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ORIGINAL PAPER

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Antioxidative activity of green and roasted coffee beans as influenced by convection and microwave roasting methods and content of certain compounds

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Abstract Antioxidative activity of green and roasted coffee beans and its correlation with the concentrations of certain substances (polyphenols, caffeine, 5-hydroxymethylfurfural) and the roasting method (convection and microwave) was determined. Antioxidative capacity was estimated based on the capability of synthetic free radical (1,1-diphenyl-2-picryl hydrazyl, DPPH$^{\cdot}$) scavenging and counteracting an oxidation of the model system composed of β-carotene and linoleic acid. Green coffee appeared to display the highest effectiveness. In the case of roasted coffee samples, the results of neither test showed distinct correlation; however they indicated that an application of microwave heating for coffee roasting better protected its antioxidative properties. This relationship corresponded to the polyphenol content in roasted beans.

Keywords Coffee · Roasting · Microwave · Polyphenol · Caffeine

Introduction

Coffee has been one of the most popular beverages all over the world, and its consumption continues to increase, due to its physiological effects as well as its pleasant taste and aroma. Antibacterial activity of coffee against Gram-positive and Gram-negative species has been reported. Both arabica and robusta display similar effectiveness in this respect, and the most active is lightly roasted coffee [1].

Due to the fact that numerous food components, underestimated in the past, have recently been accepted as important compounds in cancer and coronary disease prevention, the antioxidative activity of coffee and its free radical scavenging capability have also attracted the attention of researchers. The comparison of the antioxidative efficacy of coffee with that of other popular beverages, such as fruit juices, wines, cocoa and tea, known to contain antioxidants, has revealed the high relative activity of coffee [2, 3, 4].

The antioxidative effectiveness of coffee beans results from the presence of polyphenols, whose main component is chlorogenic acid. Polyphenols are widespread in plants, and their role in preventing oxidation has been well documented for numerous plant foodstuffs. Sanchez-Moreno et al. detected the antioxidative activity of many polyphenol compounds [5]. Similar results were reported by Chen and Ho, who carried out studies on selected hydroxycinnamic acids, and found that some polyphenols present in coffee beans, such as chlorogenic, caffeic and ferulic acids, had high antioxidative efficacy [6]. Ohnishi et al. detected the complex of caffeic acid and tryptophan in green coffee beans. Its activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was similar to that of chlorogenic and caffeic acids [7]. Kroll and Rawel found that plant polyphenols combining with proteins, usually via binding to side chains of lysine and tryptophan, slow down enzymatic degradation of proteins in vitro [8]. However, coffee may also promote oxidation since it can diminish the availability of other antioxidants present in food [9]. Changes in the antioxidative capacity of coffee upon roasting are associated with the degradation of chlorogenic acid. Some polyphenol derivatives, such as phenylindans formed upon roasting, display very high antioxidative activity [10]. Many authors attribute the antioxidative effectiveness of roasted coffee to Maillard reaction products [11, 12]. The antioxidative efficacy of volatile compounds formed by pyrolysis upon coffee roasting was studied by Fuster et al. [13]. Many of the volatile substances, such as furans and pyrroles, appeared to display very high activity with this respect. Similar research by Macku and Shibamoto [14] and Krings and Berger [15] on the antioxidative capacity of some foodstuffs subjected to roasting, such as wheat, nuts, almonds and coffee, proved the highest activity of the

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latter. Also two alkaloids of coffee beans, i.e. caffeine and trigonelline have antioxidative properties [9, 16].

Apart from the assays of antioxidative activity of individual components of green and roasted coffee beans, the influence of the degree of roasting on this enhanced activity has also been studied [11, 12]. However, there is still a paucity of reports on the effect of the roasting method on the antioxidative effectiveness of coffee. Coffee beans have been usually roasted by using the convection method. Studies on coffee microwaving have also been carried out. Coffee beans roasted using the latter method contain more soluble substances and had no burnt aroma [17].

The present study was aimed at estimation of the antioxidative activity of coffee beans roasted by using either convection or microwave methods, and determination of the correlation of this activity with the beans' humidity before roasting, as well as with concentrations of selected antioxidative substances, such as polyphenols, caffeine and 5-hydroxymethylfurfural (HMF).

