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FLAVONOIDS AND OTHER NATURAL ANTIOXIDANTS IN RAW AND ROASTED ARABICA COFFEE BEANS

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ABSTRACT

Flavonoids (Rutin, Quercetin, Kaempferol and Isorhamnetin), and other natural antioxidants (Vitamins E and C) contents in two Abyssinian coffee (Berry and Harar beans) which are dominating the coffee market in the Gulf area were determined.

In the green state, Harar beans had a higher flavonoids content than Berry beans. Although vitamin C processed the same trend like in flavonoids, vitamin E showed a higher content in Berry beans. The dominant species of flavonoids was Rutin. In green and roasted Harar beans, the amount of Rutin were 0.27 and 0.23 g/100g respectively.

The highest content of vitamin C (229.9 mg/kg) was found in green Harar beans, while it was 33.2 mg/kg for vitamin E in Green Berry beans.

It is observed that roasting significantly diminished the content of these antioxidant components.

The contribution of coffee beverage to the Recommended Daily Allowance (RDA) of flavonoids, vitamin E and C in the daily meal pool was evaluated. According to the actual consumption of coffee beverage per day in the arabian diet, it was found that 35.9% and 41.9% of flavonoids, 12.6% and 14.7% of vitamin C and 11.8% and 15.3% of vitamin E could be supplied by drinking (≈1 liter) roasted Berry and Harar coffee, respectively.

Keywords: Harar; Berry; Arabica coffee; Roasting effect; antioxidants; flavonoids; vitamin C; vitamin E.

INTRODUCTION

Coffee, together with tea, are the most popular drinks across the world. Its commercial and social importance is obvious. Coffee production is located mainly in South America, Brazil being the first producing country (42%). Africa accounts for 20.4% of the total production and Asia produces 18.5%. Nevertheless, Europe is the main coffee consumer (Smith, 1985). There are two varieties of the coffee plant with economic importance: *Coffea arabica* and *Coffea canephora*, known in the trade as *arabica* and *robusta*, respectively.

Coffee beverages are made from roasted beans belonging to one of these two varieties or blends of them. The better quality coffees and thus the most expensive ones, are considered to be the Arabica (Briandet *et al.*, 1996).

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Regarding physiological and metabolic activities, coffee is known for its notifying qualities, which activate the nervous system, enhance perception and reduce fatigue. Most of these activities are associated with caffeine (Gonzalez *et al.*, 2004). These activities could be associated with compounds other than caffeine that raise blood pressure and activate the sympathetic nervous system (Corti *et al.*, 2002; Klag *et al.*, 2003).

It is known that coffee has antioxidant properties (Castillo *et al.*, 2002; Natella at al., 2002; Pojjana *et al.*, 2002, & Yanagimoto *et al.*, 2002). These properties have a role in the prevention of diabetes, arteriosclerosis, neurodigestive disease and cancer, which will depend partly on the lack of oxidation balance in the body (McBrian & Slater, 1982; Meyer *et al.*, 1998).

Sources of antioxidative activity in coffee are the phenolic compounds (Lajolo *et al.*, 2001). Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E (Buhler and Miranda, 2004). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Buhler and Miranda, 2004). In addition to the recognized dietary antioxidants: vitamin C, and vitamin E, selenium and carotenoides; it is demonstrated that flavonoids found in fruits and vegetables also act as antioxidants. Like alpha-tocopherol (vitamin E), flavonoids contain chemical structural elements that might be responsible for their antioxidant activities (Buhler and Miranda, 2004). Flavonoid compounds have 2-5 fold greater antioxidant and free radical scavenging activities than vitamins C and E on an equimolar basis (Vinson *et al.*, 1995; Wiseman *et al.*, 1996).

The object of this study was to determine flavonoids class including: rutin, quercetin, kaempferol, and isorhamentin and other natural antioxidants (alpha-tocopherol, vitamin E and ascorbic acid, vitamin C) in green and roasted Berry and Harar arabica coffee. Further, the contribution of roasted coffee to flavonoids and antioxidants daily intake is estimated.

MATERIALS AND METHODS

Coffee samples

Raw (green) and roasted Berry and Harar coffee beans were obtained from Riyadh commercial market. The samples were powdered before analysis and kept at refrigerated temperature (4°C) during the analysis.

