Effect of roasting on antioxidant and anticholinesterase capacities of coffee

PELIN KÖSEOĞLU YILMAZ – IŞIL HACIBEKIROĞLU – UFUK KOLAK

Summary

The aim of the study was to investigate the effect of the roasting degree on total phenolic and flavonoid contents, antioxidant and anticholinesterase activities of the hexane, acetone, ethanol and water extracts of coffee. The antioxidant capacity of the water extracts prepared according to the brewing procedure of Turkish coffee was measured for the first time by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and cupric ion reducing antioxidant capacity (CUPRAC) assays. The in vitro anticholinesterase activity of the coffee extracts and caffeine was determined for the first time. Water extracts of the coffee samples exhibited the highest 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation radical scavenging, DPPH free radical scavenging and CUPRAC effects. The hexane extract of coffee roasted for 25 min showed the highest acetylcholinesterase inhibitory activity among the tested extracts. Caffeine, which is known to have therapeutic effect against Alzheimer's disease, was found to possess almost the same anticholinesterase activity as galanthamine. The roasting degree had an irregular effect on the antioxidant and anticholinesterase activities of the coffee extracts. The caffeine contents in extracts of coffee roasted for 25 min, which had strong anticholinesterase effect, were determined by HPLC, and the water extract was found to possess the highest caffeine content.

Keywords

coffee; antioxidant; anticholinesterase; caffeine; high-performance liquid chromatography; Alzheimer

The genus *Coffea* (Rubiaceae family) has over sixty different species. Three of them, namely, *Coffea arabica* L. (arabicas), *C. robusta* L. (robustas) and *C. liberica* L., have economical value [1]. *C. arabica* and *C. robusta* originate from the highlands of Ethiopia and from lower altitudes across the Ivory Coast and Uganda, whereas *C. liberica* originates from Liberia [2].

The chemical composition of green beans (green coffee) depends on *Coffea* species [3]. Green coffee has a complex chemical composition of mono- and polysaccharides, lipids, sterols, fatty acids, phenolic acids, alkaloids, diterpenes, diterpenic and triterpenic esters and ceramide [2, 4]. Green coffee also contains several elements such as potassium, magnesium, calcium and phosphorus. [5].

The chemical composition and biological activity of coffee are influenced by the roasting process. During roasting, phenolic compounds partially degrade and/or form polymer compounds in the Maillard reactions, and caramelization of saccharides as well as pyrolysis of organic compounds take place [6]. Coffee possesses strong antioxidative compounds such as chlorogenic acids, hydroxycinnamic acids, caffeine and melanoidins [7]. During roasting, particularly with the degradation of chlorogenic acids, the content of phenolic compounds in coffee decreases. There is also some loss in the caffeine content by sublimation but most of it quantitatively transfers to the brew [2]. Despite the decrease of the phenolic content in the roasted coffee, the antioxidant activity of coffee may remain the same or even be

Pelin Köseoğlu Yilmaz, Ufuk Kolak, Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, Beyazit 56, 34116 Istanbul, Turkey.
Işil Hacibekiroğlu, Department of Analytical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, Fatih 105, 34093 Istanbul, Turkey.

Correspondence author:
Ufuk Kolak, e-mail: ufukkolak@yahoo.com, tel.: +90 212 4400258; fax: +90 212 4400254

improved by the Maillard reactions' final products, melanoidins, which possess very high antioxidant activity [8].

The consumption of caffeine, which is the primary purine alkaloid of coffee, may cause positive or negative effects on human health depending on the daily intake, the consumer's health condition and age. Some epidemiologic studies suggest that caffeine might be an effective therapeutic agent against Alzheimer's disease (AD), which is one of the health problems among the elderly people, and its consumption might reduce the risk factors. Also coffee/caffeine, but not decaffeinated coffee, has been associated with a lower relative risk of Parkinson's disease [9].

