STUDY ON THE FLAVONOIDS, TOCOPHEROL AND ASCORBIC ACID CONTENT IN RAW AND ROASTED ARABICA COFFEE BEANS

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ABSTRACT

In this study we examined flavonoids, alpha- tocopherol and ascorbic acid content in raw and roasted Arabica coffee beans. Rutin, Quercetin, Kaempferol and Isorhamnetin are the various flavonoids determined in this study and it has been found that the roasted coffee exhibited lesser content of flavonoids than unroasted coffee and the concentration of Rutin was highest among all identified flavonoids. Similarly α-tocopherol and ascorbic acid was also highest in green beans. Green Legamy Arabica coffee showed highest concentration of all flavonoids and ascorbic acid and also provide maximum percentage of Recommended Dietary Allowance for flavonoids, ascorbic acid and alpha tocopherol.

Keywords: Ascorbic acid, beverage, coffee, flavonoids, tocopherol.

INTRODUCTION

Free radicals are very perilous due to their potential to attack structures of cell, hinder their normal functions, and thus in turn lead to toxic and deleterious effects on human health (Hudakova et al., 2016). Free radicals and ROS are derived from two sources, i.e. firstly from external sources such as exposure to radiation, smoke pollutants and secondly is endogenous metabolic process in human body such as mitochondrial respiration, inflammation, peroxisomal metabolism, exercise and so on (Langseth,1996). Antioxidants work as potential therapeutic agents to avert free radical generated damage in the human body (Chandra et al., 2014). An antioxidant is capable of blocking the chain propagation reaction produced by oxidants and considerably prevents or slows down the oxidation of other molecules or substrates (Rangan and Bulkley, 1993). The contribution of antioxidants is extensive; they protect human bodies against premature ageing, weakening of the immune system, and other health problems caused by free radicals (Hudakova et al., 2016). There are two types of antioxidants: (1) natural and (2) synthetic. Natural antioxidants are gaining attraction of the public and researchers because of the instability and possible activity as carcinogens of the synthetic antioxidants (Ramalakshmi et al., 2008; Namiki, 1990). Fruits, vegetables, chocolates, nuts are the various sources of antioxidant. Research shows that coffee is much more than combination of just caffeine and water. Polyphenols, mainly, phenolic acids and flavonoids are of great abundance in coffee and contribute to its flavor and health properties (Wang and Ho, 2009). Flavonoids are hydroxylated phenolic substances and their chemical nature depends on their structural class, degree of hydroxylation and polymerization and other substitutions and conjugations (Kelly et al., 2002) and they arbitrate their antioxidant effects by either by chelating metal ions or by scavenging free radicals (Kumar et al., 2013). Flavonoids can be categorized into various classes such as flavones, flavonols, flavanones, flavanonol, isoflavones and flavan-3-ols. They have been reported to have antiviral, anti-allergic, anti-inflammatory and anti-tumor and antioxidant activities (Buhler and Miranda, 2004).

Coffee is one of the world's most popular beverages. It is also the most important consumed and traded food commodity worldwide and ranks second, after crude oil, among all commodities (Fujioka and Shibamoto, 2008). Unique flavor increases the popularity of coffee and makes it one of the most pleasing and regularly consumed beverages (Hecimovic et al., 2011). It cannot be grown everywhere; the place where coffee can be planted is tropical and subtropical zone. The best place for coffee growth also known as coffee belt or coffee zone is nearby the equator between 25°S and 25°N. Coffee beans are produced from two different species of Coffea genus: Coffea arabica and Coffea canephora syn. Coffea robusta (Hecimovic et al., 2011). This two species of coffee are different in many ways, such as their ideal growing climates, physical aspects, chemical composition and characteristics of the brew made with the ground roasted seeds. Concerning the physiological and metabolic activities of coffee, it is known for its notifying qualities, as it activates the nervous system, enhance perception and reduce fatigue. Most of these activities are associated with caffeine (Gonzalez et al., 2004). Coffee is very