Analytical methods

Flavonoids analysis by HPLC

The extraction and determination of flavonoids in coffee were done according to Lunn (2000). Coffee samples (2 gm) were extracted with 50 ml extracting solution (methanol-water-acetic acid, 55:40:5 (v/v)). For Rutin determination, the extracts were put in sonication bath at 60°C for 15 min.

The resultant mixtures were filtered throught micro-filter. For Quercetin, Keampferol and Isorhmnetin, 4 g of coffee samples were dissolved in 100 ml methanol, 6% HCl and extracted with ethyl ether. All the extracts were concentrated by rotary evaporater to 10 ml.

The coffee solutions were analysed by HPLC (Shimadzu, Japan), using Shim- Pack CLC- ODS column. The mobile phase was water- methanol- acetic acid (50: 50: 1). The flow rate was 1 ml/ min. The detector (Shimadzu, Japan) was UV set at 254 nm for Rutin and 465 nm for Quercetin, Keampferol and Isohamnetin. An auto injector was used to inject 20 µl of the test solution into the HPLC system. Flavonoids standards of Hyperside (Quercetin), Kaempferol, Isorhammetin, and Rutin were purchased from Sigma (Germany). The standards were dissolved in the extraction solution.

Vitamin C analysis by HPLC

Coffee samples were extracted with a water – acetonitrile – acetic acid mixture (94:5:1 (v/v)). The suspension sonicated for 15 min at 50°C. The extracted was centrifuged (6000 *g* for 12 min) and the 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 × 4,6 mm). The spectra were recorded at 280 nm. The flow rate was 1.0 ml/ min. The mobile phase was methanol 300 ml / buffer 700 ml (1.36 g potassium dihydrogen phosphate + 1.08 g sodium octylsulfonate + 915 ml water + 5 ml triethylamin).

Vitamin E analysis by HPLC

The coffee solutions were analysed using a Pinnacle ODS 3 µm 125 × 4mm 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) (Lunn, 2000).

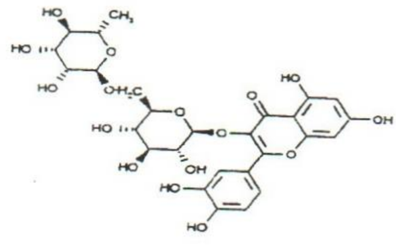
Statistical analysis

Quantitive data from HPLC analysis were compared using analysis of variance (ANOVA). For variables where significant F- values ($P< 0.05$) were found, Fisher's Least Significant Difference (LSD) has been used to compare means (Walpole, 1990).

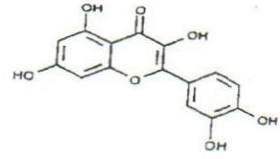
RESULTS AND DISCUSSION

The chromatogram of four types of detected flavonoids in coffee samples is shown in Fig. 2, while Fig. 1 shows the structures of these flavonoids. Table (1) shows the percentage of flavonoids found in green and roasted arabic coffee (Berry and Harar beans). The HPLC results show that rutin compound was the first one in the detected flavonoids in arabic coffee.

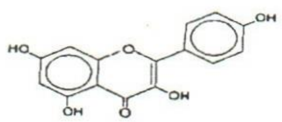
The levels of rutin were higher in Harar coffee beans than in Berry, while roasting process led to decrease its content. It is known that the capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. 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).



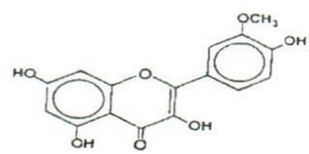
Rutin



Quercetin



Kaempferol



Isorhamentin

Fig. 1. Chemical structures of investigated flavonoids.

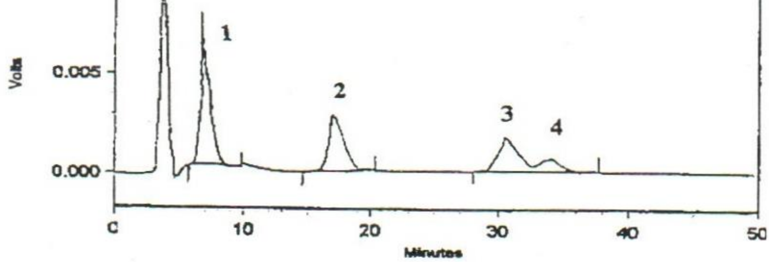


Fig. 2. HPLC chromatograms of flavonoids determined in coffee arabica; 1. Rutin; 2. Quercetin; 3. Kaempferol; 4. Isorhamentin.

