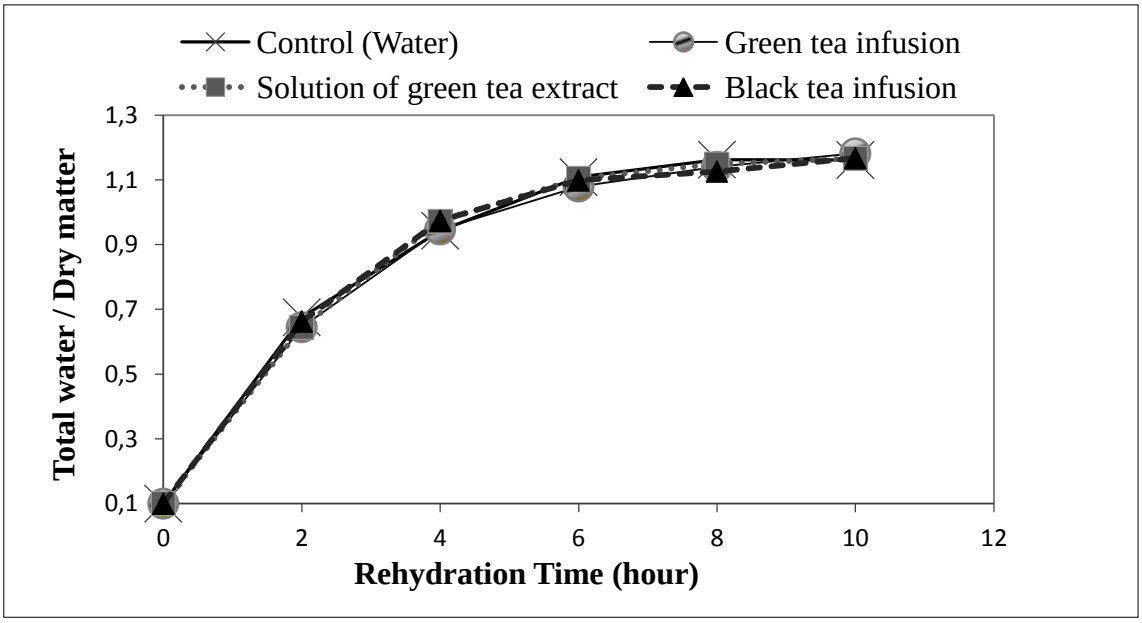


Figure 5.7 The rehydration curves of chickpeas in water, tea infusions and green tea extract at 30°C

Table 5.6 Moisture content and ratio of total water to dry matter of rehydrated chickpeas

Time (hour)	Rehydration medium							
	Control (Water)		Black Tea Infusion		1% Solution of Green Tea Extract		Green Tea Infusion	
	Moisture (%)	Total water / Dry matter	Moisture (%)	Total water / Dry matter	Moisture (%)	Total water / Dry matter	Moisture (%)	Total water / Dry matter
0	9.14	0.1	9.14	0.1	9.14	0.1	9.14	0.1
2	40.31	0.68	39.86	0.66	39.20	0.64	39.20	0.64
4	48.53	0.94	49.34	0.97	49.29	0.97	48.64	0.95
6	52.57	1.11	52.35	1.10	52.49	1.10	51.91	1.08
8	53.76	1.16	52.96	1.13	53.46	1.15	53.28	1.14
10	53.73	1.16	53.86	1.17	53.84	1.17	54.17	1.18

As shown in Figure 5.8, the rehydration profiles obtained were almost same for all mediums. The results showed that chickpeas rehydrated rapidly in the first two hours. In fact, the total weight of chickpeas increased 50.6% on average at the end of two hours. The rehydration rate slowed down considerably starting from 6th hour and it was almost ended at the 8th hour.

5.4. The Use of Rehydration Process in Phenolic Rich Medium as a Tool for Phenolic Enrichment of Chickpeas

5.4.1 Effect of Rehydration in Tea Infusions and Green Tea Extract on Phenolic Content, Antioxidant activity and Antidiabetic activity of Chickpeas

The antioxidant potential of chickpeas were determined by testing the total phenolic and flavonoid content and antioxidant activity based on free radical scavenging capacity. After the determination of total phenolic and flavonoid contents and antioxidant potential of chickpeas for five different rehydration times, further test were conducted on selected chickpeas to characterize their antidiabetic activity.

5.4.1.1 Total Phenolic Content of Chickpeas

The total phenolic contents were determined for control chickpeas and for chickpeas rehydrated in three different phenolic rich solutions (green and black tea infusions and 1% solution of green tea extract) for different incubation periods (2-10 hours) (Table 5.7). The total phenolic content in chickpeas rehydrated in black tea infusion, 1% solution of green tea extract and green tea infusion ranged between 164.6 and 196.9, 256.1 and 369.1, 235.4 and 444.6 mg catechin / 100g dry matter of chickpeas, respectively.

Table 5.7 Total phenolic contents of chickpeas rehydrated in different medium

Rehydration Time (hour)	Total phenolic content (mg catechin / 100g chickpeas (d.m.))			
	Control (Water)	Black Tea Infusion	1% solution of Green Tea Extract	Green Tea Infusion
2	97.8 ± 1.7 a	164.6 ± 1.2 d	256.1 ± 2.5 g	235.4 ± 2.8 l
4	112.8 ± 1.2 b	179.2 ± 0.5 e	287.6 ± 5.1 h	332.2 ± 1.8 j
6	98.2 ± 0.2 a	179.4 ± 1.7 e	302.2 ± 5.5 i	337.5 ± 7.1 j
8	112.7 ± 1.9 b	196.9 ± 1.3 f	334.3 ± 5.9 j	358.9 ± 1.7 m
10	128.3 ± 4.0 c	185.5 ± 2.5 e	369.1 ± 2.0 k	444.6 ± 10.5 n

a-n: Different letters at each column indicate significantly different results at P<0.05.

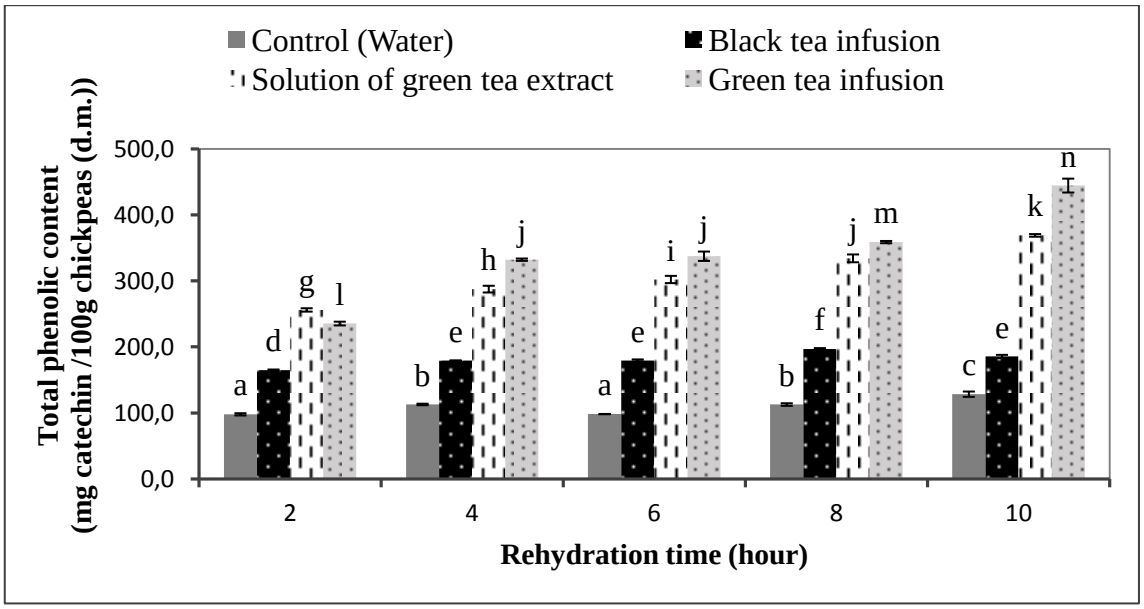


Figure 5.8 Total phenolic contents of chickpeas rehydrated in different medium. Different letters at the column indicate significantly different results at P<0.05.

