J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 2 (1): 31 - 47, 2011

POLYPHENOLS AND CAFFEINE OF GREEN AND ROASTED COFFEE BEANS, AS NATURAL ANTIOXIDANTS

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ABSTRACT

Green, light, medium and dark roasted coffee beans were used in the powder form to identify polyphenols by HPLC. Eleven components were identified in green, light and dark coffee roasted powder. Para Coumaric, Ferulic, Salicylic and Cinnamic acids were absent in medium roasted coffee powder. Polyphenol extract was more effective for the inhibition of lipid peroxidation than caffeine and mixture of polyphenols and caffeine. Antioxidant capacity by 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid cation radical ($ABTS^{+}$) showed a higher percentage of inhibition for polyphenols of green coffee (86.09 %) than light (82.67 %), medium (81.14 %) and dark (79.43 %). Caffeine of dark coffee samples has the lowest value of inhibition (72.0 %) followed by medium coffee sample (72.95 %) then light sample (75.05 %), finally the green coffee sample (77.34 %). It seems that oxidative activity was affected with two factors: temperature and period of roasting.

INTRODUCTION

Antioxidants play an important role in preventing or delaying autooxidation and have attracted a lot of attention as food additives. Both synthetic and natural antioxidants are widely used in many food products. Natural antioxidants have been developed since past decade, mainly because of the increasing limitations on the use of synthetic antioxidants. In general, natural antioxidants are prefered than synthetic ones because of their safety and friendship to the environment. There has been a growing interest in replacing them with natural ingredients because of the toxicity of synthetic antioxidants. Now, it is well known that the use of some common synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) has become controversial issue because of adverse toxological effect. Hence, in recent years, the evaluation of antioxidative activity of naturally occurring substances has been of interest (Imaida *et al*., 1983).

The coffee plant belongs to the genus of Coffea (Rubiaceae family). Coffee is a giant global industry and ranks second only to petroleum in terms of dollars traded worldwide. Presently, coffee production is about 6.3 million tons, with Brazil and Colombia contributing to nearly 44 % of these figures (Madhava Naidu *et al*., 2008).

Coffee is known to be a rich source of compounds with potent antioxidant activity. In a study performed with rats, it was observed that feeding rats with coffee brew resulted in an increase of the total antioxidant capacity of the plasma (Somoza *et al*., 2003).

A major contributor to the antioxidant activity was identified as N-Methylpyridinium, a recently discovered alkaloid that is present in roasted coffee in concentrations of up to 0.25 % on a dry weight basis (Stadler *et al*.,

2002). Moreover, coffee consumption has been associated with reduced incidences of several types of cancer (Leitzmann *et al*., 2007); Tavani and La-Vecchia, 2000), liver Cirrhosis (Tverdal and Skurtveit, 2003) and type 2 diabetes (Van Dam and Feskens, 2002). The antioxidant capacity of coffee has been attributed to its content of polyphenols and melanoidins (Anese and Nicoli, 2003; Delgado-Andrade and Morales, 2005 and Yen *et al*., 2005). Acrylamide and melanoidins are both Maillard reaction products (MRPs) formed during the roasting of coffee, typically conducted at temperatures between 220 and 250 ^{o}C. Theoretically, any attempt to inhibit the Maillard reaction as a possible measure to minimize the formation of acrylamide would lead to a reduction of the antioxidant capacity of coffee (Summa *et al*., 2006).

More intense roasting, i.e. greater thermal load, of coffee beans has been considered as a way to decrease the concentration of acrylamide in coffee, albeit with a major impact on the organoleptic properties and consequently acceptability of the product. However, the reduction in the concentration of acrylamide with darker degrees of roasting is accompanied by a reduction of the radical scavenging capacity of coffee. Furthermore, temperature, time and the speed at which coffee is roasted have an important impact on the organoleptic properties of coffee and under extreme conditions could generate other "undesirable" compounds. In this study, a trial was carried out to identify and determine the major components of polyphenols in green, light, medium and dark coffee beans. Also, radical scavenging activity was expressed as the inhibition percentage. Separated caffeine, polyphenol and mixture of both, in different concentrations, were used to retard the autooxidation of sunflower oil.

MATERIALS AND METHODS

Sampling

1-Green coffee sample was purchased from local market.

2-Light roasted coffee powder: green coffee (0.5 kg) was roasted at 160 ^{o}C for 2 hrs. and milled, at the Laboratory. of Agric. Chem., Fac. of Agric., Mansoura Univ.