Materials and methods

Materials

Chemicals. Caffeine, chlorogenic acid (3-*O*-caffeoyl-D-quinic acid), 2,2-diphenyl-1-picryl hydrazyl (DPPH$^{\cdot}$), β-carotene, and HMF were purchased from Sigma (St. Louis, USA). Linoleic acid and Tween 20 were delivered by Fluka (Buchs, Switzerland). All other chemicals were of analytical purity.

Plant material. Raw beans of *Coffea canephora (robusta)* from The Ivory Coast were delivered by Agros (Leszno, Poland).

Methods

Coffee bean processing before roasting. The humidity of green beans was increased from 7.5% to 10.0% in air with a relative humidity of 100%. The process was carried out for 40 h at room temperature in a desiccator containing a vial with distilled water below the heap of beans deposited on a sieve. The humidity of green beans was reduced from 7.5% to 5.0% by thermal processing in a convection oven at 90 °C for 38 min.

Coffee bean roasting. Green coffee beans, and humidified and dried coffee beans, were roasted using either convection (230 °C) or microwave (750 W) methods. For comparison, the coupled convection-microwave roasting of the beans was executed under the same values of temperature and electric power. The process was conducted in BOSCH Gourmet 8601 oven, providing both convection and microwave heating. The weight of each of the roasted coffee beans samples was 100 g. In each of the roasting variants triplicate samples were prepared, and the process was carried out until the decrease in the samples' weight was 9.5% of solid substance. This degree of roasting provided optimum sensory properties of the roasted coffee.

Preparation of coffee extracts. Green and roasted coffee beans were ground in a grinder and sifted through a sieve with a mesh of 300×300 μm. Coffee extracts were obtained by means of extraction with distilled water in a biphasic system composed of solid substance and liquid. The ratio of the weight of ground beans to water was 1:10. The suspension of coffee in water was incubated in a flask equipped with a reflux condenser for 15 min at 100 °C. Extraction was followed by rapid cooling and filtration through a 0.45 μm filter. The extracts were stored at 20 °C before analysis. According to Nicoli et al., freezing and thawing of coffee extracts have no impact on their antioxidative capacity [11].

Antioxidative tests in vitro. Antioxidative activity of coffee extracts was determined according to the method described by Daglia et al., based on counteracting the oxidation process in a mixture of β-carotene and linoleic acid [12]. β-Carotene (5 mg) was dissolved in 50 ml of chloroform. A 3 ml aliquot of this solution was transferred to a round bottom flask, and mixed with 40 mg of linoleic acid, and 400 mg of Tween 20. Chloroform present in this emulsion was evaporated under vacuum below 30 °C, and the dry residue was resuspended in 100 ml of distilled water. Portions of the coffee extracts (50 μl) were introduced to 5 ml aliquots of the β-carotene emulsion in water, immediately mixed and subjected to A_{470} measurements using Specol 11. The mixtures were incubated in a water bath at 50 °C, and changes in A_{470} were determined after 10, 20 and 30 min. The measurements were carried out against a β-carotene-free blank. The rate of β-carotene degradation was calculated from the equation of the first-order reaction:

$$v_s = \ln(A_0/A_t)/t \quad (1)$$

where A_0 is initial absorbency (just after coffee extract addition), A_t absorbency after incubation at 50 °C for a particular period of time (10, 20 and 30 min).

The rate of β-carotene degradation in the absence of the coffee extracts was also determined. The control samples contained 5 ml of β-carotene emulsion and 50 μl of distilled water. Conditions of incubation and measurements of A_{470} were the same as for the coffee extract-containing samples. The rate of β-carotene decomposition in control samples (v_c) was calculated from Eq. 1.

Antioxidative activity (*AA*) was expressed as a percentage and denoted a decrease in the rate of β-carotene degradation in the presence of coffee extracts versus controls. It was calculated from the equation:

$$AA\% = (v_c - v_s)/v_c \times 100\% \quad (2)$$

where: v_s and v_c are β-carotene degradation rates in samples and controls, respectively.