Table 1: Flavonides (g/100g) in green and roasted Arabic coffee

Flavonids	GB	RB	GH	RH
Rutin	0.263[a]	0.222[b]	0.265[c]	0.226[b]
Quercetin	0.062[a]	0.024[b]	0.123[c]	0.053[d]
Kaempferol	0.057[a]	0.022[b]	0.082[c]	0.032[d]
Isorhamentin	0.071[a]	0.033[b]	0.080[c]	0.049[d]

Means of three replicates. Different letters in each row indicate significant differences.
GB: green Berry; RB: roasted Berry; GH: green Harar; RH: roasted Harar.

Table 2: Natural antioxidants (mg/kg) in green and roasted Arabic coffee

Natural antioxidants	GB	RB	GH	RH
Vitamin C	209.5[a]	176.4[b]	229.9[c]	205.4[d]
Vitamin E (α-tocopherol)	33.2[a]	20.0[b]	30.7[c]	26.8[d]

Means of three replicates. Different letters in each row indicate significant differences.
GB: green Berry; RB: roasted Berry; GH: green Harar; RH: roasted Harar.

In regular, the typical Arabian consumer consumes ≈1 liter of Arabic coffee daily, this amount delivers approximately 152.5 and 178 mg of flavonoids in roasted Berry and Herar, respectively. Such daily intake contributes to 35.9 and 41.9% of flavonoids of the Recommended Daily Allowance (RDA) for two coffee types. (Table 3).

Table 3: Approximate percents contribution of flavonoids and vitamins by daily cups of roasted Berry and Harar Arabic coffee*

Components	mg components in 50g	Average RDA needed (mg)	% of RDA** components in daily cups
Flavonides			
RB	152.5	425 (50-800)	35.9
RH	178	425 (50-800)	41.9
Ascorbic acid (C)			
RB	8.8	70	12.6
RH	10.3	70	14.7
α-tocopherol (E)			
RB	1.0	8.5 (7-10)	11.8
RH	1.3	8.5 (7.10)	15.3

*** Coffee prepared by adding 50g of roasted powder coffee to 1 liter boiled water and brewed for 10 min. This quantity is daily consumed by moderate consumer.**
**** Recommended Daily Allowance (RDA) according to (Buhler and Miranda, 2004).**
RB: roasted Berry; RH: roasted Harar.

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 (7-10 mg). The high consumption of tea and coffee may be most influential on total flavonoid intake in certain groups of people (Buhler and Miranda, 2004).

It is reported that tea flavonoides are water soluble. Lakenbrink *et al.*, (2000) reported that a cup of tea allowed to brew for 40-60 seconds will typically deliver approximately 140 mg of flavonoids. Also, the UK Trade Technical Committee stated that using typical British consumer brewing condition and encompassing the range of blends and bag weights commonly on the UK gives a figure of 125mg/235ml serving. The longer the tea is left brew, the higher the concentration of flavonoides (Englehardt, 1999). According to our knowledge there are no available information regarding the flavonoids in coffee even its compensate to RDA.

Table (2) shows the quantity of α-tocopherol in green and roasted arabic coffee (Berry and Herar beans). The results show that vitamin E (α-tocopherol) were 33, 20 mg/kg beans in green and roasted Berry while it was 30.7, 26.8 mg/kg in green and roasted Herar beans. Ogawa *et al.* (1989) determined the contents of tocopherols by HPLC in 14 green coffee beans, their roasted beans and infusions, and in 38 instant coffees. The maximum of total tocopherols in the green coffee beans was 15.7 mg/100g and the average was 11.9 mg/ 100g. The contents of α- and β- tocopherol were 2.3 to 4 5 and 3.2 to 11.4 mg/100g respectively, γ- and δ- tocopherol were not found.

In the present study, roasting diminishes the content of α-tocopherol significantly (Table 2), the same trend was demonstrated by Coors (1984) which 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.

Tawfik and El-Bader (2005) reported that the oil content in green and roasted Berry and Herar Arabic coffee beans were 12.6, 11.3, 12.3, 11.8%. respectively Hence, the concentration of vitamin E reached to 264.5, 176.7, 249.5, and 226.5 mg/kg oil in green Berry, roasted Berry, green Herar and roasted Herar, respectively.