3-Medium roasted coffee powder: green coffee (0.5 kg) was roasted at 160 ^{o}C for 5 hrs. exactly as described before.

4-Dark roasted coffee powder (commercial sample): green coffee (0.5 kg) was roasted and milled in the market by their own procedure.

Routine analysis:

Moisture, ash, protein, lipid and carbohydrates contents for all samples were determined according to the method of A.O.A.C. (2000).

Reliminary phytochemical tests:

Preliminary phytochemical tests were carried out on the samples under investigation, to detect the presence of terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrates, phenolic glucosides and resins as reported by Harborne (1988).

Identification of polyphenols by HPLC:

Phenolic compounds of the forementioned samples were extracted according to the method of Ben-Hammouda *et al*. (1995). Identification of individual phenolic compounds were performed on a Hewlett-Packard HPLC (Model 1100). Eleven standard phenolic compounds were used namely Catechin, P-hydroxy benzoic acid, Chlorogenic acid, Caffeic acid, Syringic acid, Caffeine, Vanillic acid, P-Coumaric acid, Ferulic acid, Salicylic acid and Cinnamic acid were obtained from Sigma (st. Louis, USA) and Merck-Schuchardt (Munich, Germany). Reagents and solvents used were specific for HPLC spectral grade.

Evaluation of antioxidant activity of caffeine and polyphenols of coffee bean samples:

Caffeine and polyphenols of green coffee beans, light, medium and dark were extracted using the method of A.O.A.C. (2000) for caffeine and the method of Singleton and Rossi (1965) for polyphenols. Separated samples of caffeine, polyphenols and mixture of caffeine and polyphenols in the ratio of 1:1 (wt/wt) were used for the evaluation of antioxidative activity by two means:

Determination of antioxidant capacity by 2,2¯-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical ($ABTS^+$) :

$ABTS^+$ assay based on the method of Re *et al*. (1999) with slight modifications. $ABTS^+$ solution was added to MnO_2 solution, then shaked and centrifuged for 10 min., clear supernatant was separated. Investigated samples were prepared (separately) as follow:

Exactly 1 mg of methanolic extract (ME) was dissolved in mixture of methanol and phosphate buffer 0.1 M, pH 7.0 in the ratio of 1:1. Resultant solution (20 μl) was added to $ABTS^+$ solution, as described before. Blank sample was prepared exactly in the same manner but differ only in the addition of 20 μl of 2mM ascorbic acid, instead of investigated sample-buffer solution.

Absorbance of resulting green-blue color was measured at 734nm. Decrease in absorbance is expressed as a percentage of inhibition which was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{A_{Blank} - A_{Test}}{A_{Blank}} \times 100$$

Effect of caffeine, polyphenols and their mixture on the stability of sunflower oil:

Extracted caffeine, polyphenols or a mixture of them were added separately to sunflower oil in concentrations of 1000, 3000 and 6000 ppm. The experiments were carried out as follows:

Exactly 25 g of crude sunflower oil sampls were placed in 120 x 30 mm Petri dishes and the calculated ppm values were added to each oil sample and well stirred to ensure uniform distribution in the oil. Another experiment was performed using the synthetic antioxidant 100 ppm BHT. A control

experiment (sunflower oil without any addition) was also conducted in the same manner.All samples were incubated at 60 ^{o}C (Alaiz *et al*., 1995) for 20 days. Peroxide values (PV) were determined daily to measure the stability of oil samples according to IUPAC (1987).

Protection index (PI) was calculated according to the following equation (Alaiz *et al*., 1995):

$$PI = 100 - [100 (PV_{ME} – PV_{BHT}) / (PV_{control} – PV_{BHT})]$$

Where: PV_{ME}, PV_{BHT} and $PV_{control}$ are peroxide values of treated oils with ME, BHT and control sample, respectively.

