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Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting

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ABSTRACT

The bioactive composition of coffee, as one of the most popular beverages in the world, has attracted interest as a potential source of beneficial bioactive compounds, especially polyphenols and caffeine. Since the content of these compounds is affected by the processing conditions, the objective of this study was to determine the content of polyphenolic compounds and caffeine in four different coffee varieties: Minas and Cioccolatato (*Coffea arabica*), and Cherry and Vietnam (*Coffea canephora* syn. *Coffea robusta*), roasted by three varying degrees (light, medium and dark). The content of the polyphenolic compounds and the antioxidant capacity of coffees were determined using UV/Vis spectrophotometric methods, while the content of chlorogenic acid derivatives was determined using HPLC analysis. The caffeine content was determined by means of two spectrophotometric methods, as well as HPLC analysis. Additionally, raw caffeine was also obtained by an isolation procedure with chloroform. Cherry coffee, a variety of *C. canephora* exhibited the highest overall content of total phenols (42.37 mg GAE/g), followed by Minas coffee, while Cioccolatato contained the lowest TPC (33.12 mg GAE/g). Cherry coffee also exhibited the highest content of individual classes of polyphenols (flavan-3-ols, procyanidins and tannins), while the highest content of chlorogenic acid (CQA) derivatives was determined in Minas and Cioccolatato coffees (*C. arabica*). The highest content of total and individual polyphenolic compounds was determined in coffees roasted in both light and medium roasting conditions, which was also observed for the content of CQA derivatives and antioxidant capacity of roasted coffees. The highest caffeine content in the coffee samples was determined by employing the HPLC analysis (0.06–2.55%). Light roasted Cherry coffee contained the highest overall content of caffeine among all coffees, which exhibited a decrease with intensified roasting.

1. Introduction

The popularity and worldwide appeal of coffee, which stems from its unique flavour, make it currently one of the most desirable and frequently consumed beverages. Also, it has a strong historical, cultural, social and economic importance. Coffee is the single most important tropical commodity traded worldwide, accounting for nearly half of total exports of tropical products. The world's largest importer of coffee is the EU, accounting for 66% of worldwide imports, or four million tonnes, in 2008, followed by the United States (24%, 1.5 million tonnes) and Japan (7%, 423 602 tonnes) (Pay, 2009). Coffee beans found on the market are produced from two different species of *Coffea* genus: *Coffea arabica* and *Coffea canephora* syn. *Coffea robusta*. Traditionally, *C. canephora*, primarily from African origins, was the dominant component in most coffee blends available in Belgium/Luxemburg, France, Portugal and the UK, while most blends available in Scandinavia, Austria, Switzerland, Germany, Italy and Spain incorporated a much higher proportion of *C. arabica* (Pay, 2009). Both species present a rich source of biologically active compounds. The coffee beverage is rich in bioactive substances, such as nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogallic acid and especially caffeine (Minamisawa, Yoshida, & Takai, 2004). Coffee also contains dietary minerals, where it can provide up to 8% of the daily intake of Cr (Santos, Lauria, & Porto da Silveira, 2004) and can be a substantial source of Mg; a mean of 63.7 mg/cup (100 ml) has been reported (Astier-Dumas & Gounelle de Pontanel, 1974). Among the polyphenolic antioxidants, coffee provides a high content of phenolic acids of the hydroxycinnamic acids family (caffeic, chlorogenic, coumaric, ferulic and sinapic acids), which contribute significantly to the total polyphenol intake (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Coffee is the major source of chlorogenic acids in human diet (Daglia, Papetti, Gregotti, Bertè, & Gazzani, 2000) and there are reports showing its antioxidant activity *in vitro* (Moreira et al., 2001). The group of chlorogenic acids include some isomer subgroups as caffeoylquinic acids, dicaffeoylquinic acids

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and feruloylquinic acids. Those acids are important for the formation of pigments, taste and aroma of coffee beverages (Olthof, Hollman, & Katan, 2001; Yen, Wang, Chang, & Duh, 2005).