In the current study, it was aimed to determine the effect of the roasting degree on total phenolic and flavonoid contents, and antioxidant as well as anticholinesterase activities of the coffee samples. The water extracts of green beans and four different roasted samples were prepared according to the brewing procedure of Turkish coffee. The hexane, acetone and ethanol extracts of the samples were also prepared. In addition, regarding the therapeutic potential of caffeine against AD, the caffeine contents of extracts from coffee roasted for 25 min, which exhibited high anticholinesterase activity, were determined by high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Materials

Green beans of *Coffea arabica* L. from Brazil and coffee samples roasted at 140–160 °C for 12 min, 20 min, 22 min, 25 min, and grounded, were purchased from a local market (Izmir, Turkey) in May 2012.

Chemical substances

Ethanol, hexane, acetone, potassium acetate, potassium thiosulfate, aluminium nitrate nonahydrate, potassium dihydrogen phosphate, sodium bicarbonate and HPLC-grade methanol, acetonitrile and ortophosphoric acid were supplied from Merck (Darmstadt, Germany); pyrocatechol, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), copper (II) chloride dihydrate, 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB), electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 $U \cdot mg^{-1}$) and horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 $U \cdot mg^{-1}$) from Sigma (St. Louis, Missouri, USA); acetylthiocholine iodide and Folin-Ciocalteu reagent from Applichem (Darmstadt, Germany); neocuproine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and galanthamine hydrobromide from Sigma-Aldrich (St. Louis, Missouri, USA); 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), butyrylthiocholine iodide and caffeine from Fluka Chemie (Fluka Chemie, Steinheim, Germany); ammonium acetate, sodium carbonate, sodium hydrogen phosphate and sodium dihydrogen phosphate from Reidel de Haen (Seelze, Germany).

Preparation of the coffee extracts

In this study, green beans and four coffee samples roasted at different degrees were used for the preparation of the extracts (Tab. 1). Approximately 4.5 g of coffee and 50 ml of water are used to prepare Turkish coffee (one cup of coffee). To determine the effect of roasting degree on the total phenolic and flavonoid contents, antioxidant and

Tab. 1. Classification of coffee extract samples and extraction yields of different solvents.

Sample codes	Roasting time [min]	Extraction yield [%]
Hexane extracts		
H1	–*	10.3
H2	12	12.8
H3	20	13.5
H4	22	14.2
H5	25	10.2
Acetone extracts		
A1	–*	10.4
A2	12	11.1
A3	20	12.1
A4	22	12.4
A5	25	10.1
Ethanol extracts		
E1	–*	8.5
E2	12	12.0
E3	20	11.8
E4	22	12.1
E5	25	9.5
Water extracts		
W1	–*	22.7
W2	12	22.1
W3	20	17.7
W4	22	19.2
W5	25	18.5

* – green beans.

anticholinesterase potentials of one cup of coffee samples, the extracts were prepared as follows: 4.5 g of the coffee sample were macerated with 50 ml of the solvent (hexane, acetone and ethanol) at room temperature for 10 min. After filtration, the solvent was evaporated under reduced pressure.

The water extracts were prepared following the brewing procedure of Turkish coffee, which is boiling 4.5 g of the coffee sample with 50 ml of distilled water for 10 min. The extract was filtered and water was evaporated under reduced pressure.

Instrumentation

Spectrophotometric analyses were performed by a Power Wave XS microplate spectrophotometer (BioTek, Winooski, Vermont, USA). HPLC analyses were done using an Agilent 1100 series system with a reverse-phase column C18 (30 cm × 3.9 mm × 10 μm, Agilent, Santa Clara, California, USA) and a photodiode array detector (Agilent).