significant source of polyphenolic compounds and studies have shown beneficial health effect of green coffee extracts such as anti hypertension effects (Kozuma et al., 2005), modulate glucose metabolism (Blum et al., 2007), beneficial to reduce the risk of type 2 diabetes (Jiang et al., 2014), Alzheimer's disease (Barranco et al., 2007), and heart failure (Mostofsky *et al*., 2012). Coffee is a blend of chemical compounds which are either occurring or formed during the roasting process (Lee, 2016). Roasting is processing of green coffee and it causes the modification of the chemical composition of the beans into hundreds of chemicals which is responsible for the complex aroma and taste of coffee (Hudakova et al., 2016; Lee, 2016).The objective of this study is to determine the natural antioxidants such as flavonoids (Rutin, Quercetin, Kaempferol and Isorhamnetin), ascorbic acid and alpha tocopherol in green and roasted berry and Legamy Arabica coffee commonly consumed in Saudi Arabia.

MATERIALS AND METHODS

Samples and Reagents

Raw (green) and roasted berry and Legamy coffee beans were obtained from Riyadh commercial market. The samples were ground into powder in a mortar, and then accurately weighed and kept at refrigerated temperature (4°C) during analysis. All the reagents used were of analytical grade.

Analytical Method

Determination of Flavonoids

The extraction and determination of flavonoids in coffee were done according to Lunn (2000). Coffee samples (2 gm) was weighed and extracted with 50 ml of extracting solution (methanol: water: acetic acid, 50:40:5 (v/v)). For Rutin determination, the extracts were put in sonication bath at 60°C for 15 minutes. The resultant mixture was filtered through micro filter. For Quercetin, Kaempferol and Isorhamnetin, 4 gm of coffee samples were dissolved in 100 ml methanol and extracted with ethyl alcohol. All the extracts were concentrated by rotary evaporator to 10 ml. Following parameters were used to determine flavonoids in coffee. The coffee solutions were analyzed by HPLC (Shimadzu, Japan), using Shim-Pack CLC-ODS column. The mobile phase was water-methanol-acetic acid (50:50:1) and flow rate was 1ml/min. The detector (Shimadzu, Japan) was UV set at 254 nm for Rutin and 465 nm for Quercetin, Kaempferol and Isorhamnetin. An auto injector was used to inject 20 µl of the test solution into the HPLC system.

Determination of ascorbic acid (Vitamin C)

Coffee samples were extracted with water: acetonitrile: acetic acid mixture (94:5:1 (v/v)). The suspension sonicated for 15 min at 50°C. The extract was centrifuged (6000 rpm for 12 min) and

vitamin C in the supernatant was evaluated by HPLC according to Lunn (2000).The separation was done using a Nucleosil 100-10 C 18 column (125 x 4.6mm). The spectra were recorded at 280 nm. The flow rate was 1.0 ml /min. the mobile phase was methanol 300 ml / 700 ml buffer (1.36 g potassium dihydrogen phosphate+ 1.08 gm sodium octylsulfonate+ 915 ml water +5 ml triethylamin).

Determination of Alpha tocopherol (Vitamin E)

Alpha tocopherol was determined according to the method proposed by Lunn (2000). The coffee solutions were analyzed using a Pinnacle 3 µm, 125x 4 mm column (Resteck). The spectra were recorded at 285 nm. The flow rate was 1.0 ml/min. The mobile phase was methanol: water (95:5 v/v).