As seen in Figure 5.8, total phenolic contents of chickpeas rehydrated in tea infusions and solution of green tea extract all had significantly higher total phenolic contents than control chickpeas rehydrated in water. The total phenolic content of chickpeas rehydrated in solution of green tea extract and green tea infusion increased considerably as rehydration period was increased from 2 to 10 h. In contrast, the total phenolic content of chickpeas rehydrated in black tea infusion did not increase considerably by increase of rehydration period (Figure 5.8). It is important to note that the increase in total phenolic content is only 68% for chickpeas rehydrated in black tea infusion while the total phenolic content of chickpeas rehydrated in green tea infusion

and solution of green tea extract increased almost 150%. On the other hand, at the end of 10 hour rehydration period the total phenolic contents of chickpeas rehydrated in black tea infusion, solution of green tea extract and green tea infusion were almost 44.5%, 188% and 247% higher than that of chickpeas rehydrated in water.

5.4.1.2 Total Flavonoid Content of Chickpeas

The results of total flavonoid contents of chickpeas rehydrated in water (control) and three different phenolic rich solution (tea infusions and solution of green tea extract) at 2, 4, 6, 8 and 10 hour are given in Table 5.8. The total flavonoid content in chickpeas rehydrated in black tea infusion, 1% solution of green tea extract and green tea infusion ranged between 56.3 and 78.2, 81.9 and 117.5, 103.3 and 123.8 mg catechin / 100g dry matter of chickpeas, respectively.

Table 5.8 Total flavonoid contents of chickpeas rehydrated in different medium

Rehydration Time (hour)	**Total flavonoid content (mg catechin / 100g chickpeas (d.m.))**			
	Control (Water)	**Black Tea Infusion**	**1% Solution of Green Tea Extract**	**Green Tea Infusion**
2	39.5 ± 1.9 a	56.3 ± 2.9 cf	92.5 ± 2.0 g	103.3 ± 1.7 j
4	46.7 ± 2.7 b	78.2 ± 1.9 e	81.9 ± 2.1 e	104.7 ± 1.4 j
6	43.7 ± 2.8 b	57.4 ± 1.7 f	102.5 ± 1.2 hj	105.8 ± 4.3 j
8	52.7 ± 2.4 c	64.6 ± 2.2 d	99.0 ± 4.6 h	116.8 ± 3.2 i
10	66.1 ± 0.6 d	59.0 ± 1.5 f	117.5 ± 2.6 i	123.8 ± 3.7 k

a-k: Different letters at each column indicate significantly different results at $P<0.05$.

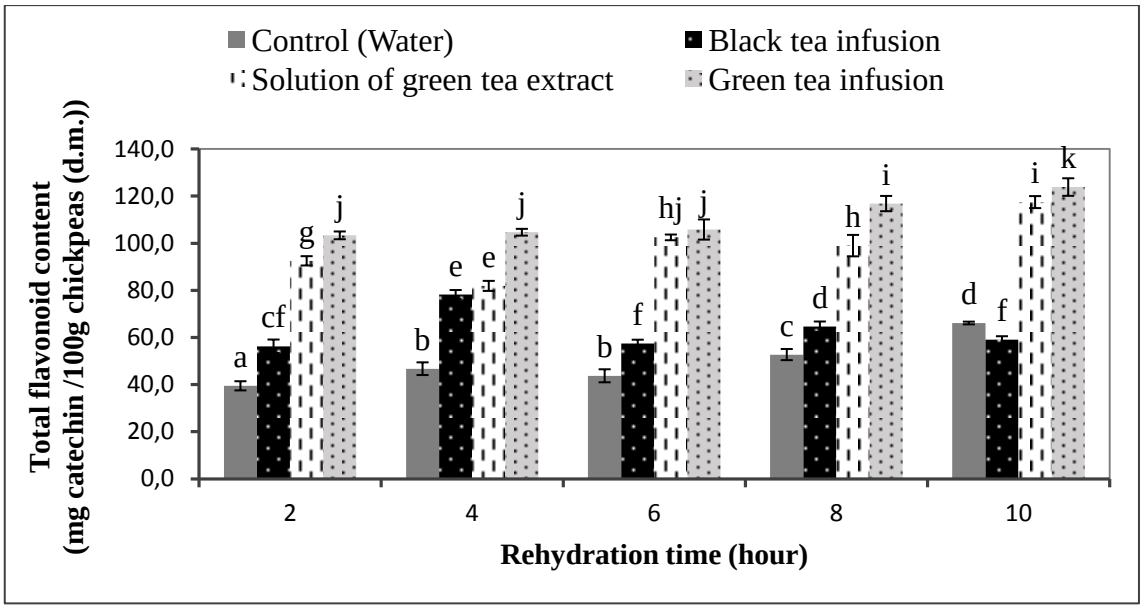


Figure 5.9 Total flavonoid contents of chickpeas rehydrated in different medium. Different letters indicate significantly different results at $P<0.05$.

As shown in Figure 5.9, the total flavonoid content of chickpeas rehydrated in green tea infusion did not change between 2th and 6th hours, but it slight increased at 8th and 10th hours. In contrast, the flavonoid content of chickpeas rehydrated in black tea infusion and solution of green tea extract showed some fluctuations. These results clearly showed that the diffusion of flavonoids from tea infusions and green tea extracts to chickpeas occurred rapidly within the first two hours. The increased rehydration periods caused slight increases or reductions in flavonoid content of chickpeas due to reversible diffusion of flavonoids from the chickpeas during formation of equilibrium concentration for the flavonoids.

Rehydration of chickpeas in black tea infusion, solution of green tea extract and green tea infusion caused almost 43%, 134% and 162% increase in total flavonoid content within 2 hour respectively. The increase in total flavonoid content of chickpeas rehydrated 10h in solution of green tea extract and green tea infusion was almost 180%. In contrast, the total flavonoid content of chickpeas rehydrated 10h in black tea infusion was slightly lower than that for the control chickpeas (Figure 5.10). This result clearly showed the possible condensation and insolubilization of blacktea flavonoids in 10h rehydrated chickpea samples.

5.4.1.3 Free Radical Scavenging Based Antioxidant Activity of Chickpeas

The antioxidant activities based on free radical scavenging capacity of chickpeas are given in Table 5.9. Free radical scavenging capacities of chickpeas rehydrated in black tea infusion, solution of green tea extract and green tea infusion ranged between 1931 and 2316, 3065 and 4163, 2724 and 5422 μmol Trolox / 100g dry matter of chickpeas, respectively.

Table 5.9 Free radical scavenging based antioxidant activity of chickpeas rehydrated in different medium

Rehydration Time (hour)	**Free radical scavenging capacity (μmol Trolox / 100g chickpeas (d.m.))**			
	Control (Water)	**Black Tea Infusion**	**Solution of Green Tea Extract**	**Green Tea Infusion**
2	1242 ± 35 a	1990 ± 88 cd	3065 ± 124 f	2724 ± 48 i
4	1165 ± 28 a	2088 ± 15 c	3667 ± 159 g	4010 ± 13 j
6	1250 ± 37 a	1931 ± 24 d	4076 ± 120 hj	4468 ± 123 k
8	1152 ± 29 a	2316 ± 13 e	3539 ± 153 g	5060 ± 46 l
10	1395 ± 42 b	2115 ± 91 c	4163± 150 h	5422 ± 61 m

a-m: Different letters at each column indicate significantly different results at P<0.05.