The presence of tocopherols in coffee oil was reported by Folstar *et al.* (1977) for the first time, α-tocopherol was clearly identified, while β- and γ-tocopherol, not being separated by TLC and GC, were considered as one group. Croos (1984) also determined total, β- and γ-tocopherol by HPLC. Folstar *et al.* (1977) found that concentrations of α-tocopherol of 89 to 188 mg/kg oil, and values for β- plus γ-tocopherol of 252-530 mg/kg oil. In the arabic coffee oil, values of 161 mg/kg α-tocopherol and 597 mg/kg β-tocopherol were found; where the robusta coffee oil contained 107 mg/kg α-tocopherol and 260 mg/kg α-tocopherol (Coors, 1984).

Tocopherols have been analysed in coffee brews. The contents of total tocopherols in coffee infusions and instant coffee solutions were determined as 0.003-0.013 and 0.001-0.013 mg/100ml, respectively (Ogawa *et al.*, 1989). Coors (1984) analyzed α-, β- and γ- tocopherols 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/100g. The predominance of α- tocopherol is a prominent feature of coffee beans, in contrast to other vegetables and fruits.

Daily consumption of several cups (≈1 liter) of Arabic coffee daily deliver approximately 1.0 mg and 1.3 mg of tocopherols for Berry and Herar coffees, respectively. This daily intake contributes to amount of 11.8-15.3% of vitamin E of the RDA (Table 3). The average daily intake of vitamin E is 7-10 mg (Buhler and Miranda, 2004).

Table 2 shows the content of vitamin C in the two types of green and roasted arabic coffee. Harar beans had a higher vitamin C content than that of Berry beans while roasting process decreased its content significantly (Table 2). No available datas about the content of vitamin C in the arabic coffee in the literatures.

The tea (*Strobilanthes crispus*) leaves contained high amounts of vitamins, especially ascorbic acid (9.8%), in the fresh leaves. Indian tea did not show any appreciable amount of ascorbic acid due to losses during processing (Ismail *et al.*, 2000).

Consumption of arabic coffee (≈1 liter) during the day deliver 8.8 mg and 10.3 mg of vitamin C for Berry and Herar coffees, respectively. This represents 12.6, and 14.7% of the RDA of vitamin C for both coffee types, respectively (Table 3).

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الفلافونيدات و مضادات الاكسدة الاخرى فى حبوب القهوة العربية الخام و المحمصة

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تم تقدير أربع مركبات من المواد الفلافونيدية (روتين ، كورستين ، كيمفيرول، ايزورامنتين) و مضادات الأكسدة الطبيعية الأخرى (فيتامين هـ ، ج) في نوعين من القهوة الحبشية (البرى، الهراري) و المنتشرة في منطقة الجزيرة العربية.

محتوى الفلافونيدات و فيتامين ج فى حبوب الهراري الخام أعلى من البرى الخام بينما كان فيتامين هـ أعلى في حبوب البرى.

تم تقدير نسب المواد الفلافونيدية و الفيتامينات التي يتم الحصول عليها يوميا كنتيجة شرب القهوة . وجد أن الفلافونويد من نوع الروتين هو النوع السائد و كانت كميته ٠,٢٧ و ٠,٢٣ جم/١٠٠جم في حبوب القهوة من نوع الهراري الخام و المحمصة على التوالي. كانت أعلى نسبة لفيتامين ج هي ٢٢٩,٩مجم/كجم في حبوب الهراري الخام ، بينما كانت أعلى نسبة من فيتامين هـ هي ٣٣,٢مجم/كجم في حبوب البري الخام. كان للتحميص تأثير معنوي في تقليل هذه النسب. تم تقييم ما يقدمه مشروب القهوة من الكمية التي ينصح بتناولها يوميا من المواد الفلافونيدية و فيتامين هـ و فيتامين ج. فوفقا للاستهلاك الفعلي اليومي من مشروب القهوة العربية (حوالي ١ لتر) في الوجبة العربية ، وجد أنها تساهم بمقدار ٣٥,٩% و ٤١,٩% من الفلافونيدات ، ١٢,٦ % و ١٤,٧% من فيتامين ج ، و ١١,٨% و ١٥,٣% من فيتامين هـ لكل نوعي البري و الهراري المحمصة على التوالي.