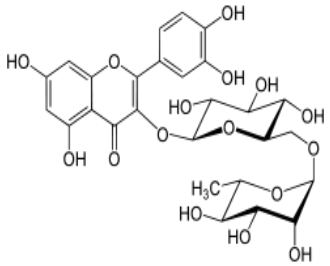
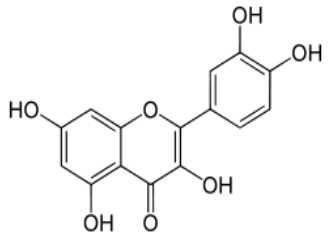
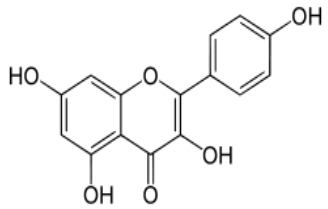
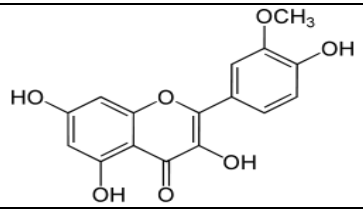
Statistical analysis

Quantitative data from HPLC analysis were compared using analysis of variance (ANOVA). For variables where significant F values ($P<0.05$) were found, Fischer's least significance Difference has been used to compare means (Walpole, 1990).

RESULTS AND DISCUSSIONS

Coffee is a worldwide popular brewed drink prepared from roasted coffee beans and is famous for its organoleptic characteristics and stimulating effects (Alves et al., 2010). The stimulant property of coffee brew is mainly accredited to caffeine (Nogaim et al., 2013). Conditions of roasting and the procedures of extraction adopted for brew preparations result in a great chemical diversity (Illy and Viani, 2005). In international market, Arabica coffee is more costly and is also considered to be superior due to its sensory properties (Gielissen and Graffland, 2009). The term "green coffee bean" refers to unroasted mature or immature coffee beans. People consume coffee or tea every day, sometimes several times a day and it is the main drink in Europe, America and Asia (Yashin et al., 2013). Rutin, Quercetin, Kaempferol and Isorhamnetin are the various flavonoids determined in this study and it has been found that the roasted coffee exhibited lesser content of flavonoids than unroasted coffee. A brief description of the analyzed flavonoids has been given in Table 1. Rutin is a flavone and Quercetin, Kaempferol and Isorhamnetin are flavonols. Flavonoids are defined by their chemical structure, which includes two aromatic rings (the ''A'' and ''B'' rings) linked by a three-carbon bridge that comprises part of a third six-member ''C'' ring (Beecher, 2003).

Table 1: A brief description of the different flavonoids analyzed in this study

Flavonoids	Class	Structure	IUPAC	Dietary Sources	Function
Rutin	Flavone		2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-({[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}methyl)oxan-2-yl]oxy}-4*H*-chromen-4-one	Buckwheat, (Kreft et al., 1999) peaches, (Chang et al., 2000), apples (Sluis et al., 2001).	Inhibits platelet aggregation (Navarro-Núñez et al., 2008), anti-inflammatory (Guardia et al., 2001), inhibits aldose reductase activity (Reddy et al, 2014).
Quercetin	Flavonol		2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	Red kidney beans, raw (powdered), Kale, plums (black), bilberry, sea buckthorn berry (Justesen and Knuthsen , 2001)	Inhibit the oxidation (Russo et al, 2014), possess estrogenic (female sex hormone like) activities (Moutsatsou 2007), act as an agonist of the G protein-coupled estrogen receptor (GPER) (Prossnitz and Barton, 2014).
Kaempferol	Flavonol		3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	onions, broccoli, Brussels sprouts, squash, cucumbers, lettuce, green beans, peaches, spinach, potatoes, blackberries, raspberries, (Calderon-Montaño et, 2011) apples (Liu,, 2013), grapes, tomatoes, green tea (Kim and Choi, 2013).	Anti-bacterial and anti viral activity, antioxidant effect, anti cancer,anti diabetic (Calderon-Montaño et al., 2011).
Isorhamentin	Flavonol		3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromen-4-one	Wine (red) (Rassouw and Marais (2004), almond (Milbury et al., 2006).	Prevent endothelial dysfunction (Sherry et al., 2012), induces the expression of neurofilaments (Sanchez et al., 2007).