Determination of total phenolic and flavonoid content

The solutions of the coffee extracts were prepared with ethanol at a concentration of 1 000 mg·l^{-1}. A volume of 180 μl of distilled water, 4.0 μl Folin-Ciocalteu reagent and 12.0 μl of 2% Na_2CO_3 solution were added to 4.0 μl of the sample solution and the mixture was kept for 2 h at room temperature. After that, the absorbance of the sample was measured at 760 nm. The calibration curve of pyrocatechol was prepared with the standard solutions in the concentration range of 0.5–4.0 μg·ml^{-1} and the total phenolic content results were expressed as pyrocatechol equivalents (PE) [10]. For the determination of total flavonoid content, 172 μl of 80% ethanol and 4 μl of 1.0 mol·l^{-1} potassium acetate were added to 20 μl of sample solution. After 1 min, 4 μl of 10% $Al(NO_3)_3$ solution was added to the mixture and, after 40 min, the absorbance was measured at 415 nm. The calibration curve of quercetin was prepared in the concentration range of 5–40 μg·ml^{-1} and the total flavonoid contents were expressed as quercetin equivalents (QE) [11].

Antioxidant activity

The antioxidant capacity of the coffee extracts and caffeine was calculated as Trolox-equivalent antioxidant capacity (*TEAC*). The calibration curve of Trolox was prepared with different concentrations in the range of 10–80 μmol·l^{-1}.

The sample solutions were prepared with ethanol for ABTS cation radical decolorization and DPPH free radical scavenging assays and with distilled water for cupric reducing antioxidant capacity (CUPRAC) assay at a concentration of 1 000 mg·l^{-1}.

ABTS cation radical decolorization assay

The solution of ABTS cation radical was generated by 19.2 mg of ABTS and 3.3 mg $K_2S_2O_3$ in 5 ml of distilled water. The solution was kept in dark for 16 h at room temperature. Then, the solution was diluted so that its absorbance at 734 nm was approximately 0.70. A volume of 10 μl of sample solution and 30 μl of distilled water were mixed with 160 μl of ABTS cation radical solution and, after 6 min of incubation at room temperature, absorbance was measured at 734 nm [12].

DPPH free radical scavenging assay

The DPPH$^{\cdot}$ radical solution was prepared by dissolving 4.0 mg of DPPH in 100 ml of ethanol and mixing the solution for half an hour in the dark. A volume of 10 μl of sample solution and 30 μl of distilled water were mixed with 160 μl of DPPH$^{\cdot}$ solution and, after 30 min of incubation at room temperature, absorbance was measured at 517 nm [13].

Cupric reducing antioxidant capacity assay (CUPRAC)

A volume of 12.5 μl of the sample solution and 54.5 ml of distilled water were added to a solution prepared by adding 61.0 μl of 10 mmol·l^{-1} $CuCl_2$, 61.0 μl 7.5 mmol·l^{-1} neocuproine and 61.0 μl of 1.0 mmol·l^{-1} NH_4CH_3COO buffer (pH 7). The absorbance at 450 nm was measured after 1 h incubation at room temperature [14].

Anticholinesterase assay

The substrates of the reaction were acetylthiocholine iodide and butyrylthiocholine iodide whereas DTNB was used for the measurement of the anticholinesterase activity. The sample solutions in ethanol were prepared at a concentration of 4 000 mg·l^{-1}. Aliquots of 130 ml of 100 mmol·l^{-1} phosphate buffer (pH 8.0), 10 μl of sample solution and 20 μl AChE (or BChE) solution were mixed and incubated for 15 min at 25 °C. Then, 20 μl of DTNB solution (prepared by adding 2.0 ml of pH 7.0 and 4.0 ml of pH 8.0 phosphate buffer to a mixture of 1.0 ml of 16 mg·ml^{-1} DTNB and 7.5 mg·ml^{-1} $NaHCO_3$ in pH 7.0 phosphate buffer) was added. The reaction was initiated by the addition of 20 μl acetylthiocholine iodide (or butyrylthiocholine iodide). In this method, the activity was measured by following the yellow colour produced as a result of the thio anion produced by the enzymatic hydrolysis of the substrate react-

ing with DTNB [15]. The hydrolysis of these substrates was monitored at 412 nm.