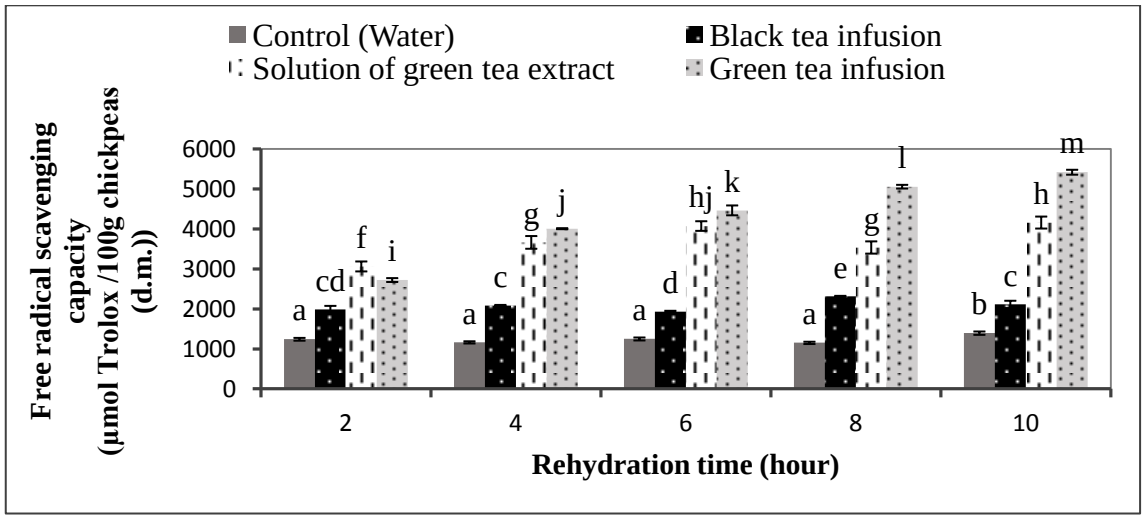


Figure 5.10 Free radical scavenging based antioxidant activity of chickpeas rehydrated in different medium. Different letters at each column indicate significantly different results at P<0.05.

Antioxidant activities of chickpeas rehydrated in phenolic rich solutions were significantly higher than those of control chickpeas rehydrated in water. The antioxidant activity of chickpeas rehydrated in green tea infusion increased by increasing rehydration period from 2 to 10 hour, but the antioxidant activity of chickpeas rehydrated in solution of commercial green tea extract and black tea infusion showed some slight fluctuations. For the chickpeas rehydrated in commercial green tea extract, the antioxidant activity increased between 2^{nd} and 6^{th} hours of rehydration, but a slight drop and a following slight increase occurred in the antioxidant activity when chickpeas were rehydrated in this phenolic rich medium for 8 and 10 hours, respectively. In contrast, it is hard to report any considerable change in antioxidant activity by increase of rehydration time of chickpeas incubated in black tea infusion. Thus, it is once more proved that the 2h rehydration period is a sufficient time to incorporate black tea phenolic compounds into chickpeas. It is important to report that the antioxidant activity of chickpeas rehydrated in black tea infusion, solution of green tea extract and green tea infusion increased almost 1.6, 2.5 and 2.2 fold within 2 hour rehydration period, respectively. Moreover, should also be reported that the rehydration of chickpeas in black tea infusion, solution of green tea extract and green tea infusion caused almost 1.5, 3 and 4 fold increases in antioxidant activity within 10 hours of rehydration, respectively (Figure 5.10).

5.4.1.4 Antidiabetic Activity of Chickpeas

Antidiabetic activities determined by assaying α-glucosidase inhibition of extracts obtained from chickpeas rehydrated in different phenolic rich medium are given in Table 5.10. Different phenolic rich solutions caused almost 2.2 – 2.6 fold increase in antidiabetic activity of chickpeas within a 2 hour rehydration period. For chickpeas rehydrated in black tea infusion, almost 35% increase was also observed in antidiabetic activity within 10 hour rehydration period. In contrast, the increase of rehydration period from 2 to 10h caused almost no differences among antidiabetic activities of control chickpeas rehydrated in water and chickpeas rehydrated in green tea infusion and solution of green tea extract. This result suggests the neutralization of antidiabetic phenolic compounds in green tea infusion and green tea extract by the extended rehydration period (10h). It was possible that the neutralization of antidiabetic activity

in indicated samples was due to the complexes formed between antidiabetic phenolic compounds in green tea infusion and extract, and chickpea proteins or polysaccharides. Alternatively, it is also possible that the antidiabetic phenolic compounds underwent some polymerization reactions which cause neutralization of their antidiabetic activities.

Table 5.10 Antidiabetic activity of chickpeas rehydrated in different medium

Rehydration time (hour)	**Antidiabetic activity (mmol Acarbose / 100g chickpeas (d.m.))**			
	Control (Water)	**Solution of Green Tea Extract**	**Black Tea Infusion**	**Green Tea Infusion**
2	112.2 ± 29.8 a	249.0 ± 24.7 b	273.0 ± 21.5 b	292.3 ± 25.8 bc
10	344.6 ± 32.1 cd	383.0 ± 58.4 d	465.2± 7.3 e	342.3 ± 9.1 cd

a-e: Different letters at each column indicate significantly different results at P<0.05.

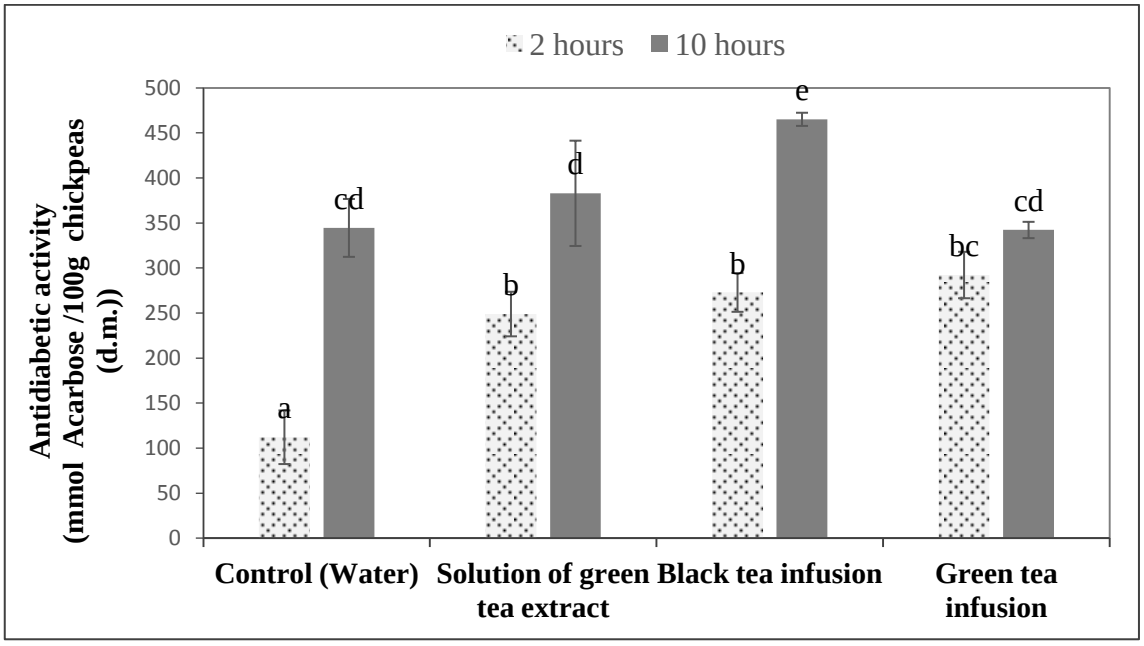


Figure 5.11 Antidiabetic activity of chickpeas rehydrated in different medium. Different letters indicate significantly different results at P<0.05.