RESULTS AND DISCUSSION

Chemical composition of green and roasted coffee beans:

Samples under investigation were used for the determination of: moisture, ash, protein, lipid, total carbohydrates and caffeine. The proximate composition of green and roasted coffee beans is shown in Table (1). Moisture of green coffee samples represents approximately 11.52 % with the highest value for the green sample and the lowest value for the dark sample. Moisture levels are within the range of 8.5-13.0 % as reported by Clarke (1985). After roasting moisture levels decreased to an average of 3.85, 2.15 and 1.63 % for light, medium and dark coffee powder, respectively. Ash contents were 5.15, 5.68, 5.98 and 5.63 % for green, light, medium and dark coffee respectively. Protein levels for green coffee sample were 15.93 %, 16.19 % for light coffee, 17.96 % for medium and 16.84 % for dark coffee powder. These values are within the range of 11.0-16.5 % as reported by Macrae (1985) and Oliveira *et al*., (2006). Difference in protein levels may be due to the difference in moisture contents as described before. It is worthy to state that these results were based on the determination of crude nitrogen, so they include caffeine and other nitrogenous compounds. The lipid contents were found to be different among green, light, medium and dark coffee. Medium and dark coffee powder had higher oil contents than those of light and green coffee. The lipid contents ranged from 9-15 % as stated in literature (Turatti, 2001 and Oliveira *et al*., 2006). Total carbohydrates were ranged between 53.5 and 60.00% as shown in Table (1). From the same table caffeine was represented 1.37, 1.47, 1.55 and 1.97 % for green, light, medium and dark coffee, respectively. These levels of caffeine were in agreement with those obtained by Paulo Mazzafera (1999). Differences in the levels of caffeine may be due to differences of moisture contents.

Table (1): Some chemical composition content (gm/100g dry basis).

Some chemical composition / Sample	Green	Roasted coffee beans		
		Light	Medium	Dark
Moisture	11.52	3.85	2.15	1.63
Ash	5.15	5.68	5.98	5.63
Protein	15.93	16.19	17.96	16.84
Lipid	13.91	15.15	16.67	15.91
Total carbohydrate	53.50	59.14	57.24	60.00
Caffeine	1.37	1.47	1.55	1.97

Preliminary phytochemical tests:

Obtained data indicated the presence of terpenes, tannins, flavonoids, alkaloids, carbohydrates, phenolic glycosides, polyphenolic compounds and caffeine. On the other hand, saponins and resins were absent in all samples as shown in table (2).

Table (2): The preliminary phytochemical tests of crude methanolic extract of coffee beans.

Tests / Sample	Terpenes	Tannins	Flavonoids	Saponins	Alkaloids	Carbohydrate or glycosides	Phenolic glycosides	Polyphenolic compounds	Caffeine	Resins
Green	+	+	+	-	+	+	+	+	+	-
Light	+	+	+	-	+	+	+	+	+	-
Medium	+	+	+	-	+	+	+	+	+	-
Dark	+	+	+	-	+	+	+	+	+	-

Identification of polyphenols of investigated samples using HPLC technique:

Technique of HPLC was used for identification and determination of polyphenolic compounds in coffee beans (green, light, medium and dark roasted samples).

Eleven polyphenolic compounds were available as authentic samples namely Catechin, Caffeine, P-hydroxy benzoic, Chlorogenic, Caffeic, Syringic, Vanillic, Coumaric, Ferulic, Salicylic and Cinnamic acids.

Obtained data revealed that 25, 26, 27 and 25 compounds with different retention times were detected in HPLC chromatograms of green coffee beans, light, medium and dark roasted samples, respectively.

Table (3) indicated clearly that all investigated samples contained Catechin, Caffeine, P. hydroxy benzoic, Chlorogenic, Caffeic, Syringic and Vanillic acids with different concentrations. From the same table it could be noticed that P. Coumaric, Ferulic, Salicylic and Cinnamic acids were found in green and roasted coffee at the market and also in the roasted light sample, while they were absent in the medium roasted sample. This absence of previous mentioned compounds may be attributed to the effect of roasting time (5 hrs.) as reported by Sacchetti *et al*., (2009); Parliment (2000); Steinhart *et al*., (2002) and Duarte *et al*., (2005). They concluded that polyphenols content was observed decreasing with roasting. It is worthy to mention here that , dark roasted coffee bean sample obtained from the local market was prepared by heating at higher temperature for shorter time.

Also, Del Castillo *et al*. (2002) found that, during the roasting process, the naturally occurring polyphenol constituents are transformed to a complex mixture of Maillard reaction products.

Table (3): Identification of polyphenols of green, light, medium and dark roasted coffee beans using HPLC technique.