Determination of the caffeine content

The solutions of the extracts of coffee roasted for 25 min were prepared at a concentration of 0.05 g·ml^{-1} in ultra pure water. Samples were filtered through a 0.45 μm filter before analysis. Ten microlitres of each sample were injected to an Agilent 1100 series HPLC system with a reverse-phase column and a photodiode array detector. The mobile phase consisted of 100 ml of HPLC grade acetonitrile and 900 ml of a solution prepared by dissolving 1.7 mg of potassium dihydrogen phosphate in 950 ml ultra pure water, adjusting pH to 3.5 with 5% ortophosphoric acid, and the volume to 1.0 l with ultra pure water. The flow rate was 1.0 ml·min^{-1} and the column temperature was kept at 40 °C. Detection was performed at 214 nm [16].

Statistical analysis

All of the analyses were made in triplicate. The statistical analysis was performed using the Excel software (Microsoft Office 2007, Microsoft, Redmond, Washington, USA) to calculate the means and the standard deviations, correlation and one-factor ANOVA test.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

The water extracts prepared as Turkish coffee were found to be the richest in phenolic contents, followed by the ethanol extracts (Tab. 2). These results suggest that most of the phenolics in the coffee extracts were relatively polar compounds. The flavonoid contents in the hexane extracts of the coffee samples roasted to different degrees were slightly higher than those of the other extracts, which might be caused by the presence of nonpolar flavonoids. It was also observed that the total phenolic content of the extracts was influenced irregularly by the roasting degree, which might be the consequence of non-destructed, destructed and/or re-formed phenolics through roasting. A disordered relationship was also observed between the roasting degree and the total flavonoid content.

Antioxidant activity

A literature survey showed that the roasting process may influence the antioxidant capacity of coffee, but there were no regular results indicating the net affect of the roasting degree [7, 17]. VIGNOLI et al. reported that the antioxidant activity of coffee was unaffected by the roasting degree because of the balance of degraded and newly formed compounds [7]. HEČIMOVIĆ et al. obtained fluctuated results regarding the effect of the roasting degree using ABTS cation radical assay, whereas the authors found the highest antioxidant potential at light roasting degree, using the ferric reducing ability of plasma (FRAP) assay [17]. It was also reported that the antioxidant capacity might vary between different coffee brewing techniques and also with the addition of milk [4, 18].

In this study, significant differences were determined between the antioxidant capacity of the green beans and the coffee samples roasted to dif-

Tab. 2. Total phenolic and flavonoid contents of the coffee extracts.

Samples	Total phenolics [g·kg^{-1}]	Total flavonoids [g·kg^{-1}]
Hexane extracts		
H1	12.66 ± 2.33	34.81 ± 6.00
H2	12.66 ± 2.33	42.21 ± 2.12
H3	11.02 ± 0.00	44.80 ± 1.16
H4	10.20 ± 1.16	32.53 ± 1.91
H5	12.66 ± 2.33	34.81 ± 6.00
Acetone extracts		
A1	15.41 ± 0.95	34.58 ± 0.58
A2	17.60 ± 1.65	31.90 ± 2.73
A3	14.31 ± 0.00	30.26 ± 0.79
A4	15.13 ± 1.16	28.81 ± 2.47
A5	20.89 ± 2.33	39.21 ± 0.96
Ethanol extracts		
E1	63.65 ± 1.65	31.90 ± 1.03
E2	43.09 ± 3.49	25.00 ± 3.00
E3	40.63 ± 2.33	24.63 ± 3.08
E4	60.36 ± 4.65	31.63 ± 3.10
E5	67.76 ± 8.14	27.77 ± 0.19
Water extracts		
W1	64.47 ± 3.49	30.35 ± 4.64
W2	90.79 ± 1.16	25.72 ± 4.24
W3	65.30 ± 0.00	11.28 ± 0.77
W4	59.81 ± 3.42	12.51 ± 1.73
W5	71.88 ± 2.33	26.13 ± 0.58

The results are average of three measurements ± standard deviation.
By statistical analysis the differences in total phenolic and flavonoid contents of the differently roasted coffee extracts were found to be significant ($p < 0.05$).
Phenolic and flavonoid contents were expressed as pyrocatechol equivalents per kilogram of dry extract and quercetin equivalents per kilogram of dry extract, respectively.