5.5. Effect of Drying and Refrigerated Storage on Phenolic content and Antioxidant Activity of Phenolic Enriched Chickpeas

Chickpeas rehydrated in the solution of green tea extract, black tea infusion and green tea infusion were dried at 80°C for 5 hours following rehydration in these

medium for 2 hours. The samples were then stored at 4°C for 3 months. Total phenolic content of samples rehydrated in black tea and green tea infusion showed almost 25% decrease after drying. In contrast, chickpeas rehydrated in solution of green tea extract were stable during drying. Interestingly, total phenolic content of chickpeas rehydrated in black tea and green tea infusion showed no decrease during storage, while total phenolic content of chickpeas rehydrated in solution of green tea extract decreased almost 16% at the end of the storage. These results suggested the high thermal stability and high storage stability of commercial green tea phenolics and tea infusion (green or black) phenolics, respectively. It is also important to note that the chickpea phenolics are also highly stable since total phenolic content of control chickpea sample did not change both after drying and at the end of the storage (Table 5.11).

Table 5.11 Effect of drying and storage on total phenolic content of chickpeas rehydrated 2 hours in different phenolic rich medium

Processes applied in sequence	**Rehydration medium / Total phenolic content (mg catechin / 100g chickpeas (d.m.))**			
	Control (Water)	**Black Tea Infusion**	**Solution of Green Tea Extract**	**Green Tea Infusion**
1.Rehydration (for 2h)	115.3 ± 2.4 a	176.0 ± 2.5 b	199.5 ± 6.3 e	304.2 ± 2.5 g
2. Drying (at 80°C for 5h)	109.9 ± 5.3 a	131.4 ± 8.7 c	218.7 ± 20.8 f	233.4 ± 5.2 h
3.Storage (at 4°C for 3 months)	108.5 ± 10.5 a	150.9 ± 5.1 d	184.0 ± 9.3 b	256.6 ± 0.7 i

a-i: Different letters at each column indicate significantly different results at $P<0.05$.

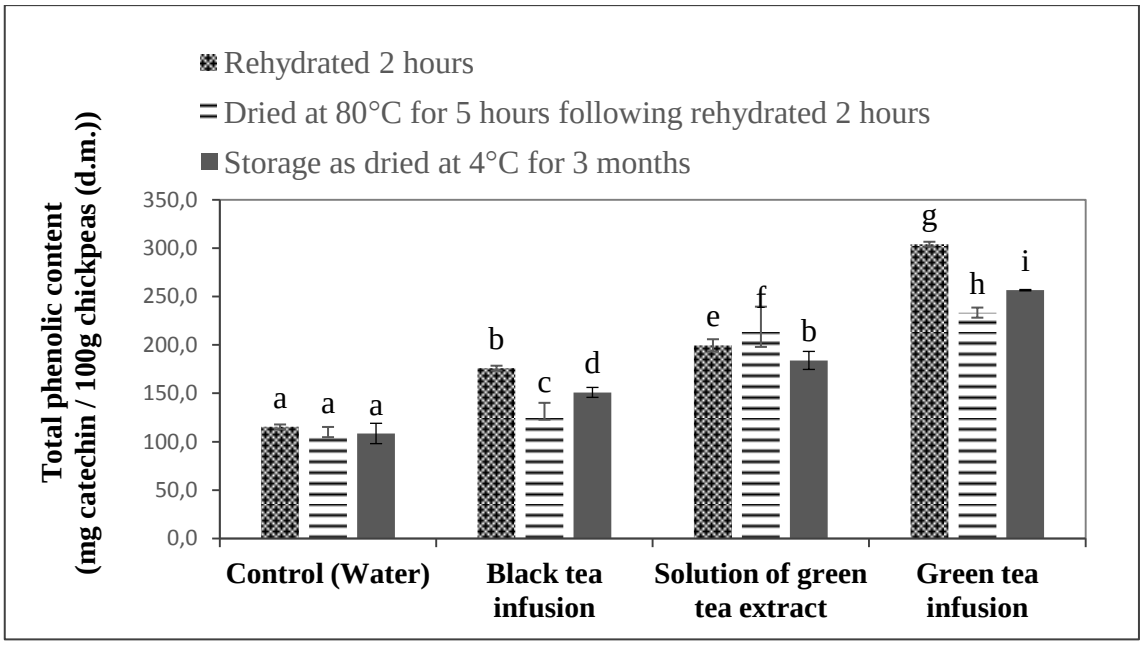


Figure 5.12 Effect of drying and storage on total phenolic content of chickpeas rehydrated 2 hours in different phenolic rich medium. Different letters indicate significantly different results at $P<0.05$.

As shown in Figure 5.13, total flavonoid content of chickpeas rehydrated in solution of green tea extract and black tea infusion were more stable than that of chickpeas rehydrated in green tea infusion. The total flavonoid content of the chickpeas rehydrated in green tea infusion was reduced by almost 30% after drying. Interestingly, total flavonoid content of control samples increased almost 2 fold after drying. It appeared that the temperature applied during drying caused the release of some protein-bound or polysaccharide-bound chickpea flavonoids and this caused increase of free flavonoid content. It is also possible that the condensed flavonoids in control chickpeas are destabilized by the heating applied during drying and this affected the detectability of chickpea flavonoids.

At the end of the 3 months, total flavonoid content in control chickpea samples decreased almost 2 fold. Thus, it appeared that the released flavonoids in control by heating are not stable. In contrast, total flavonoid content of chickpeas rehydrated in phenolic rich medium increased slightly at the end of the storage. The increased phenolic content of phenolic enriched chickpeas could be explained simply by increased solubility of incorporated phenolics during storage.

Table 5.12 Effect of drying and refrigerated storage on total flavonoid content of chickpeas rehydrated 2 hours in different phenolic rich medium

Processes applied in sequence	**Rehydration medium / Total flavonoid content (mg catechin / 100g chickpeas (d.m.))**			
	Control (Water)	**Black Tea Infusion**	**Solution of Green Tea Extract**	**Green Tea Infusion**
1.Rehydration (for 2h)	19.86 ± 0.75 a	36.21 ± 2.04 b	46.88 ± 2.35 e	75.64 ± 3.53 g
2. Drying (at 80°C for 5h)	36.50 ± 0.33 b	33.29 ± 2.65 c	44.76 ± 1.67 e	53.14 ± 1.05 f
3.Storage (at 4°C for 3 months)	16.96 ± 0.99 a	40.29 ± 1.08 d	52.73 ± 2.28 f	59.18 ± 0.19 h

a-h: Different letters at each column indicate significantly different results at $P<0.05$.

The effect of drying and storage on antioxidant activity of chickpeas showed some differences. For example, the drying increased the antioxidant activities of control chickpeas rehydrated in water and chickpeas rehydrated in green tea extract slightly (Table 5.13). A slight reduction was observed in the antioxidant activity of chickpeas rehydrated in black tea infusion by drying. However, at the end of 3 months storage the antioxidant activity of indicated chickpea samples were almost same with their initial antioxidant activity. It was only the chickpeas rehydrated in green tea infusion that showed a slight decline both after drying and storage. However, it is important to report that there are no major losses in antioxidant activity by drying and storage, thus, drying could be a potential method to obtain shelf-life stable phenolic enriched chickpeas.