The quality of coffee used for the preparation of a beverage is related to the chemical composition of the roasted beans, which, in turn, is affected by the chemical composition of green beans and by post-harvest processing conditions (drying, storage, roasting and grinding). During roasting, the green beans are heated at 200–240 °C for 10–15 min depending on the degree of roasting required, which is generally evaluated by colour (Andriot, Le Quéré, & Guichard, 2004). The characteristic flavour of coffee represents a combination of numerous chemical compounds produced by the chemical and physical changes that occur during roasting (Franca, Oliveira, & Vitorino, 2002). The roasting process, especially at temperatures above 180–200 °C, leads to profound changes in the chemical composition and biological activities of coffee as a result of the generation of compounds deriving from the Maillard reactions (Czerny, Mayer, & Grosch, 1999), and organic compounds resulting from pyrolysis (Daglia et al., 2000). In quantitative terms, major chemical alterations have been described for chlorogenic acid (Trugo & Macrae, 1984), sucrose (Trugo & Macrae, 1982), trigonelline (Casal, Oliveira, & Ferreira, 2000) and amino acids (Macrae, 1987; Nehring & Maier, 1992).

It has been previously established that phenolic antioxidants naturally occurring in coffee are lost during the roasting process (Delgado-Andrade & Morales, 2005; Steinhart, Luger, & Piost, 2002). This decrease of polyphenolic compounds is associated to the degradation of chlorogenic acid, which influences the antioxidant capacity of the roasted coffee. However, the antioxidant content and efficiency of roasted coffee can be maintained, or even enhanced, by the formation of compounds with antioxidant activity, such as Maillard reaction products (Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997; Del Castillo, Gordon, & Ames, 2005). Some polyphenol derivatives, such as phenylindans formed upon roasting, display a very high antioxidant activity (Guillot, Malno, & Stadler, 1996).

Coffee's most studied component, caffeine, varies substantially depending on the coffee species, method of bean-roasting and beverage preparation. The widespread natural occurrence of caffeine in a variety of plants undoubtedly played a major role in the long-standing popularity of caffeine-containing products, especially coffee. Although there are considerable fluctuations in the caffeine content of different coffee beverages, the caffeine content of caffeine-containing coffees ranges from 58 to 259 mg/serving (Bell, Wetzel, & Grand, 1996). McCusker, Goldberger, and Cone (2003) reported a wide range of caffeine concentrations (259–564 mg/dose) in the same coffee beverage obtained from the same outlet on six consecutive days. Caffeine exerts pharmacological effects on the central nervous system, the heart, the renal system, the peripheral and central vasculature, the gastrointestinal system, and the respiratory system (Lawrence, 1986). The strong pharmacological effects of caffeine have led to consumer demand for caffeine-free coffee beverages. Due to the widespread consumption of caffeine and its potential physiological effects, it is important for both health professionals and consumers to know the exact caffeine content in food. It is therefore important to precisely determine the caffeine content in different coffee types, as a way to assess their content in order to find a more precise relationship between the amounts of consumed caffeine and its physiological effects.

Despite the worldwide use of coffee, especially in Western cultures (Pay, 2009), there is a lack of studies addressing the issues related to the changes of biologically active compounds occurring during coffee roasting. Therefore, the aim of this study was to determine caffeine and polyphenols content, as well as the antioxidant capacity of four coffee varieties roasted in three roasting degrees – light, medium and dark. For that purpose, the content of total polyphenols and several polyphenolic classes as well as the antioxidant capacity, was determined in raw and roasted coffee beans. Determination of caffeine content was conducted by means of four different methods: chloroform extraction, two different spectrophotometric methods (micro-method and lead-acetate method) and high performance liquid chromatography (HPLC) as the most precise method.

2. Materials and methods

2.1. Chemicals

Analytical grade of Folin–Ciocalteu, formic acid, potassium peroxodisulphate, sodium carbonate, sodium chloride, formaldehyde, ferric chloride hexahydrate, ferrous sulphate heptahydrate, lead acetate, chloroform and hydrochloric acid were supplied by Kemika (Zagreb, Croatia). Methanol (HPLC grade) was supplied by J.T. Baker (Deventer, Netherlands). Vanillin, 4-dimethylaminocinnamaldehyde, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt) and caffeine were purchased from Fluka (Switzerland). Benzene and sulphuric acid were supplied by Carlo Erba Reagents (Milano, Italy).

2.2. Preparation of coffee extracts

In this paper, the polyphenol and caffeine contents in *C. arabica* and *C. canephora* were studied. Four coffee varieties analysed in this study (Cherry, Minas, Vietnam, Cioccolatato) were obtained from a local coffee manufacturer (Table 1). Green beans of each coffee variety were roasted for 10 min in three roasting degrees (Pacorini coffee roaster, Italy), under the conditions displayed in Table 2. All coffee varieties were processed in triplicate for each temperature regime of lab-scale roasting used to mimic industrial processing of coffee. Each batch consisted of 0.5 kg of green coffee beans. Roasted beans were packed in airtight plastic bags. The

Table 1
General information and description of coffee varieties analysed in the study.