More than 4000 varieties of flavonoids have been recognized, and are known to influence the quality and stability of foods by acting as flavorants, colourants, and antioxidants (Kumar et al., 2012; Craig, 1999) and are also responsible for the attractive colours of the fruits, flowers and leaves (De Groot and Rauen, 1998). The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Buhler and Mirinda, 2004).

From the Figure 1 it can be depicted that the levels of Rutin was highest among all flavonoids. It works as a strong radical scavenger and inhibitor of lipid peroxidation in vitro (Kerry and Abbey, 1997) but roasting process led to decrease its content. Hudakova et al. (2016) and Cheong et al. (2013) also reported highest content of flavonoids in unroasted coffee Arabica. In a study by Lee, (2016), it has been found that the sample roasted at lowest temperature exhibited highest total flavonoids content. Hecimovi et al. (2011) in their study showed that roasting affects polyphenolic compounds of coffee and confirmed that light and medium roasting are more favorable roasting. Katsube et al. (2009) mentioned that polyphenolic compounds are highly thermolabile compounds that easily decompose under the effect of high temperature (above 80°C). Lower levels of polyphenols in roasted coffee can be attributed to their degradation, polymerization, or auto oxidation during roasting (Cammerer and Kroh, 2006). Yashin et al. (2013) revealed that microwave roasting protects antioxidants better than convection roasting, and the wet method (probably due to lesser oxidation of the antioxidants) for separating the beans from the fruits is preferable to the dry method. Phenolics and Flavonoids are commonly composed of a group of compounds, which act as primary antioxidants (Adesegun et al., 2009) and are known to react with lipid peroxy radicals (Torel et al., 1986) and hydroxyl radicals (Afanas'ev et al., 1989).

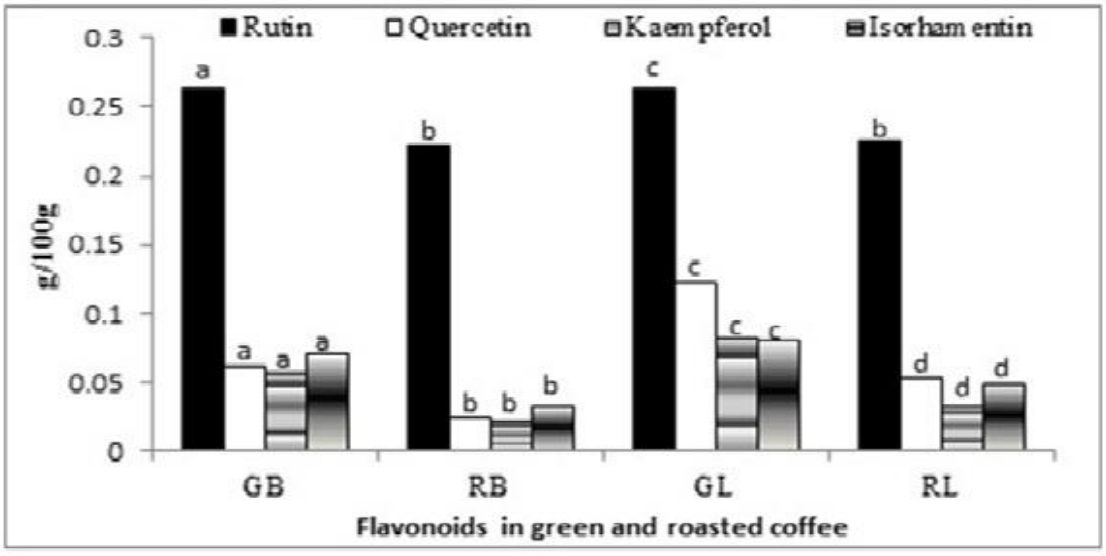


Figure 1: Flavonoids (g/100g) in green and roasted Arabic coffee. Means followed by different letters (for each flavonoids) are significantly different ($p \leq 0.05$).GB: green Berry, RB: roasted Berry, GL: green Legamy, RL: roasted Legamy.

Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity (Buhler and Miranda, 2004). Quercetin in particular is known for its iron chelating and iron stabilizing properties and to produce cell cycle arrest in proliferating lymphoid cells. Beside its antineoplastic activity,

Quercetin also exerts growth inhibitory effects in several malignant tumor cell lines in vitro (Kumar and Pandey, 2013).

Some antioxidants like ascorbic acid are hydrophilic, while others like carotenoids and vitamin E are clearly liphophillic (Pulido et al., 2003). Figure 2 depicts the content of ascorbic acid in green and roasted Arabica coffee. The result shows that ascorbic acid was 209.5 mg/kg and 176.4 mg/kg in green and roasted berry while it was 230.1 mg/kg and 205.5 mg/kg in green and roasted Legamy Arabica coffee (Fig.2.). Highest level of ascorbic acid was found in green Legamy coffee. Roasting significantly decreased the content of ascorbic acid (Fig. 2.). Authors didn't found any previous data on the ascorbic acid content and effect of roasting on its content in Arabic coffees. The result shows that α tocopherol was 33.2 mg/kg and 20mg/kg in green and roasted berry while it was 30.8 mg/kg and 26.9mg/kg in green and roasted legamy Arabica coffee (Fig.2.). Highest level of α tocopherol was found in green berry coffee. In the present study, roasting diminishes the content of alpha tocopherol significantly (Fig. 2.). The same trend was demonstrated by Coors (1984), who found that roasting decreased the content of α tocopherol, β-tocopherol and total tocopherols to 79 to 100%, 84 to 100% and 83 to 99% respectively. For the first time, the presence of tocopherols in the unsaponifiable matter of green coffee beans oil was described by Folstar et al. (1977). In a study performed by Ogawa et al. (1989) in 14 kinds of green coffee beans, total tocopherol of 12 kinds of them were more than 10 mg/g, the maximum was 15.7/100 g and the average was 11.9mg/100g. The average content of alpha tocopherol mentioned by him was 3.5 mg/100g which is quite similar to our study. Tocopherols are known to be relatively stable to roasting procedure. Ogawa et al. (1989) reported mean value of 94% (83-99%) for total tocopherols remaining after roast. Coors (1984) analyzed α, β, and γ tocopherol in different varieties of coffee beans. They were contained in a ratio of approximately 2:4:0.1, the total content being about 5.5 to 6.9 mg/100 gm. The prevalence of α tocopherol is a major feature of coffee beans in contrast to other vegetables and fruits. In different vitro studies, vitamins have shown less antioxidant activity than polyphenols (Sanchez-Moreno et al., 2000; Pulido et al., 2000). In humans vitamin E acts as an integral part of the primary intracellular defense system and it has been correlated with the prevention of several diseases: cancer, age-related cataract, Parkinson's disease, atherosclerosis, low density lipoproteins, Parkinson's disease, coronary heart disease and some immunologic diseases (Bramley et al., 2000; Nelis et al., 2000).