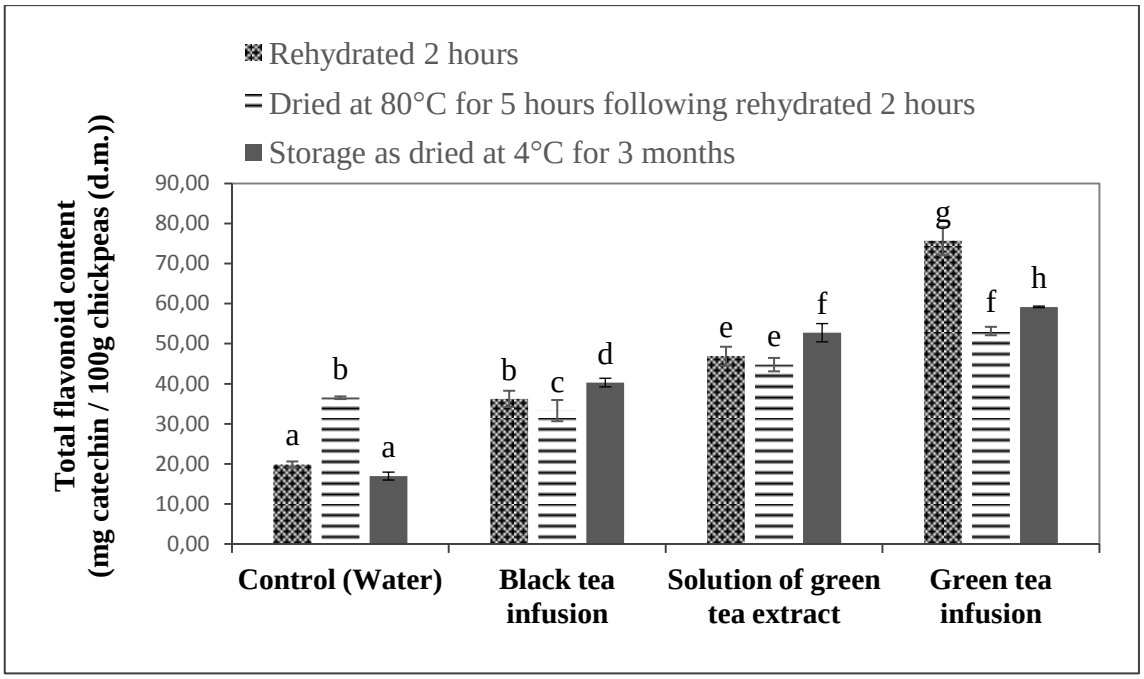


Figure 5.13 Effect of drying and refrigerated storage on total flavonoid content of chickpeas rehydrated 2 hours in different phenolic rich medium. Different letters indicate significantly different results at P<0.05.

Table 5.13 Effect of drying and refrigerated storage on free radical scavenging based antioxidant activity of chickpeas rehydrated 2 hours in different phenolic rich medium

Processes applied in sequence	**Rehydration medium / Free radical scavenging capacity (µmol Trolox / 100g chickpeas (d.m.))**			
	Control (Water)	**Black Tea Infusion**	**Solution of Green Tea Extract**	**Green Tea Infusion**
1.Rehydration (for 2h)	1344 ± 61 a	1856 ± 20 c	2091 ± 64 e	3820 ± 63 f
2. Drying (at 80°C for 5h)	1542 ± 57 b	1691 ± 37 d	2462 ± 102 d	3258 ± 87 g
3.Storage (at 4°C for 3 months)	1286 ± 19 a	1837 ± 36 c	2040 ± 17 e	3137 ± 62 h

a-h: Different letters at each column indicate significantly different results at P<0.05.

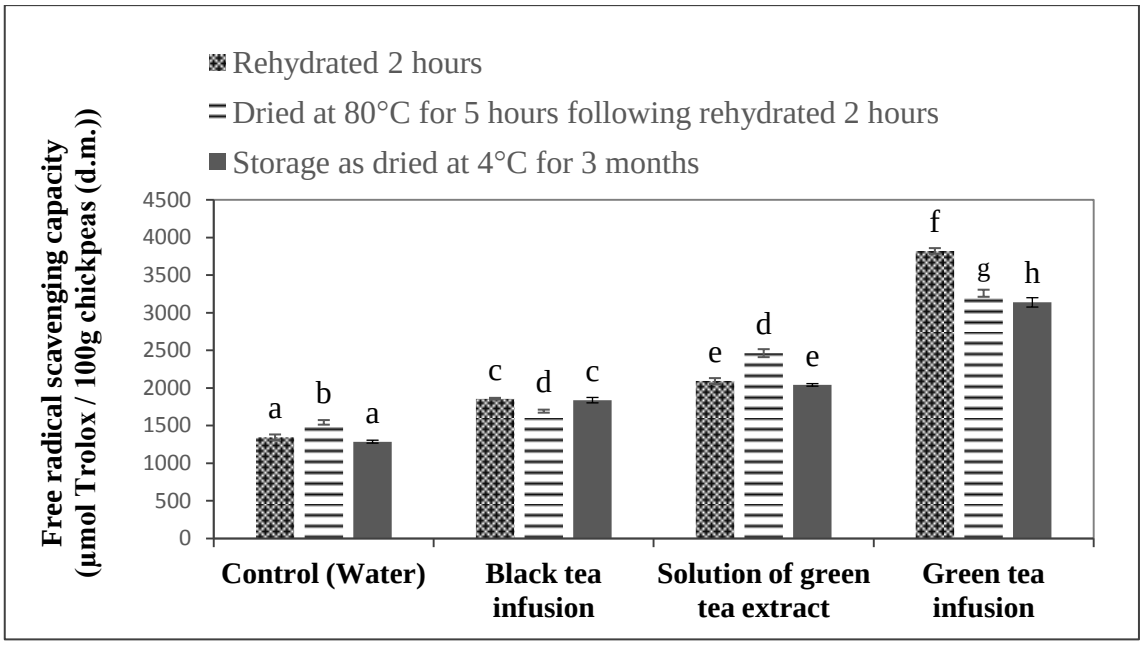


Figure 5.14 Effect of drying and refrigerated storage on free radical scavenging based antioxidant activity of chickpeas rehydrated 2 hours in different phenolic rich medium. Different letters indicate significantly different results at $P<0.05$.

5.6. Effect of Frozen Storage on Total Phenolic and Flavonoid Content and Free Radical Scavenging Based Antioxidant Activity of Phenolic Enriched Chickpeas

After rehydration in phenolic rich solutions for 10 hours, the chickpeas were frozen and stored at − 18°C for 6 months. As given in Table 5.15, the total phenolic content of controls did not change considerably, while a slight decrease was observed in the total phenolic content of chickpeas rehydrated in black tea infusion and solution of green tea extract at the end of 6 months. In contrast, total phenoic content slightly increased for the chickpeas rehydrated in green tea infusion (Figure 5.15). The increased phenolic content could be due to the increased extractability (solubility) of the phenolic compounds in these chickpeas after freezing-thawing. On the other hand, the total flavonoid content of chickpeas rehydrated in the solution of green tea extract and green tea infusion increased by frozen storage, while the total flavonoid content of chickpeas rehydrated in black tea infusion almost unchanged similar to the free radical scavenging capacity of chickpeas in the black tea infusion. On the contrary, a slight

decrease was observed in the free radical scavenging capacity of chickpeas rehydrated in the solution of green tea extract and green tea infusion. Since the free radical scavenging capacity of control sample also decreased, the ratio of increase in the free radical scavenging capacity was almost preserved for all used antioxidant solutions during storage (Figure 5.17).

Table 5.14 Effect of freezing and frozen storage on total phenolic and flavonoid content and free radical scavenging based antioxidant activity of chickpeas rehydrated 10 hours in different phenolic rich medium

Processes applied in sequence	**Rehydration medium**			
	Control (Water)	**Black Tea Infusion**	**Solution of Green Tea Extract**	**Green Tea Infusion**
Total phenolic content (mg catechin / 100g chickpeas (d.m.))				
1. Rehydration	132.8 ± 4.7 a	233.1 ± 16.4 b	318.6 ± 7.7 d	449.1 ± 12.9 f
2.Freezing and frozen storage at −18°C for 6 months	122.5 ± 7.9 a	208.5 ± 3.7 c	300.8 ± 5.3 e	478.9 ± 5.1 g
Total flavonoid content (mg catechin/ 100g chickpeas (d.m.))				
1. Rehydration	31.31 ± 4.09 a	49.51 ± 2.78 c	74.42 ± 5.06 d	103.36 ± 4.25 f
2.Freezing and frozen storage at −18°C for 6 months	23.7 ± 0.4 b	50.39 ± 0.55 c	80.87 ± 0.55 e	115.78 ± 1.90 g
Free radical scavenging capacity (mg catechin / 100g chickpeas (d.m.))				
1. Rehydration	1701 ± 19 a	2517 ± 55 c	3590 ± 85 d	6186 ± 145 f
2.Freezing and frozen storage at −18°C for 6 months	1500 ± 11 b	2454 ± 79 c	3381 ± 23 e	6017 ± 56 g

a-g: Different letters at each column indicate significantly different results at $P<0.05$.