Coffee samples	Arabica[a] Minas, Cioccolatato	Robusta[b] Vietnam, Cherry
Date species described	1753	1895
Optimum temperature	15–24 °C	20–30 °C
Optimal rainfall	1500–2000 mm	2000–3000 mm
Optimum altitude	1000–2000 m	0–700 m
Time from flower to ripe cherry	9 months	10–11 months
Ripe cherry	Fall	Stay
Bean shape	Flat	Oval
Caffeine content of bean	0.8–1.4%	1.7–4.0%
First flowering	4–5 years	2–3 years
Chromosomes (2n)	44	22
Yield (kg beans/ha)	1500–3000	2300–4000
Typical brew characterictics	Acidity	Bitterness, full

[a] Clifford and Willson (1985).
[b] Wrigley (1988).

Table 2
Temperature regime for coffee roasting.

Roasting degree	Coffee species			
	Minas	Cioccolatato	Vietnam	Cherry
Light roasting (°C)	162	145	168	185
Medium roasting (°C)	181	167	185	195
Dark roasting (°C)	195	195	198	205

coffee extracts were prepared according to a procedure described by Sacchetti, Di Mattia, Pittia, and Mastrocola (2009), with some modifications. Prior to analysis, samples were grounded in a coffee mill (Mazzer Luigi srl N, Italy). 2 g of ground coffee was extracted with 20 ml of deionised water at 100 °C for 15 min with occasional stirring. After extraction, the extracts were cooled in cold water and centrifuged for 15 min at 2500 rpm. The resulting supernatant was used for all analytical procedures.

2.3. Determination of total phenol and flavonoid content

The total phenol content (TPC) of each of the coffee extracts was determined spectrophotometrically according to a modified method of Lachman, Hosnedl, Pivec, and Orsák (1998). To determine the content of total flavonoids (TFC), these compounds were precipitated using formaldehyde, which reacts with C-6 or C-8 atoms of 5,7-dihydroxy flavonoids to form methyl derivates that further react with other flavonoid compounds, also at C-6 and C-8 positions. The condensed products of these reactions were removed by filtration and the remaining non-flavonoid phenols were determined as previously described. Flavonoid content was calculated as the difference between total phenol and non-flavonoid phenols content. Gallic acid was used as the standard and the results were expressed as mg gallic acid equivalents (GAE)/g of coffee (Kramling & Singleton, 1969). All measurements were performed in triplicate.

2.4. Determination of flavan-3-ol content by different assays

2.4.1. Vanillin assay

Coffee extracts were analysed for their flavan-3-ol content using a method described by Di Stefano, Cravero, and Gentilini (1989). Briefly, a volume of 500 μl of extract was added to 3 ml of the freshly prepared vanillin reagent (4% methanolic solution) and after 5 min 1.5 ml of concentrated HCl was added. After incubation for 15 min in a cold water bath, absorbance of the sample was measured at 500 nm against a blank sample. The blank sample was prepared by replacing the 4% vanillin solution with methanol. Absorbance of the blank sample was subtracted from the absorbance of the corresponding vanillin-containing sample (ΔE). The content of flavan-3-ols was calculated according to the formula: (+)-catechin = $290.8 \times \Delta E$ and the results were expressed as mg (+)-catechin/g.

2.4.2. Reaction with 4-dimethylaminocinnamaldehyde

A standard procedure, reported by Di Stefano et al. (1989), was used. The reagent was prepared by dissolving 100 mg 4-dimethylaminocinnamaldehyde (4-DAC) in a mixture of concentrated hydrochloric acid (25 ml) and methanol (70 ml), and the resulting solution was made up to 100 ml with methanol. For the analysis, 1 ml of extract was added to 5 ml of 4-DAC reagent in a glass test tube and thoroughly shaken. After 10 min an absorbance reading was taken at 640 nm, along with two blank samples prepared separately for each sample. The first blank sample consisted of 5 ml 4-DAC reagent and 1 ml of distilled water, and the second one consisted of 5 ml of distilled water and 1 ml of extract. The content of flavan-3-ols was calculated according to the formula: (+)-catechin = $32.1 \times \Delta E$, where ΔE is the difference of absorbance between the tested extract and appropriate blanks. The results were expressed as mg (+)-catechin/g.