Tab. 3. Antioxidant capacity of the coffee extracts and caffeine.

Samples	$TEAC_{ABTS}$ [mol·kg^{-1}]	$TEAC_{DPPH}$ [mol·kg^{-1}]	$TEAC_{CUPRAC}$ [mol·kg^{-1}]
Hexane extracts			
H1	0.02 ± 0.007	NA	0.18 ± 0.018
H2	0.03 ± 0.009	NA	0.24 ± 0.041
H3	0.04 ± 0.007	NA	0.27 ± 0.035
H4	0.02 ± 0.007	NA	0.24 ± 0.011
H5	0.03 ± 0.002	NA	0.23 ± 0.042
Acetone extracts			
A1	0.12 ± 0.004	0.07 ± 0.008	0.30 ± 0.020
A2	0.05 ± 0.002	0.02 ± 0.000	0.26 ± 0.024
A3	0.09 ± 0.004	0.02 ± 0.002	0.30 ± 0.007
A4	0.06 ± 0.003	0.01 ± 0.003	0.24 ± 0.011
A5	0.07 ± 0.007	0.01 ± 0.004	0.24 ± 0.010
Ethanol extracts			
E1	0.09 ± 0.009	0.03 ± 0.002	0.28 ± 0.002
E2	0.04 ± 0.004	0.02 ± 0.005	0.23 ± 0.018
E3	0.05 ± 0.001	0.04 ± 0.008	0.21 ± 0.012
E4	0.05 ± 0.001	0.02 ± 0.010	0.27 ± 0.010
E5	0.07 ± 0.003	0.03 ± 0.004	0.22 ± 0.035
Water extracts			
W1	0.30 ± 0.079	0.24 ± 0.027	0.83 ± 0.039
W2	0.40 ± 0.046	0.27 ± 0.020	0.92 ± 0.044
W3	0.33 ± 0.013	0.14 ± 0.022	0.63 ± 0.030
W4	0.38 ± 0.015	0.21 ± 0.017	0.79 ± 0.036
W5	0.41 ± 0.010	0.21 ± 0.034	0.82 ± 0.032
Caffeine	NA	0.30 ± 0.016	NA

The results are average of three measurements ± standard deviation.
TEAC – Trolox equivalent antioxidant capacity (expressed as moles of Trolox per kilogram of dry extract), $TEAC_{ABTS}$ – *TEAC* value in ABTS assay, $TEAC_{DPPH}$ – *TEAC* value in DPPH assay, $TEAC_{CUPRAC}$ – *TEAC* value in CUPRAC assay. NA – not active.

ferent degrees ($p < 0.05$), except for the results of water extracts by ABTS assay and hexane extracts by CUPRAC assay ($p > 0.05$). Some small fluctuations were observed in the data obtained by all of the three assays (Tab. 3). The water extracts showed much higher antioxidant capacity than those of the hexane, acetone and ethanol extracts. Caffeine was found to be not active in ABTS and CUPRAC assays, whereas it had an average *TEAC* value in DPPH assay (Tab. 3).

To observe the contribution of the phenolic compounds to the antioxidant capacity of the extracts (Tab. 3), the total phenolic contents were correlated with the antioxidant capacity results at 95% confidence level. CUPRAC assay yielded the strongest correlation ($r = 0.68$) with the reduction of Cu(II)-neocuproine chelate cation to Cu(I)-neocuproine chelate by phenolic compounds. It was determined that the total flavonoid contents had no good correlation with antioxidant capacity.