Free radical scavenging. This test enabled determination of the activity of coffee extracts against the stable free radical DPPH$^{\cdot}$. The samples contained 0.1 ml aliquots of coffee extracts and 3.9 ml of DPPH$^{\cdot}$ solution (5 mg per 100 ml of methanol) [5, 18]. A_{517} was measured immediately after the mixture was prepared, and after 10, 20 and 30 min incubation at room temperature. Respective blanks were composed of 3.9 ml of methanol and 0.1 ml of coffee extract. The control sample contained 3.9 ml of the DPPH$^{\cdot}$ solution and 0.1 ml of water. In this case, measurements of A_{517} were done against a mixture of 3.9 ml of methanol and 0.1 ml of water. The activity in DPPH$^{\cdot}$ scavenging (SE) was expressed as a percentage and calculated from the following equation:

$$SE\% = (\ln A_c/t - \ln A_s/t)/\ln A_c \times 100\% \quad (3)$$

where: A_s/t and A_c/t are the absorbencies of samples and controls respectively, after particular periods of incubation.

Determination of polyphenols content. Total polyphenol content was determined by using both the spectrophotometric method and HPLC. The spectrophotometric method of polyphenols determination employs the Folin-Ciocalteu reagent [19]. A 0.1 ml aliquot of the extract prepared for the antioxidative activity estimation was mixed with 0.5 ml of the Folin-Ciocalteu reagent and 5 ml of the saturated $CaCO_2$ solution. The volume of the mixture was filled up to 50 ml with distilled water before 30 min incubation at room temperature, followed by A_{700} measurement. The content of total polyphenols was expressed in milligrams of chlorogenic acid.

For the polyphenols determination by using HPLC, a 6 ml aliquot of the extract prepared for the antioxidative activity assays was purified on C_{18} Sep-Pak cartridge 51910 (Millipore Waters, Milford, USA), previously washed with 5 ml of methanol and 3 ml