Components	Green		Roasted coffee beans					
			Light		Medium		Dark	
	Rt.	Area%	Rt.	Area%	Rt.	Area%	Rt.	Area%
Catechin	2.42	6.63	2.43	7.41	2.46	7.05	2.48	4.80
P.oH Benzoic acid	2.65	1.55	2.64	1.06	2.63	0.87	2.59	0.47
Chlorogenic acid	2.73	35.11	2.73	21.74	2.73	8.80	2.73	7.37
Unknown	2.85	7.40	2.15	0.39	2.14	0.83	2.15	0.26
Unknown	3.30	0.91	2.32	1.12	2.27	0.68	2.38	1.80
Caffeic acid	3.31	2.25	3.32	2.89	3.32	4.03	3.32	2.57
Syringic acid	3.85	0.51	3.85	1.29	3.82	0.42	3.85	1.21
Caffeine	4.02	3.55	4.02	3.61	4.00	2.28	4.02	3.49
Unknown	4.36	14.04	4.37	27.48	4.37	25.37	4.36	40.74
Vanillic acid	4.40	11.30	4.49	5.00	4.40	3.05	4.44	17.49
Unknown	4.66	7.80	3.37	1.90	2.77	1.12	3.39	1.23
Unknown	5.19	0.44	3.62	2.08	2.84	6.02	3.64	2.33
Unknown	5.67	4.44	2.85	6.90	3.42	0.52	2.82	4.66
Unknown	6.10	0.40	4.57	7.58	3.50	2.40	4.99	3.25
P. Coumaric acid	6.23	0.26	6.25	0.47	---	---	6.24	0.55
Unknown	6.30	0.11	4.97	3.18	3.63	1.67	5.36	1.20
Ferulic acid	6.41	0.02	6.35	0.16	---	---	6.33	0.49
Unknown	6.54	0.39	5.68	3.19	3.70	1.04	5.67	1.86
Unknown	6.71	0.52	6.72	0.38	3.77	0.40	6.08	0.58
Salicylic acid	6.85	0.35	6.86	0.04	---	---	6.87	0.31
Unknown	6.99	0.37	7.03	0.72	4.12	0.41	7.03	1.17
Unknown	7.59	0.28	8.55	0.22	4.16	0.31	7.58	1.38
Unknown	8.70	0.60	8.62	0.10	4.18	0.26	8.53	0.42
Cinnamic acid	8.82	0.11	8.89	0.13	---	---	8.82	0.13
Unknown	9.07	0.69	9.06	0.14	4.20	0.25	8.61	0.27
Unknown	---	---	9.11	0.82	4.23	0.30	---	---
Unknown	---	---	---	---	4.41	25.50	---	---
Unknown	---	---	---	---	4.97	2.98	---	---
Unknown	---	---	---	---	5.28	0.31	---	---
Unknown	---	---	---	---	5.35	1.02	---	---
Unknown	---	---	---	---	5.68	2.12	---	---

Chlorogenic acid was the main component of polyphenols and reached 35.11, 21.74, 8.80 and 7.37 % for green coffee beans, light, medium and dark roasted samples, respectively. Difference in concentrations of chlorogenic acid may be due to the effect of heat and time of roasting. Our findings about identification of polyphenols from green coffee beans, roasted coffee obtained from local market, roasted coffee in Laboratory for different periods i.e. 2 and 5 hrs. were in agreement with those reported by Naidu *et al*. (2007). They stated that green coffee contained polyphenols such as ferulic and caffeic acids, while chlorogenic acid was the main compound and their concentrations varied according to the species and roasting time.

Clifford (1999) and Daglia *et al.,* (2000) concluded that every 1 % reduction in dry matter, 8-10 % of chlorogenic acid is lost subsequently. Green coffee beans had a higher chlorogenic acid content than roasted

beans which was then reflected in higher antioxidant activity of green coffee beans as compared to roasted coffee beans.

Antioxidant capacity determined by ($ABTS^+$) cation radical :

The capacity of caffeine and polyphenols extracts of green coffee, light, medium and dark roasted samples to scavenge the $ABTS^+$ radical were determined separately and compared with the reduction of ascorbic acid as control sample which is known as a strong reducing agent.

From table (4) and Figures (1 and 2), it could be seen that all extracts showed different degrees of inhibition capacity, but their capacities were less than ascorbic acid which had the maximum inhibition (91.41 %).

The same table indicated clearly that extracts of polyphenols for all samples had the absorbance values of 0.073, 0.091, 0.099 and 0.108 with the values of 86.09, 82.67, 81.14 and 79.43 as percentage of inhibition, for green coffee, light, medium and dark roasted samples, respectively.

On the contrary, the absorbance values of caffeine for all samples had the values of 0.119, 0.131, 0.142 and 0.147 with the values of 77.34, 75.05, 72.95 and 72.00 as percentages of inhibition for green, light, medium and dark roasted samples, respectively.