Anticholinesterase activity

It was proposed that β-amyloid, the abnormal protein, might be one of the key factors in the progress of AD [19]. Cholinesterases promote the aggregation of β-peptides and amyloid formation. Arendash and Cao indicated the ability of moderate caffeine intake (the human equivalence of 500 mg or 5 cups of coffee per day) for protection or treatment of AD in a mouse model. It was reported that caffeine given in drinking water to AD mice was protective against memory impairment and also lowered brain levels of β-amyloid [20].

In this study, the anticholinesterase activity of

the extracts was measured as inhibition of AChE and BChE. Values of BChE inhibitory activity of the extracts were generally higher than those of AChE inhibitory activity (Tab. 4). The results for roasting degree were found to be significantly different from each other ($p < 0.05$), except for BChE data of the water and acetone extracts ($p > 0.05$). The effect of roasting degree on both AChE and BChE inhibitory activities of the samples was found to be irregular. The hexane extract of coffee roasted for 25 min (H5) exhibited the highest AChE inhibitory activity among the tested extracts.

Tab. 4. Anticholinesterase activity of the coffee extracts and caffeine.

Samples	Inhibition [%]	
	AChE	BChE
Hexane extracts		
H1	58.2 ± 0.5	84.1 ± 0.7
H2	76.6 ± 1.4	83.1 ± 1.2
H3	78.3 ± 0.7	80.6 ± 0.8
H4	52.5 ± 0.2	78.8 ± 0.3
H5	81.0 ± 0.1	77.3 ± 2.3
Acetone extracts		
A1	67.1 ± 0.8	83.5 ± 1.3
A2	56.9 ± 1.8	81.7 ± 5.2
A3	67.0 ± 1.9	81.9 ± 0.3
A4	66.7 ± 1.7	81.7 ± 2.2
A5	66.4 ± 2.8	83.1 ± 0.1
Ethanol extracts		
E1	39.2 ± 1.2	78.4 ± 0.7
E2	57.6 ± 0.5	76.8 ± 0.3
E3	38.7 ± 1.4	66.5 ± 1.2
E4	50.2 ± 1.2	77.8 ± 2.0
E5	50.6 ± 2.8	79.3 ± 0.4
Water extracts		
W1	68.7 ± 3.2	88.0 ± 0.3
W2	59.8 ± 1.0	86.7 ± 2.0
W3	70.2 ± 2.3	86.6 ± 1.5
W4	69.9 ± 2.8	87.8 ± 0.5
W5	65.3 ± 2.2	88.3 ± 0.6
Caffeine	72.5 ± 1.4	85.0 ± 0.7
Galanthamine	80.9 ± 0.3	86.1 ± 0.5

The results are average of three measurements ± standard deviation. Galanthamine is a standard drug.
AChE – acetylcholinesterase, BChE – butyrylcholinesterase.

Caffeine content

Since the anticholinesterase activity of the coffee samples roasted for 25 min was found to be higher than the values for other extracts, the caffeine content was determined by HPLC (Fig. 1). The water extract (W5) had the highest caffeine content (3.1% ± 0.1%). Both of the AChE and BChE inhibitory activities of caffeine were found to be similar with galanthamine, which was used as the standard compound. In contrast, the hexane extract of coffee roasted for 25 min (H5) possessed the highest anticholinesterase activity with the lowest caffeine content (0.5% ± 0.0%; Tab. 5). This might be caused by the presence of nonpolar compounds having higher anticholinesterase activity than caffeine.