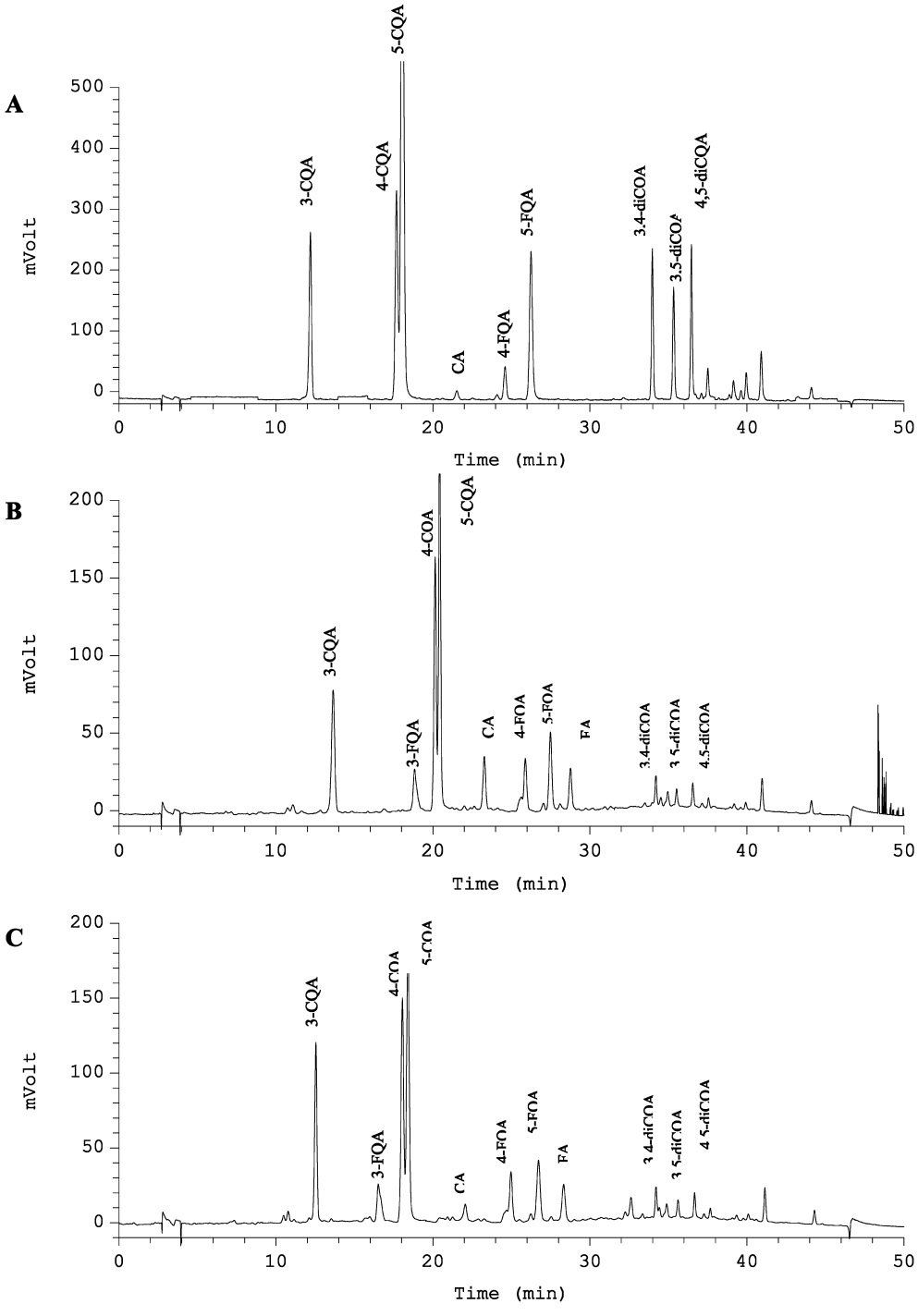

Fig. 1A—C The HPLC chromatograms of polyphenols from: **A** green coffee (G), **B** convection roasted coffee (CR), **C** microwave roasted coffee (MR). Conditions are described in Materials and methods. *CQA* Caffeoylquinic acid, *FQA* feruloylqinic acid, *diCQA* dicaffeoylquinic acid, *CA* caffeic acid, *FA* ferulic acid

of distilled water. The extract applied onto the column was eluted with 20 ml of methanol:water (40:60, v/v). The eluate was diluted up to a final volume of 50 ml with distilled water, and 20 μl of the diluted sample was applied onto the HPLC column.

The analysis was performed by using a liquid chromatograph (Knauer, Germany), equipped with 250×4 mm LiChrospher 100 RP 18 (5 μm) column and UV-VIS detector. Elution was carried out with two solvents, acetonitrile (A) and redistilled water, acidified with 85% phosphoric acid to pH 2.6 (B). The samples were eluted for 45 min at a flow rate of 1 ml per min, with a linear gradient of acetonitrile in water, prepared from the A:B mixture (3:97) and pure acetonitrile. Chlorogenic acid isomers were identified based on literature data [20, 21, 22]. Concentrations of individual isomers were calculated from the surface area under corresponding peaks, and compared with that of standard 5-chlorogenic acid solution. Next, the surface areas were summed to calculate the total polyphenol content. Patterns of the analysis are presented in Fig. 1.

Caffeine content. Caffeine content was assayed according to the modified method of Levin [23]. Ground coffee beans were extracted in a milieu of ammonia, and the extract was separated on two Celite columns: acidic and basic, using diethylene ether. Purified caffeine was eluted with chloroform. Measurements of the absorbency of the eluate were carried out at a wavelength of

276 nm, and at 246 and 306 nm, in order to determine the correct base line and eliminate the effect of other compounds, interfering with caffeine assays.

HMF assays. HMF assays in coffee extracts were carried out after removal of colloids with Carez reagents and filtration through 0.45 μm filter. The filtrate (10 ml) was adjusted to 100 ml with distilled water. Next, a 10 ml aliquot was transferred to a 100 ml flask, and the volume was filled up with methanol. Than 20 μl of the sample was applied onto the HPLC column.

HMF content was determined by using reverse-phase HPLC and a UVD 170S detector (Dionex, Germany). Hypersil ODS 5 μm (250×4.6 mm,) column was applied as a stationary phase, and methanol:water (10:90 v/v) as an isocratic mobile phase. The flow rate was 1 ml min^{-1}. Absorbency of the samples was measured at 283 nm [24].

All presented results are average values of triplicate assays. The standard deviation has been taken into consideration.

Results and discussion

Coffea robusta beans were roasted by using two different methods, such as convection heating and microwaving. Green beans, as well as those dried prior to roasting and humidified ones were subjected to the processing.