2.5. Determination of tannin content

The content of tannins was determined according to a procedure described by Schneider (1976). A volume of 2 ml of coffee extract was mixed with 8 ml of water and 10 ml of acetate buffer. The mixture prepared in such a way represents the solution 1 (S1). A volume of 10 ml of the solution S1 was shaken with 50 mg of casein for 60 min and then filtered. This filtrate represents the solution S2. A quantity of 1 ml of each solution (S1 and S2), was mixed separately with 0.5 ml of Folin–Ciocalteu reagent and then both solutions were diluted to 10 ml with 33% sodium carbonate decahydrate solution. The absorbance of such prepared solutions was measured against a blank sample at 720 nm. The content of tannins was evaluated upon three independent analyses. Absorbance values obtained for S1 correspond to total polyphenol content. Differences between absorbance of S1 and S2 correspond to the content of casein-adsorbed tannins in coffee samples. The content of tannins was expressed as mg of tannic acid/g.

2.6. Quantitative determination of proanthocyanidins

Proanthocyanidins (i.e. condensed tannins) were analysed by the procedure described by Porter, Hrstich, and Chan (1986), with some modifications. Briefly, butanol/HCl assay was carried out by mixing 2 ml of coffee extract with 4 ml of a solution of *n*-BuOH–conc. HCl (95:5, v/v) and 0.2 ml of a 2% solution of $NH_4Fe(SO_4)_2 \times 12H_2O$ in 2 M HCl. The solution was capped and thoroughly mixed and heated for 45 min at 95 °C in a water bath. The sample was cooled and the visible spectrum recorded at $\lambda = 550$ nm. The blank value of the BuOH–HCl–Fe(III) solvent was subtracted. The quantity of condensed tannins was determined from a standard curve of cyanidin chloride treated with BuOH–HCl–Fe(III) mixture, and expressed as mg cyanidin chloride equivalents (CyE)/g.

2.7. Determination of antioxidant capacity of extracts

2.7.1. Ferric reducing/antioxidant power

The ferric reducing/antioxidant power (FRAP) assay was carried out according to a standard procedure by Benzie and Strain (1996). All measurements were performed in triplicate. Aqueous solutions of $FeSO_4 \times 7H_2O$ (100–1000 μM) were used for the calibration curve and the results are expressed as mmol/l Fe (II).

2.7.2. Free radical scavenging assay

The Trolox equivalent antioxidant capacity (TEAC) of coffee extracts was estimated by the ABTS radical cation decolourisation assay (Re et al., 1999). The results, obtained from triplicate analyses, were expressed as Trolox equivalents, and derived from a calibration curve determined for Trolox (100–1000 μmol/l).

2.8. Determination of caffeine content

2.8.1. Caffeine isolation with chloroform

The caffeine isolation procedure was performed according to a modified method described by Rapić (1995). Briefly, 20.0 g of coffee and 90 ml of distilled water was refluxed for 30 min, and filtered under vacuum. The residue was again refluxed and filtered. The obtained filtrates were combined, then 12.5 ml of $Pb(CH_3COO)_2$ solution was added, boiled (5 min), and filtered through a Büchner funnel with silica gel layer. The filtrate was extracted four times with chloroform (40 ml). Combined chloroform phases were washed with KOH solution and then with distilled water. Chloroform was removed from the extracts by a rotary evaporator. After evaporation, extracted caffeine was weighed and expressed as a percentage.

2.8.2. Caffeine determination using the lead acetate solution

This procedure is based on international standards with some modifications (Yao, Chen, Cheng, & Liu, 1993; Yao, Cheng, Chen, & Liu, 1992). Coffee extract (10 ml), HCl solution (5 ml) and $Pb(CH_3COO)_2$ solution (1 ml) were mixed and diluted to 100 ml with distilled water. The solution was filtered through Whatman

No. 1 filter paper. The filtrate (25 ml) and H_2SO_4 solution (0.3 ml) were combined, diluted to 50 ml with distilled water and filtered again. Absorbance of the filtrate was measured at 274 nm. The content of caffeine (%) was calculated using a standard curve derived from caffeine (0–250 mg/l). All measurements were performed in triplicate.

2.8.3. The micro-method for the determination of caffeine

Coffees were also analysed for their caffeine content according to the method reported by Groisser (1978). Briefly, the pH of the coffee extracts was adjusted to 8–9. Ten ml of benzene, 100 μl of extract and 0.5 g of NaCl were mixed, shaken (1 min), and centrifuged (10 min, 3500 rpm). Five ml of benzene layer was combined with 5 ml of 5 N H_2SO_4, mixed and centrifuged (5 min, 3500 rpm). The bottom (H_2SO_4) layer was pipetted into a quartz cuvette and the absorbance was read at 273 nm against a blank (H_2SO_4). Results, obtained from triplicate analyses, were calculated using a standard curve and expressed as a percentage.