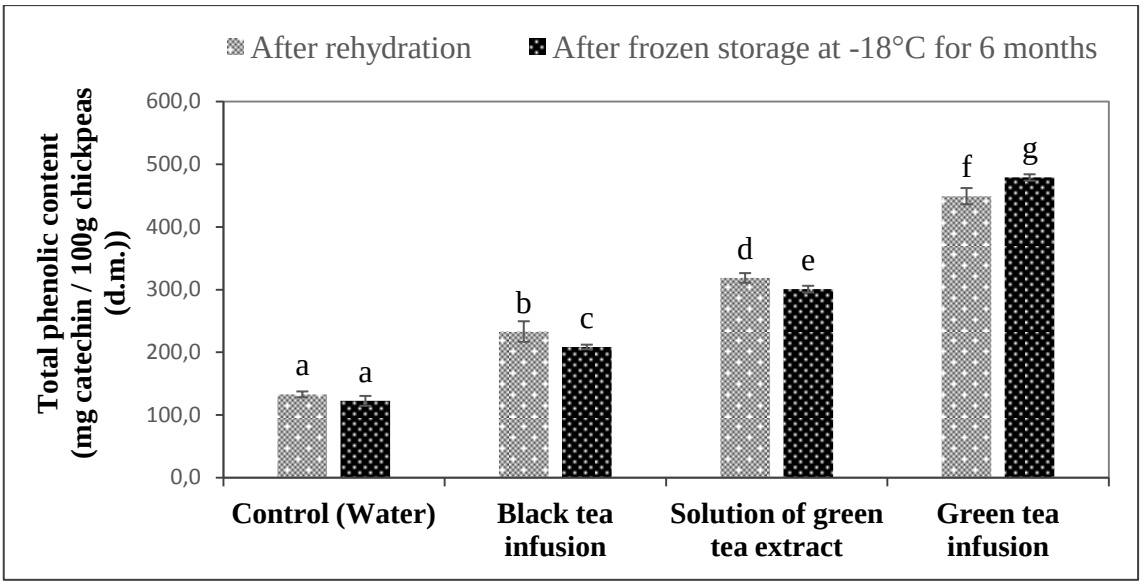


Figure 5.15 Effect of frozen storage on total phenolic content of chickpeas rehydrated 10 hours in different phenolic rich medium. Different letters indicate significantly different results at P<0.05.

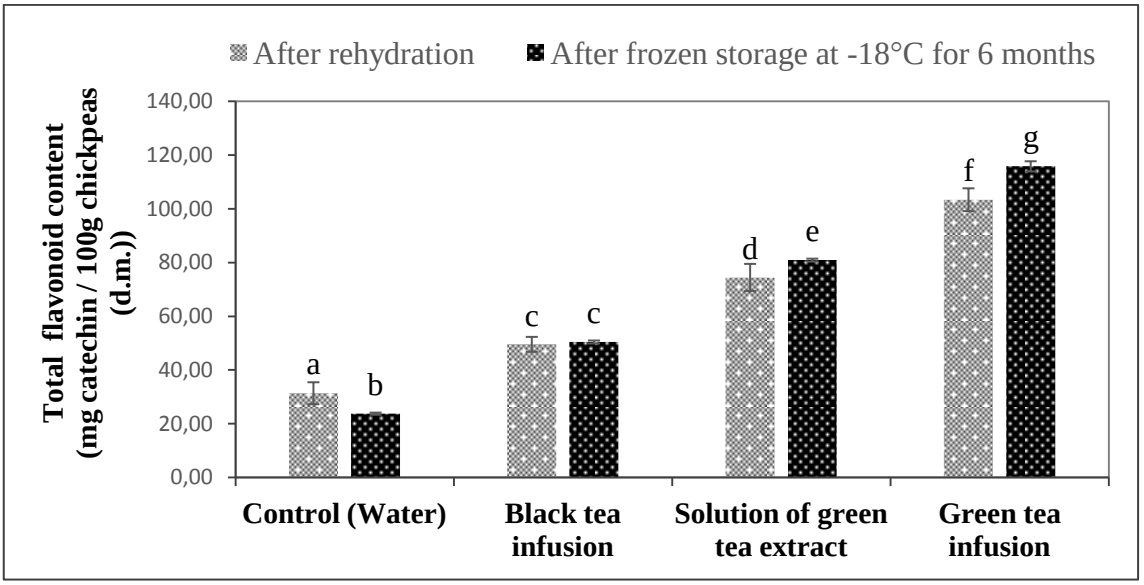


Figure 5.16 Effect of frozen storage on total flavonoid content of chickpeas rehydrated 10 hours in different phenolic rich medium. Different letters indicate significantly different results at P<0.05.

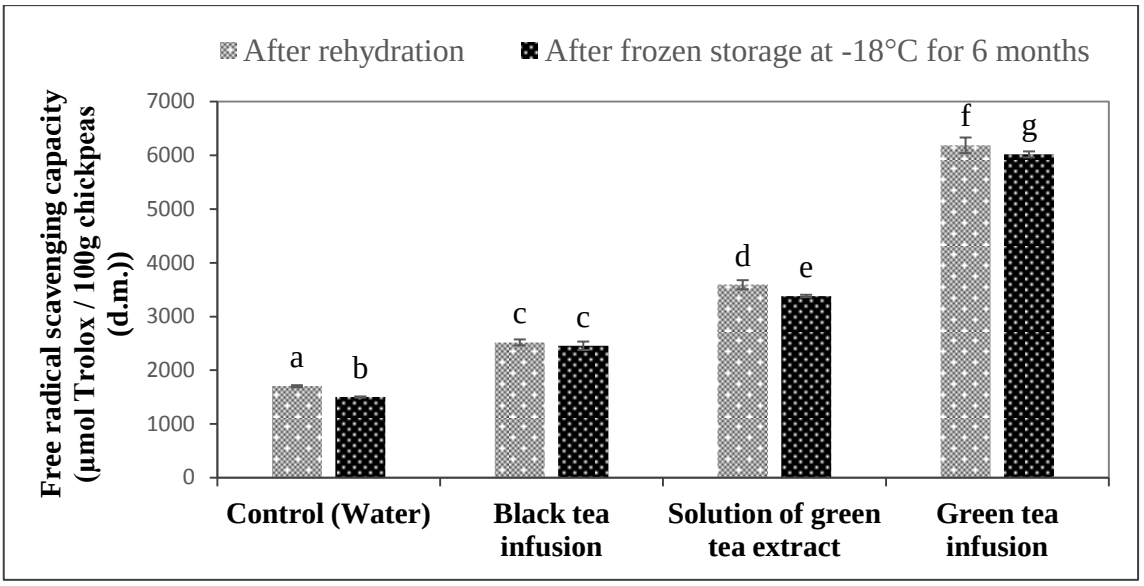


Figure 5.17 Effect of frozen storage on free radical scavenging based on antioxidant activity of chickpeas rehydrated 10 hours in different phenolic rich medium. Different letters indicate significantly different results at $P<0.05$.

5.7. The Use of Phenolic Enriched Chickpeas for Production of Phenolic Enriched Chickpea Protein Isolates

5.7.1 Comparison of Total Phenolic and Flavonoid Content, Antioxidant and Antidiabetic Potential of Protein Isolates from Standard Chickpeas and Phenolic Enriched Chickpeas

The total phenolic and flavonoid content, antioxidant activity and the antidiabetic activity of chickpea protein were determined for control chickpeas rehydrated in water and phenolic enriched chickpeas rehydrated in green tea infusion for 10 hour. According to the previous studies conducted in our laboratory, the chickpea protein isolates obtained with the method used in the current thesis contains almost 0.73 (g/g) total protein content (Aydemir and Yemenicioğlu 2013). Therefore, all results based on protein were corrected by the factor of 0.73 to calculate contribution of protein and protein bound phenolics in different parameters (total phenolic and flavonoid content, antioxidant and antidiabetic activity) and express results per 100g of chickpea (d.m.).