The initial humidity of the beans markedly affected the time of roasting (Table 1): roasting time increased with humidity. Microwaving required a longer processing time compared to convection heating for the same level of the beans' initial humidity. The difference in time between the processes decreased with increasing humidity of the green coffee, as a result of the fact that wetter material better absorbs microwave energy. The initial humidity of green beans, the duration of heating, and the method used for roasting influenced the final temperature of the roasted beans. The application of microwaving gave rise to a significant decrease in this temperature (Table 1). Microwaves operate directly in the core of the beans, so that the process of roasting is intensified throughout the whole interior of the bean. In the convection method, roasting of the bean's core brings about burning of the surface layer.

Polyphenol content

The polyphenols were extracted with hot water in order to determine the amount of polyphenols present in coffee infusions as prepared by consumers. Comparison of results obtained by using two different methods of polyphenols assessment in green and roasted beans, spectrophotometric determination and HPLC, revealed that the first method gave higher values. This was probably caused by the presence of other compounds, such as Maillard condensation products and tryptophan, also giving positive reactions with the Folin-Ciocalteu reagent.

Roasted beans appeared to contain less polyphenols than the green ones, since more than 60% of chlorogenic acid present in green coffee was degraded upon roasting.

The initial humidity of green beans had a stronger impact on final polyphenol concentration in roasted beans, than the roasting method. The lowest polyphenols content was detected in coffee beans roasted after initial drying, and the highest in beans roasted using the combined convection-microwave method, presumably due to the relatively short time of processing (Table 1 and Table 2). Although the degree of polyphenols decomposition was to a lesser extent influenced by the roasting method, microwaving caused a smaller decrease in their amount, as assayed for roasted coffee beans

Table 1 Roasting time and final temperature of coffee beans under different roasting conditions. Valuesare ±standard deviation (n=3)

Humidity of coffee beans prior to roasting		Roasting method	Roasting time min	Beans temperature °C
Green	7.5%	Convective roasting	9.75±0.21	238±3
Green	7.5%	Micowave roasting	11.08±0.17	207±2
Wetted	10%	Convective roasting	12.32±0.08	255±5
Wetted	10%	Micowave roasting	12.37±0.15	231±2
Predried	5%	Convective roasting	3.78±0.07	228±4
Predried	5%	Micowave roasting	7.42±0.06	203±1
Green	7.5%	Convective microwave roasting	5.33±0.12	228±2

Table 2 Total polyphenols, caffeine and 5-hydroxymethylfurfural (*HMF*) contents in green coffee beans roasted under different conditions. Values are ±standard deviation (n=3)

Coffee beans	Polyphenols g/100 g db spectrophotometric method	Polyphenols g/100 g db HPLC method	Caffeine g/100 g db	HMF mg/kg db
Green	7.88±0.13	7.15±0.15	2.12±0.03	Nd
Convective roasting	2.26±0.14	2.07±0.11	1.82±0.02	33±0.6
Micowave roasting	2.31±0.03	2.19±0.12	1.90±0.05	28±1.1
Wetted convective roasting	2.58±0.01	2.36±0.23	1.89±0.11	30±0.4
Wetted micowave roasting	2.75±0.02	2.64±0.26	2.07±0.08	26±0.9
Predried convective roasting	2.06±0.19	1.97±0.14	1.86±0.04	35±0.2
Predried micowave roasting	2.11±0.18	2.03±0.20	2.08±0.05	32±1.5
Convective microwave roasting	3.03±0.10	2.87±0.29	1.95±0.08	43±0.9

samples with the same initial humidity before thermal processing. A similar relationship between the heating method and polyphenol concentration was noted by Friedman and Dao, who determined changes in chlorogenic acid content in ergot, thermally processed using either the convection or the microwave method. Convection heating caused stronger degradation of chlorogenic acid in ergot samples [25].

Caffeine content was estimated in green and roasted coffee beans. Green beans contained 2.1% of this alkaloid, and the roasted ones 1.8–2.1%. Similar results were obtained by Daglia et al. [16], who determined caffeine concentration in five varieties of robusta coffee, using HPLC. They detected 1.8–3.0% caffeine in green beans, 1.7–2.1% in medium roasted beans, and 1.6–1.9% in strongly roasted ones. The decrease in caffeine content in microwaved beans was lower than that in coffee roasted using the convection method (Table 2). The stability of ergot alkaloids upon convection heating and microwaving was also studied by Friedman et al. [25]. They found that microwaving caused the lower loss in content of these alkaloids. The initial humidity of green coffee beans affected the final caffeine content in roasted beans to a lesser extent.