CONCLUSION

The antioxidant capacity of the coffee extracts and caffeine was determined by ABTS cation radical decolorization assay and, for the first time in Turkish coffee samples, by DPPH free radi-

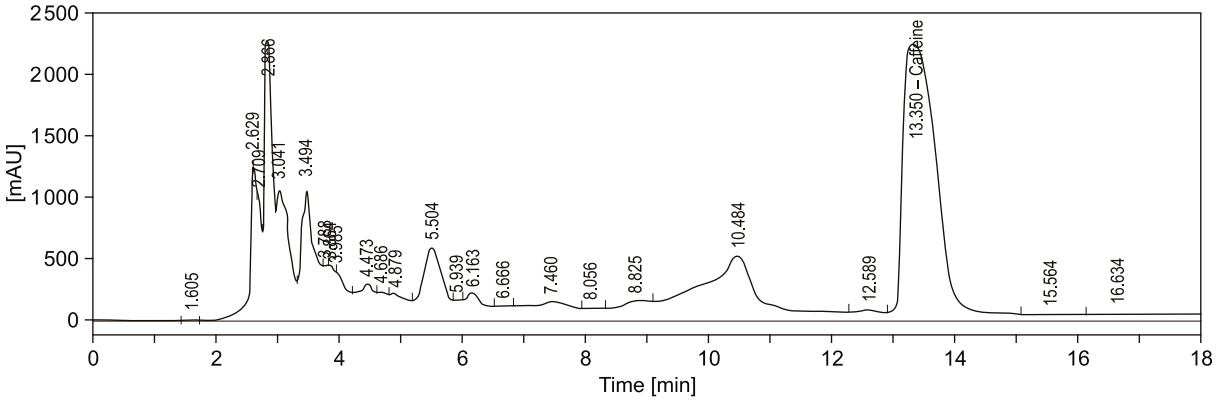

Fig. 1. HPLC chromatogram of the water extract of coffee roasted for 25 min (W5).

Tab. 5. Caffeine content of the extracts of coffee roasted for 25 min.

Samples	Caffeine content [%]
H5	0.5 ± 0.0
A5	1.2 ± 0.1
E5	2.0 ± 0.0
W5	3.1 ± 0.1

The results are average of three measurements ± standard deviation.

cal scavenging and CUPRAC assays. The anticholinesterase activity of the coffee extracts and caffeine contents were determined also for the first time. In the present work, it was found that the roasting degree had an irregular effect on the antioxidant and anticholinesterase activities of the coffee samples. In addition, there was no relationship between the caffeine content and anticholinesterase effect of the coffee samples. Since caffeine has a strong anticholinesterase activity, its effects on other neurodegenerative diseases may be enlightened by pharmacological investigations. Also, further phytochemical studies could be performed to find out active compounds in coffee extracts exhibiting the strong anticholinesterase potential.

ACKNOWLEDGEMENTS

This study was supported by the Research Fund of Istanbul University (Project number: BYP-26210). We thank Küçük Avci (Izmir, Turkey) for the coffee samples.