Table (4): Reducing power of caffeine and polyphenols extracts from green, light, medium and dark roasted coffee beans.

Extract	Sample	Absorbance at 734 nm	% Inhibition
Caffeine	Green	0.119	77.34
	Light for 2hrs	0.131	75.05
	Medium for 5hrs	0.142	72.95
	Dark (local market)	0.147	72.00
Polyphenols	Green	0.073	86.09
	Light for 2hrs	0.091	82.67
	Medium for 5hrs	0.099	81.14
	Dark (local market)	0.108	79.43

Finally it could be concluded that all extracts of polyphenols had a higher inhibition capacity as percentages compare with caffeine. Also, polyphenols and caffeine extracts from green coffee had the highest values of inhibition as percentage than those of all roasted samples.

From the same table it could be observed that polyphenols and caffeine extracts of roasted coffee at the market (dark) had the lowest values of inhibition, this may be attributed to the effect of high temperature which used in roasting. Del Castillo *et al*. (2002) reported that during the roasting process, the naturally occurring polyphenolic constituents are transformed to a complex mixture of Maillard reaction products. Castellucio *et al*. (1995) when they used the $ABTS^+$ method, stated that light or medium roasted coffee have been shown higher antioxidant activity, which was in agreement with our finding.

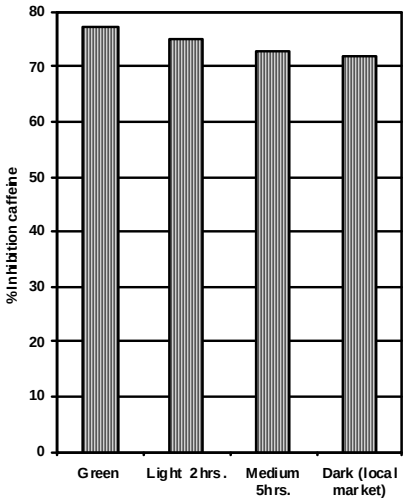

Fig.(1): Antioxidant activity of caffeine extract of green and roasted coffee beans by the reaction with ($ABTS^{+}$) cation radical.

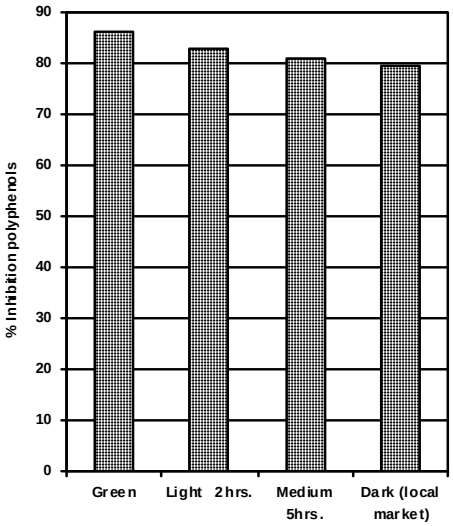

Fig. (2): Antioxidant activity of polyphenols extract of green and roasted coffee beans by the reaction with ($ABTS^{+}$) cation radical.

Caffeine and polyphenols extracted from coffee beans as natural antioxidants:

This trial was carried out to evaluate the effect of caffeine and polyphenols extracted from roasted coffee beans obtained from local market on the inhibition of lipids peroxidation. Peroxide value was taken as a good parameter to evaluate the stability of sunflower oil during incubation at 60 ^{o}C for 20 days. Peroxide value for fresh sunflower oil sample was 6.30 meq O_2/kg oil.

Tables (5, 6 and 7), show the peroxide values of control and treated samples with 100 ppm BHT as synthetic antioxidant, caffeine, polyphenols and mixture of caffeine and polyphenols methanolic extract as natural antioxidants in different concentrations, i.e. 1000, 3000 and 6000 ppm. Obtained data of peroxide values were divided by the peroxide value of fresh sunflower oil sample (6.30).

From the same table, it could be concluded that calculated peroxide value for the untreated sample (control) increased gradually from 1.09 after one day to a maximum value of 286 after 13 days from the beginning of incubation at 60 ^{o}C.

Peroxide values of the control sample were continually decreased after 14 days till the end of the experiment i.e. (259.84, 215.81, 194.35, 165.58, 158.61, 154.06 and 111.25 after 14, 15, 16, 17, 18, 19 and 20 days, respectively).