HMF content in samples of roasted coffee was found to be 28–43 mg kg^{-1} (Table 2). According to Daglia et al., HMF concentration in a medium roasted coffee ranges from 30 to 80 mg kg^{-1}, and in a strongly roasted one, it does not exceed 40 mg kg^{-1} [16]. Kinetics of HMF formation throughout roasting was studied by Kanjahn and Maier [26], who detected that this compound was intensively formed during the first stages of this process, when its concentration achieved a value of 1,000 mg kg^{-1}, and thereafter it decreased, to 150 and 20 mg kg^{-1}, for medium and strongly roasted beans, respectively. In the present study, humidity of raw coffee beans affected HMF content in roasted beans. Its higher concentrations were detected in the coffee samples dried prior to roasting (Table 2). Model studies of Yeo and Shibamoto [27], which focused on the influence of water content on the synthesis of Maillard reaction products during microwaving, were in accordance with our results. The authors found that a decline in humidity in the studied system gave rise to more pronounced browning. This process was initiated after a longer period of time due to the lower efficiency of microwave absorption, but finally it was more intensive. The method of roasting also influences HMF production. Our experiments showed that less HMF is formed upon microwaving than upon convection heating. MacLeod and Coppock [28], who studied the rates of volatile compounds synthesis during microwaving and convection heating of beef meat, found that more furans were formed in the case of the latter method [28].

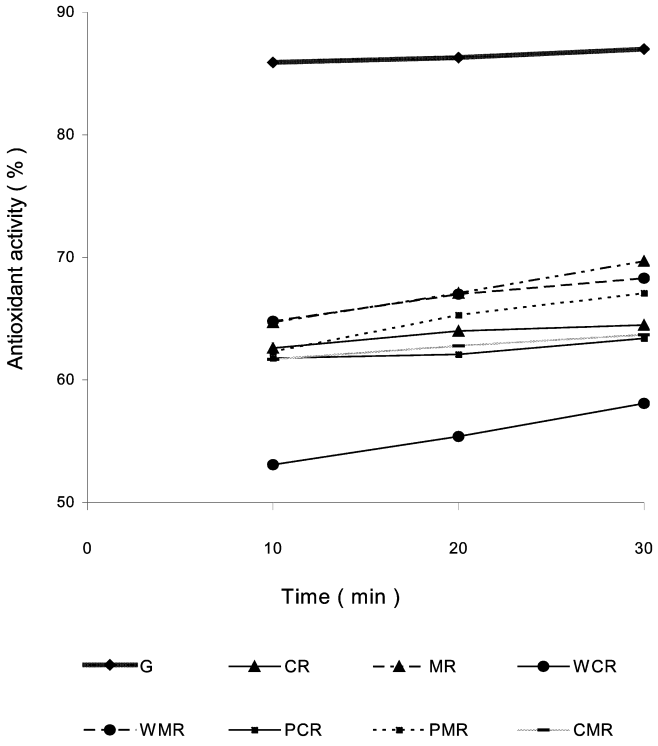

Fig. 2 Effect of roasting method on the antioxidative activity of coffee extracts in retarding linoleic oxidation. *G* Green coffee, *CR* convection roasted coffee, *MR* microwave roasted coffee, *CMR* convective microwave roasting, *PMR* predried micowave roasting, *WMR* wetted micowave roasting, *WCR* wetted convective roasting, *PCR* predried convective roasting

Antioxidative test in vitro

Green coffee beans prevented oxidation of β-carotene mixed with linoleic acid most efficiently (Fig. 2). The process of coffee bean roasting resulted in lower antioxidative activity. The microwaved samples retained more of their initial capacity, due to higher contents of polyphenols and caffeine (Table 2). The best correlation was observed between antioxidative activity and polyphenol content, determined by using both the spectrophotometric method and HPLC (Table 2).