REFERENCES

1. Montero, M. – Marqués, M. – Muniz, F.: Consumo de café y colesterol sérico. Grasas y Aceites, *50*, 1999, pp. 141–152. DOI 10.3989/gya.1999.v50.i2.649.
2. Crozier, A. – Ashihara, H. – Tomás-Barbéran, F.: Teas, cocoa and coffee, plants secondary metabolites and health. Chichester : John Wiley & Sons, 2012. ISBN-13 978-1-4443-3441-8.
3. Brezová, V. – Šlebodová, A. – Staško, A.: Coffee as a source of antioxidants: An EPR study. Food Chemistry, *114*, 2009, pp. 859–868. DOI 10.1016/j.foodchem.2008.10.025.
4. Parras, P. – Martínez-Tomé, M. – Jiménez, A. M. – Murcia, M. A.: Antioxidant capacity of coffees of several origins brewed following three different procedures. Food Chemistry, *102*, 2007, pp. 582–592. DOI 10.1016/j.foodchem.2006.05.037.
5. Jaganyi, D. – Madlala, S. P.: Kinetics of coffee infusion: a comparative study on the extraction kinetics of mineral ions and caffeine from several types of medium roasted coffees. Journal of the Science of Food and Agriculture, *80*, 2000, pp. 85–90. DOI 10.1002/(SICI)1097-0010(20000101)80:1<85::AID-JSFA489>3.0.CO;2-#.
6. López-Galilea, I. – Paz de Peña, M. – Cid, C.: Correlation of selected constituents with the total antioxidant capacity of coffee beverages: Influence of the brewing procedure. Journal of Agricultural and Food Chemistry, *55*, 2007, pp. 6110–6117. DOI 10.1021/jf070779x.
7. Vignoli, J. A. – Bassoli, D. G. – Benassi, M. T.: Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. Food Chemistry, *124*, 2011, pp. 863–868. DOI 10.1016/j.foodchem.2010.07.008.
8. Guillot, F. L. – Malno, A. – Stadler, R. J.: Antioxidant properties of novel tetraoxygenated phenylindan isomers formed during thermal decomposition of caffeic acid. Journal of Agricultural and Food Chemistry, *44*, 1996, pp. 2503–2510. DOI 10.1021/jf9508155.
9. Ascherio, A. – Zhang, S. M. – Hernan, M. A. – Kawachi, I. – Colditz, G. A. – Speizer, F. E. – Willett, W. C.: Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Annals of Neurology, *50*, 2001, pp. 56–63. DOI 10.1002/ana.1052.
10. Slinkard, K. – Singleton, V. L.: Total phenol analysis automation and comparison with manual methods. American Journal of Enology and Viticulture, *28*, 1977, pp. 49–55.
11. Park, Y. K. – Koo, M. H. – Ikegaki, M. – Contado, J. L.: Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Brazilian Archives of Biology and Technology, *40*, 1997, pp. 97–106.
12. Re, R. – Pellegrini, N. – Proteggente, A. – Pannala, A. – Yang, M. – Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, *26*, 1999, pp. 1231–1237. DOI 10.1016/S0891-5849(98)00315-3.
13. Blois, M. S.: Antioxidant determinations by the use of a stable free radical. Nature, *181*, 1958, pp. 1199–1200. DOI 10.1038/1811199a0.
14. Apak, R. – Guclu, K. – Ozyurek, M. – Karademir, S. E.: Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 7970–7981. DOI 10.1021/jf048741x.
15. Ellman, G. L. – Courtney, K. D. – Andres, V. – Featherston, R. M.: A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, *7*, 1961, pp. 88–95. DOI 10.1016/0006-2952(61)90145-9.
16. Prodolliet, J. – Bruelhart, M.: Determination of acesulfam-K in foods. Journal of AOAC International, *76*, 1993, pp. 268–274.
17. Hečimović, I. – Belščak-Cvitanović, A. – Horžić, D. – Komes, D.: Comparative study of polyphenols and

caffeine in different coffee varieties affected by the degree of roasting. Food Chemistry, *129*, 2011, pp. 991–1000. DOI 10.1016/j.foodchem.2011.05.059.
18. Niseteo, T. – Komes, D. – Belščak-Cvitanovič, A. – Horžič, D. – Budeč, M.: Bioactive composition and antioxidant potential of different commonly consumed coffee brews affected by their preparation technique and milk addition. Food Chemistry, *134*, 2012, pp. 1870–1877. DOI 10.1016/j.foodchem.2012.03.095.
19. McGleenon, B. M. – Dynan, K. B. – Passmore, A. P.: Acetylcholinesterase inhibitors in Alzheimer's disease. British Journal of Clinical Pharmacology, *48*, 1999, pp. 471–480. DOI 10.1046/j.1365-2125.1999.00026.x.
20. Arendash, G. W. – Cao, C.: Caffeine and coffee as therapeutics against Alzheimer's disease. Journal of Alzheimer's Disease, *20*, 2010, pp. 117–126. DOI 10.3233/JAD-2010-091249.

Received 26 October 2013; 1st revised 10 December 2013; accepted 12 December 2013; published online 18 June 2014.