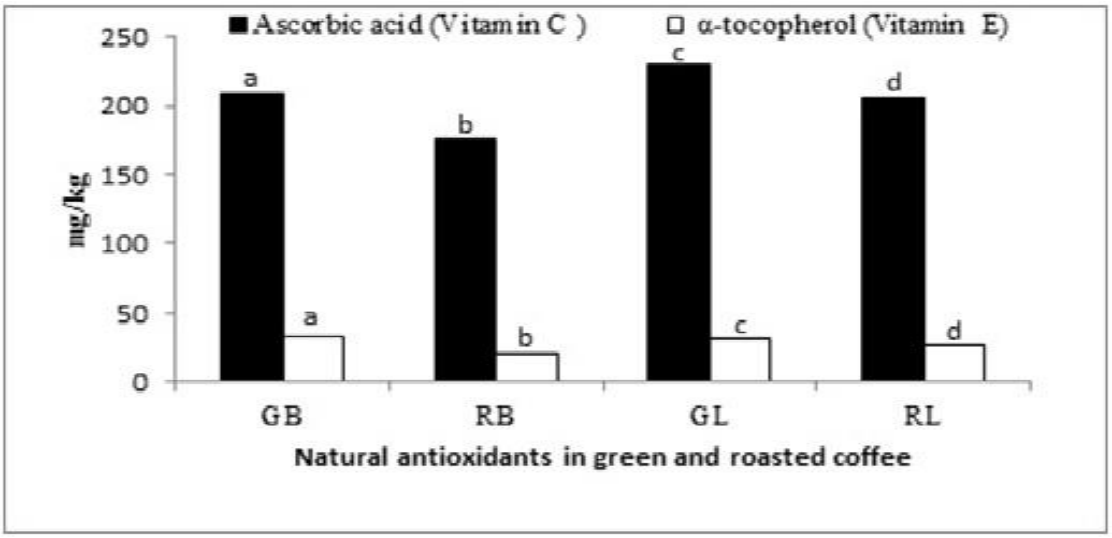


Figure 2: Natural antioxidants (mg/kg) in green and roasted Arabic coffee. Means followed

by different letters (for each flavonoids) are significantly different ($p \leq 0.05$).GB: green Berry, RB: roasted Berry, GL: green Legamy, RL: roasted Legamy.

In regular, the typical Arabian consumer consumes almost 1 liter of Arabic coffee everyday and this amount delivers approximately 152.5 and 178 mg of flavonoids in roasted berry and Legamy coffee respectively (Table 2). Such daily intake contributes to 35.9 and 42.12% of flavonoids of the recommended daily allowances (RDA) for two coffee types. The contribution of flavonoids to the antioxidant defense system may be substantial considering that the total daily intake of flavonoids can range from 50 to 800 mg. This intake is high compared to the average daily intake of other dietary antioxidants like vitamin C (70 mg), and vitamin E (7-10mg). The high consumption of tea and coffee may be most influential on total flavonoids intake in certain group of people (Buhler and Miranda, 2004).Lakenbink et al. (2000) reported that a cup of tea allowed to brew for 40 -60 sec will typically deliver approximately 140 mg of flavonoids. The longer the tea is left brew, the higher the concentration of flavonoids (Englehardt, 1999). In a study by Richelle et al. (2001), the beverages were prepared as 0.7-2.5% soluble coffee and 1.5-3.5% cocoa; teas (green, black, or herbal) were prepared as one tea bag infused over 5 min in 220 ml of hot water. They concluded that these commonly consumed beverages have a significant antioxidant activity, the highest being soluble coffee on a cup-serving basis.

Table2: Approximate percents contribution of flavonoids and vitamins by daily cups of roasted Berry and Legamy coffee*

Components	Mg components in 50 gm	Average RDA needed (mg)	% of RDA**
Flavonoids			
RB	152.5	425 (50-800)	35.9
RL	179	425 (50-800)	42.12
Ascorbic acid (Vitamin C)			
RB	8.8	70	12.6
RL	9.9	70	14.1
α-tocopherol (Vitamin E)			
RB	1.0	8.5 (7-10)	11.8
RL	1.2	8.5 (7-10)	14.12

*Coffee prepared by adding 50g of roasted powder coffee powder to 1 liter boiled water and brewed for 10 minutes. This quantity is daily consumed by moderate consumer.

**Recommended Daily Allowance (RDA) according to Buhler and Miranda (2004).

RB: roasted Berry, RL: roasted Legamy

CONCLUSION

The best level of flavonoids, ascorbic acid and α- tocopherol content was observed in green Arabica coffee (maximum in Legamy coffee) as compared to roasted Arabica coffee. These results lead us to conclude that these natural antioxidants decrease with roasting and coffee is a good source of flavonoids, ascorbic acid and α- tocopherol as it contributes a great percentage of their RDA. According to the obtained results, coffee is a valuable source of antioxidants with a great potential health benefits.

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