The results of phenolic and flavonoid determination clearly showed the presence of high amounts of phenolic compounds within the protein isolates (Fig 5.18 and 5.19). The protein isolates were obtained by isoelectric precipitation and extensive washing. Thus, it is clear that the phenolic compounds are bound by the protein and could not be removed by precipitation and washing. This result was expected since it is a well-known and proved truth that the hydroxyl groups of phenolic compounds could form hydrogen bonds with the carboxyl group of the protein (Damodaran 1986). The Protein–phenolic binding mechanism is also affected from different factors such as molecular weight, and degree of methylation and hydroxylation of the phenolic compounds (Ozdal et al. 2013). The results for the control samples showed that the protein could bind almost half of the total phenolic and flavonoid compounds in the chickpeas. On the other hand, the rehydration in green tea infusion caused significant changes in the amount of protein bind phenolic compounds (Table 5.15 and 5.16). It is clear that the chickpea protein had an extremely high flavonoid binding capacity. It is worth no note that the flavonoids bind by protein is equivalent to almost 90% of total flavonoid incorporated into chickpeas during phenolic enrichment.

Table 5.15 Total phenolic contents of different chickpea and chickpea protein isolates

	Rehydration medium / Total phenolic content (mg catechin / 100g chickpeas (d.m.))	
	Control (Water)	**Green Tea Infusion**
Chickpeas	132.9 ± 4.7 a	449.1 ± 12.9 c
Chickpea Protein Isolates	56.4 ± 1.2 b	255.7 ± 3.1 d

a-d: Different letters at each column indicate significantly different results at $P<0.05$.

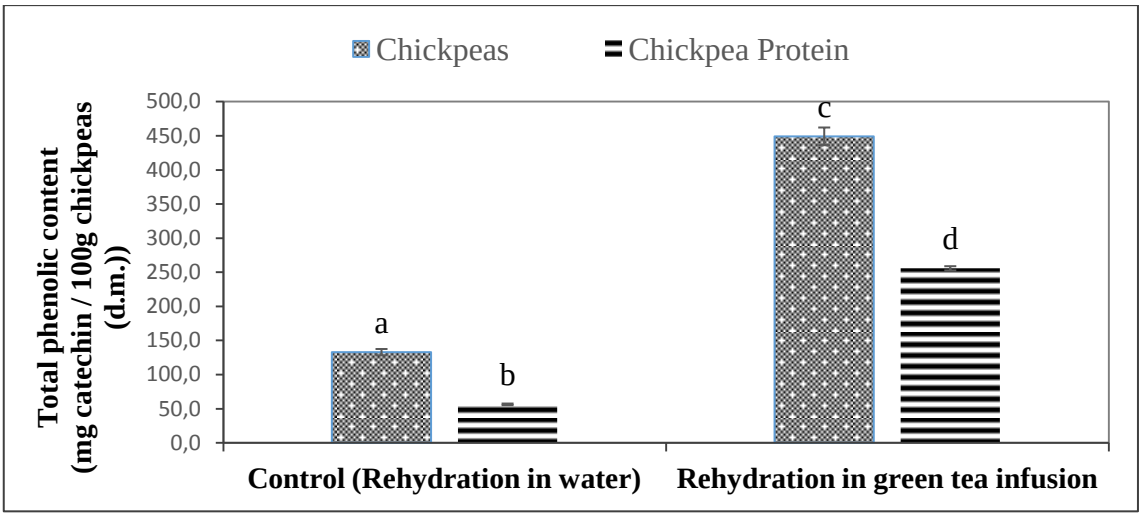


Figure 5.18 Total phenolic content of different chickpeas and chickpea protein isolates. Different letters at each column indicate significantly different results at P<0.05.

Table 5.16 Total flavonoid content of different chickpea and chickpea protein isolates

	Rehydration medium / Total flavonoid content (mg catechin / 100g chickpeas (d.m.))	
	Control (Water)	**Green Tea Infusion**
Chickpeas	31.1 ± 4.1 a	103.4 ± 4.3 c
Chickpea Protein Isolates	15.0 ± 1 b	94.4 ± 1.3 d

a-d: Different letters at each column indicate significantly different results at P<0.05.

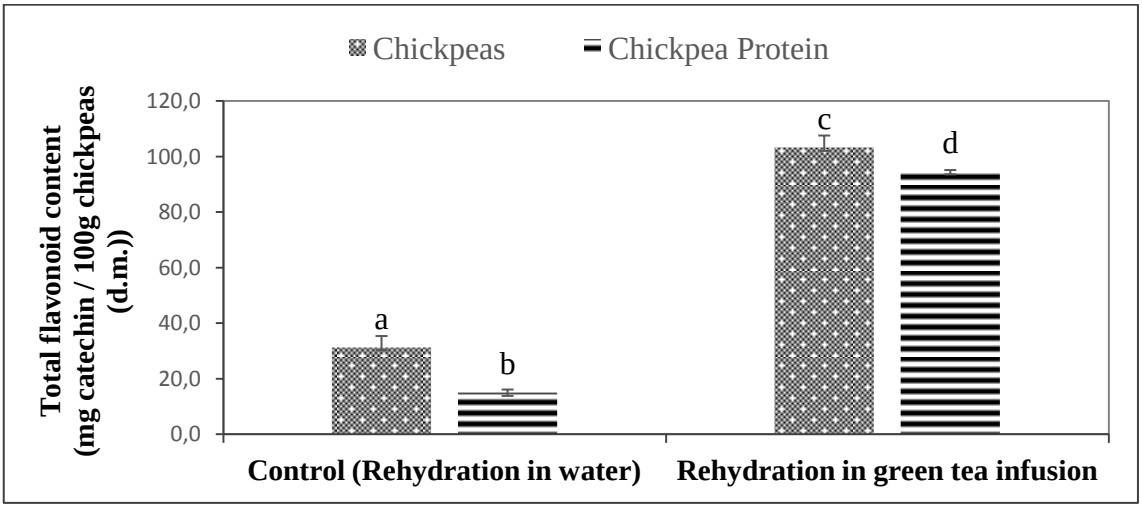


Figure 5.19 Total flavonoid content of different chickpea and chickpea protein isolates. Different letters at each column indicate significantly different results at P<0.05.

Although the chickpea proteins bind significant amounts of phenolic compounds, this did not reflect to their free radical scavenging capacity. The results clearly showed that the antioxidant activity of proteins was equivalent to almost 15% of the antioxidant capacity of chickpeas. The phenolic enrichment of chickpeas using green tea infusions increased the antioxidant activity of chickpea protein. In fact, antioxidant activity for the proteins in the phenolic enriched chickpeas is equivalent to 28% of the antioxidant activity of chickpeas. The high phenolic binding capacity, but low antioxidant activity of protein could be related with masking of the reactive phenolic hydroxyl groups by the protein tertiary or quaternary structures. It is also likely that the protein-protein interaction might also cause entrapment of reactive phenolic hydroxyl groups.

The effect of phenolic enrichment of chickpeas on α-glucosidase inhibition based antidiabetic activity of protein was also investigated. It is clear that the protein from control chickpeas did not show any antidiabetic activity. In contrast, the phenolic enrichment of chickpeas using green tea infusion caused a significant increase in the antidiabetic activity of chickpea protein. It is worth to note that the protein isolate in phenolic enriched chickpeas showed almost 1.4 fold higher antidiabetic activity than the chickpeas. It is clear that the protein-phenolic complexes in the protein isolate are much more effective antidiabetic compounds than the free phenolic compounds in the chickpea extract.

Table 5.17 Free radical scavenging based antioxidant activity of chickpeas and chickpea protein isolates

	Free radical scavenging capacity (µmol Trolox / 100g chickpeas (d.m.))	
	Control (Water)	**Green Tea Infusion**
Chickpeas	1701 ± 19 a	6186 ± 145 c
Chickpea Protein Isolates	262 ± 4 b	1727 ± 21 a

a-c: Different letters at each column indicate significantly different results at P<0.05.