Free radical scavenging

The effectiveness of green and roasted coffee beans at scavenging the synthetic free radical $DPPH^{\cdot}$ was determined. An advantage of this method is the relatively fast response compared to other assays applied for the same purpose, e.g. the Rancimat method [6]. Reduction of the $DPPH^{\cdot}$ radical, which is an acceptor of hydrogen atoms from antioxidants, and is thus converted to DPPH-H, results in a decline in molar absorbency coefficient at 517 nm from 9,600 to 1,640 M^{-1} cm^{-1}, and a simultaneous change in the color of the solution from purple to yellow. The strongest ability to scavenge $DPPH^{\cdot}$ radical was detected in green coffee beans (Fig. 3). Amongst the

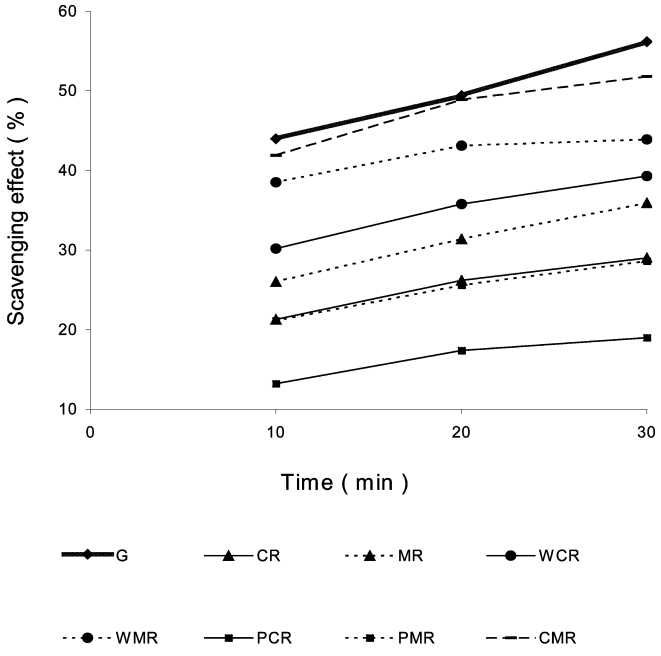

Fig. 3 Effect of roasting method on DPPH scavenging activity of coffee extracts. G Green coffee, CR convection roasted coffee, MR microwave roasted coffee, CMR convective microwave roasting, PMR predried micowave roasting, WMR wetted micowave roasting, WCR wetted convective roasting, PCR predried convective roasting

roasted beans, the highest efficacy was displayed by the sample roasted using the coupled convection-microwave method, presumably due to its having the highest residual polyphenols content. Humidification of coffee beans before roasting provided higher activity in DPPH$^{\cdot}$ scavenging, in contrast to the initial drying that resulted in the lowest final effectiveness. Microwaving caused a lesser decline in DPPH$^{\cdot}$ scavenging capacity, coinciding with the higher total polyphenol content, determined by using both the applied methods (Table 2).

The results of both tests applied for the studies were not strictly correlated, but utilizing microwaves for coffee roasting was shown to be less harmful to the antioxidative capacity of the beans. Our results are in accordance with the conclusions of other researchers who also detected the highest antioxidative efficacy in raw coffee beans. Nicoli et al. claims that the larger concentration of polyphenols in green beans brings about their higher antioxidative activity [11]. The polyphenols undergo chemical modification or degradation upon roasting, and some products of their decomposition, like phenylindans have high antioxidative activity [10]. However, the minor concentration of the latter compounds cannot compensate for the diminished concentration of polyphenols, mainly chlorogenic acid. Maillard reaction products and volatile products of pyrolysis as well as caffeine, also contribute to the antioxidative effectiveness of roasted coffee. Goodman et al. studied the activity in TMPO (a free radical) scavenging of traditional and caffeine-free coffee samples. Individual, isolated coffee components were also subjected to this test. They found that caffeine was less active against TMPO than chlorogenic and caffeic acids, but caffeine-free coffee displayed lower antioxidative capacity than the traditional one. According to the results presented, the decrease in caffeine content upon roasting did not exceed 15%, so this loss does not seem to be responsible for the final decline in antioxidative activity.

Our results indicate that the antioxidative efficiency and activity in DPPH$^{\cdot}$ scavenging of coffee extracts are predominantly dependent on the polyphenols concentration (Table 2, Fig. 2 and Fig. 3). Coffee infusions are a basic source of hydroxycinnamic acids and their derivatives in the human diet [27]. In chemical tests these compounds display very high antioxidative activity [12]. However, their effectiveness after ingestion and metabolism in liver cells still remains unknown and therefore further studies on their behavior in biological systems should be carried out.