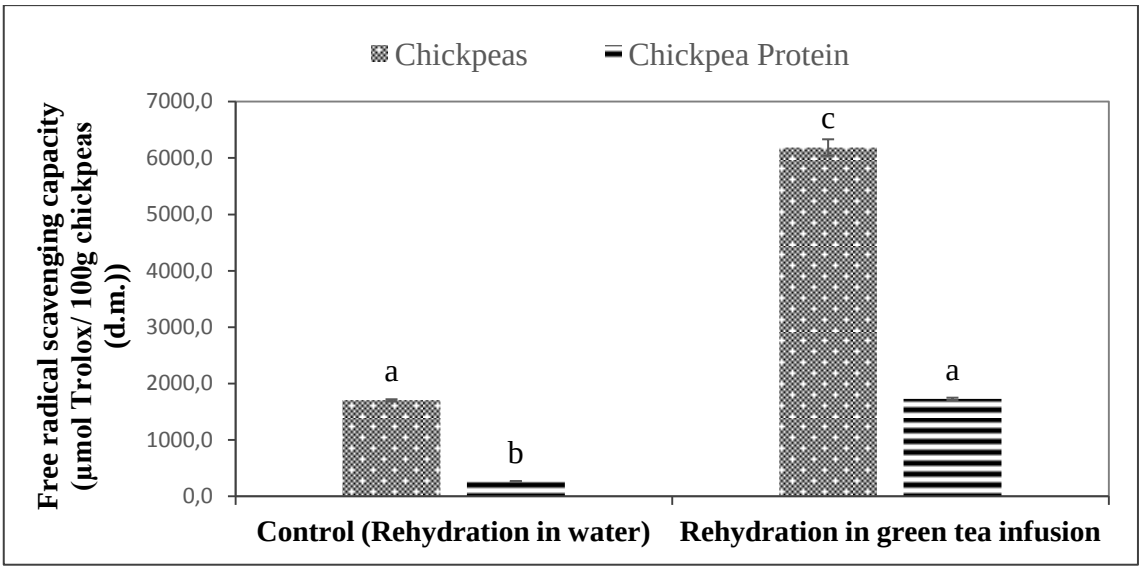


Figure 5.20 Free radical scavenging based on antioxidant activity of different chickpea and chickpea protein isolates. Different letters at each column indicate significantly different results at P<0.05.

Table 5.18 Antidiabetic activity of different chickpea and chickpea protein isolates

	Antidiabetic activity (mmol Acarbose / 100g chickpeas (d.m.))	
	Control (Water)	**Green Tea Infusion**
Chickpeas	344.6 ± 32.1 a	342.3 ± 9.1 a
Chickpea Protein Isolates	–	466.1 ± 50.7 b

a-b: Different letters at each column indicate significantly different results at P<0.05.

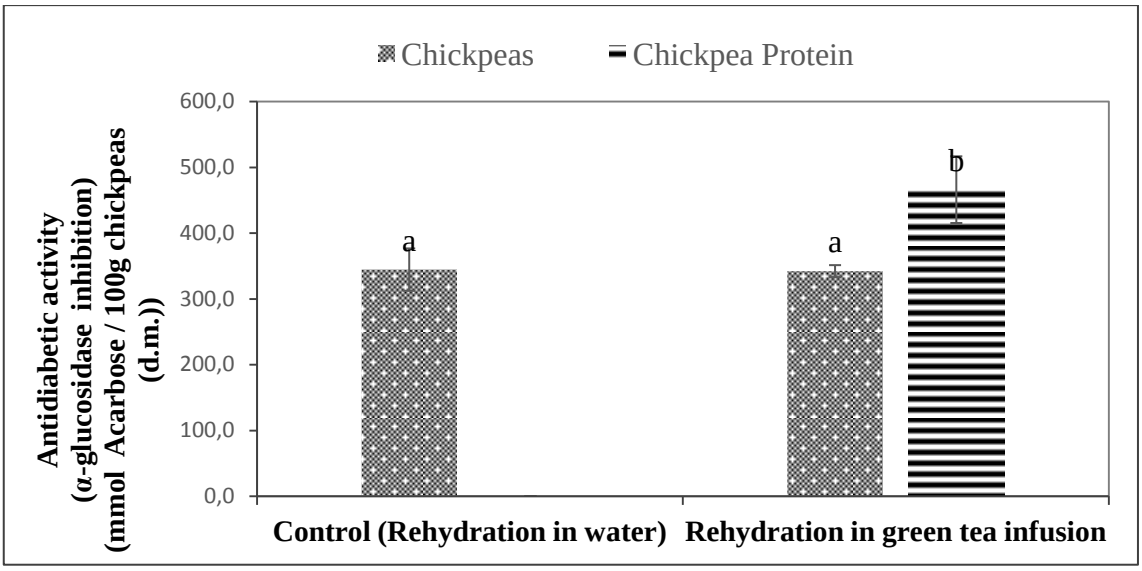


Figure 5.21 Antidiabetic activity of different chickpea and chickpea protein isolates. Different letters at each column indicate significantly different results at $P<0.05$.

CHAPTER 6

CONCLUSIONS

Phenolic enriched chickpeas as functional food products

- The controlled rehydration process was successfully employed for incorporation of green tea and black tea phenolics having historically well-known and characterized health benefits into dry chickpeas.

- The phenolic rich rehydration mediums were obtained simply by preparation of concentrated green and black tea infusions or by using solution of commercial green tea extract.

- The optimal conditions for preparation of phenolic rich tea infusions were 20 min brewing at 80°C in distilled water containing 5% (w/v) green tea, and 7.5 min brewing at 90°C in distilled water containing 5% (w/v) black tea. The 1% (w/v) soulution of a commerical green tea extract was employed in all rehydraiton processes.

- The rehydration processes continued in green or black tea infusions, or in solutions of commercial green tea extracts between 2 to 10 hours is sufficient to obtain significant increases in total phenolic content, total flavonoid content and free radical scavenging based antioxidant activity of chickpeas.

- The rehydration processes continued in green or black tea infusions, or in solutions of commercial green tea extracts for almost 2 hours is sufficient to obtain significant increases in α-glucosidase inhibiton based antidiabetic activity of chickpeas.

- The drying and then refrigerated storage at $+4^{o}C$ or freezing and then frozen storage at $-18^{o}C$ could be employed successfully to obtain commercially shelf-stable black or green tea phenolic enriched chickpeas.

Phenolic enriched chickpea protein isolates as functional food additives or ingredients

- The phenolic enriched chickpeas obtained by using green tea infusion during rehydration could successfully be employed to obtain phenolic enriched protein isolates which could be used as functional food additives or ingredients.

- The protein isolates were produced simply by using classical alkaline extraction and following isoelectric precipitation method which is extensively used in the food industry for purification of vegetable proteins.

- The results of phenolic assays conducted in extracted protein isolates clearly showed the binding of a considerable portion of incorporated green tea phenolics by the chickpea proteins.

- The amount of total phenolic and total flavonoid compound bound by protein isolated from green tea enriched chickpeas equal almost 50% and 90% of the total extractable phenolic and flavonoid compounds from dry green tea enriched chickpeas, respectively.

- The antioxidant activity of protein isolated from green tea enriched chickpeas is equal almost 28% of the measurable antioxidant activity of dry green tea enriched chickpeas.

- The antidiabetic activity of protein isolated from green tea enriched chickpeas is almost %30 higher than the measurable antidiabetic activity of dry green tea enriched chickpeas.

- These results clearly showed that the green tea enriched chickpea protein isolates could be alternative to traditional vegetable protein isolates. With their high phenolic content, and antioxidant and antidiabetic activity, the green tea enriched chickpea protein isolates are perfect candidate as functional food additives or ingredients.

FUTURE STUDIES

- The use of developed rehydration technique for phenolic enrichment of alternative dry legumes and cereals
- Characterization of technological properties of green tea enriched protein isolates
- Further in-vitro and in-vivo tests to show alternative bioactive properties of phenolic enriched chickpeas and chickpea protein isolates.

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