Our results indicate that coffee beans roasted by using either the traditional (convection) or the microwave methods, display strong activity in DPPH$^{\cdot}$ scavenging and protect linoleic acid from oxidation. However, the process of roasting resulted in a decline in the initial antioxidative capacity. The antioxidative effectiveness is, to the highest extent, correlated with polyphenols concentration. Microwaving brought about a smaller decrease in their content and therefore coffee roasted this way showed higher antioxidative activity. The activity of roasted coffee beans in synthetic free radical scavenging was significantly influenced by the humidity of the beans before roasting. The wettest beans retained the highest activity after roasting. The test based on the linoleic acid oxidation did not show such a distinct correlation, but it revealed that microwaved samples were more active as antioxidants. HMF production and caffeine degradation were not directly correlated with the antioxidative efficiency.

References

1. Daglia M, Cuzzoni MT, Dacarro C (1994) J Agric Food Chem 42:2270–2272
2. Yamaguchi T, Takamura H, Matoba T, Terao J (1998) Biosci Biotechnol Biochem 62:1201–1204
3. Vinson JA, Jang J, Yang J, Dabbagh Y, Liang X, Serry M, Proch J, Cai S (1999) J Agric Food Chem 47:2502–2504
4. Richelle M, Tavazzi I, Offord E (2001) J Agric Food Chem 49:3438–3442
5. Sanchez-Moreno C, Larrauri J A, Saura-Calixto F (1998) J Sci Food Agric 76:270–276
6. Chen JH, Ho C-T (1997) J Agric Food Chem 45:2374–2378
7. Ohnishi M, Morishita H, Toda S, Yase Y, Kido R (1998) Phytochemistry 47:1215–1218
8. Kroll J, Rawel HM (2001) J Food Sci 66:48–58
9. Goodman BA, Glidewell SM, Deidhton N, Morrice AE (1994) Food Chem 51:399–403
10. Guillot FL, Malnoë A, Stadler R (1996) J Agric Food Chem 44:2503–2510
11. Nicoli MC, Anese M, Manzocco L, Lerici CR (1997) Lebensm-Wiss Technol 30:292–297
12. Daglia M, Pepetti A, Gregotti C, Berte F, Gazzani G (2000) J Agric Food Chem 48:1449–1454

13. Fuster MD, Mitchell AE, Ochi H, Shibamoto T (2000) J Agric Food Chem 48:5600–5603
14. Macku C, Shibamoto T (1991) Volatile antioxidants isolated from brewed coffee. In: Scientific Symposium on Coffee, 14th Colloque, San Francisco, pp 146-155
15. Krings U, Berger RG (2001) Food Chem 72:223–229
16. Daglia M, Cuzzoni MT, Dacarro C (1994) J Agric Food Chem 42:2273–2277
17. Viet LT, Truchement B (1998) Swiss Patent CH 665 754 A5
18. Yen G-C, Chen, H-Y (1995) J Agric Food Chem 43:27–32
19. Fogliano V, Verde V, Randazzo G, Ritieni A (1999) J Agric Food Chem 47:1035–1040
20. Guerrero G, Suarez M (2001) J Agric Food Chem 49:2454–2458
21. Bicchi CP, Binello AE, Pellegrino GM, Vanni AC (1995) 43:1549–1555
22. Ky C-L, Noirot M, Hamon S (1997) J Agric Food Chem 45:2273–2277
23. ISO 4052 :1983. Coffee. Determination of caffeine content (routine method)
24. Albalá-Hurtado S, Veciana-Nogues MT, Izquierdo-Pulido M, Vidal-Carou MC (1997) J Agric Food Chem 45:2128–2133
25. Friedman M, Dao L (1990) J Agric Food Chem 38:805–808
26. Kanjahn D, Maier HG (1997) Dtsch Lebensm Rundsch 93:44–46
27. Yeo H, Shibamoto T (1991) J Agric Food Chem 39:1860–1862
28. MacLeod G, Coppock BM (1976) J Agric Food Chem 24:835–843
29. Clifford MN (2000) J Sci Food Agric 80:1033–1043