2.8.4. HPLC analysis of caffeine and chlorogenic acid derivatives (CQA)

Filtered coffee extracts were injected for HPLC analysis according to the method reported in our previous study (Horžić et al., 2009). Equipment used consisted of a Varian Pro Star Solvent Delivery System 230 (Varian, Walnut Creek, USA) and a Photodiode Array detector Varian Pro Star 330 (Varian, Walnut Creek, USA) with a reversed-phase column Pinnacle II C-18 (Restek, USA) (250 × 4.6 mm, 5 μm i.d.). The samples were filtered through a 0.45 μm membrane filter (Nylon Membranes, Supelco, USA) and 20 μl of each sample was injected for HPLC analysis. The mobile phase consisted of 3% formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 ml/min. The elution was performed with a gradient starting at 2% B to reach 32% B at 20 min, 40% B at 30 min and 95% B at 40 min, and becoming isocratic for 5 min. Chromatograms were recorded at 278 nm. PDA detection was performed by recording the absorbance of the eluate between 200 and 400 nm, with a resolution of 1.2 nm. Caffeine and chlorogenic acid derivatives was identified by comparing the retention times and spectral data with those of authentic standards. All analyses were repeated three times.

2.9. Statistical analysis

All measurements and analyses were carried out in triplicate. The results were analysed statistically using the Statistica 7.0 program to determine the average value and standard error. Variance analysis, with a significance level of $\alpha = 0.05\%$, was performed to determine the differences in the phenolic content due to different extraction conditions, as well as to establish the differences in the content of these compounds among the coffee extracts. Correlation analysis was also ran with the same statistical package.

3. Discussion

This study presents the content of polyphenols and caffeine of Minas and Cioccolatato coffees (*C. arabica*), as well as Vietnam and Cherry coffees (*C. canephora*). Green coffee beans, described in Table 1, as well as coffee beans roasted in three roasting degrees (light, medium and dark) were analysed.

Table 1 provides an overview of the external characteristics and cultivation conditions of *C. Arabica* (Arabica) and *C. canephora* (Robusta). According to the data displayed in Table 1, *C. arabica* is more perceptible to the outer conditions, tolerates lower temperature and needs lower rainfall content. *C. canephora* varieties grow at lower altitude and require a longer period of time for ripening. Besides the external conditions which affect the plant, the differences between these species are also evident in the coffee bean appearance. Compared to the bean of *C. arabica*, which is larger in size and flat in shape, the *C. canephora* produces smaller and oval-shaped beans. Caffeine content is much higher in *C. canephora* (1.7–4.0%) than in *C. arabica*. Typical brew characteristics of Robusta are bitterness and fullness of taste in comparison to Arabica, where acidity is more emphasised. Some sensory properties of coffee beans are attributed to volatile substances developed during roasting and brewing, which in turn result in a variety of choices for preparing beverages (Lewis, 2004).

Botanical differences between these varieties dictate the different roasting conditions necessary to obtain the desired properties of roasted coffee. The same roasting conditions do not necessarily result in coherent quality of the final product, i.e. roasted coffee of the desired sensory properties in terms of colour, aroma and acidity. The roasting degrees (light, medium and dark) were determined on an empirical basis by an experienced technologist specialised in coffee roasting and quality control. Also, the criterion of weight loss was included in the study, similar to the study of Franca, Oliveira, Oliveira, Agresti, and Augusti (2009), where roasting degrees were established based on weight loss measurements (percent difference in sample weight before and after roasting) and visual inspection of the external colour of the beans, since these are the most common procedures employed by the coffee roasting industry. According to their study, average mass loss values per roasting degree were 14%, 15%, and 19% at 200 °C, corresponding to light, medium, and dark roasts, respectively. Since in our study a lower temperature regime was employed for a constant time, we have chosen a slightly lower percentage of weight loss as a characteristic of each roasting degree (light – 9–11%, medium – 11–14%, dark – 14–16%).

It is well established that green coffee beans contain efficient plant antioxidants, such as chlorogenic acids, phenolic acids, polyphenols and alkaloids; and their content depends mainly on the coffee species (*C. arabica*, *C. canephora* syn. C. *robusta*) and their origin (Belay, Ture, Redi, & Asfaw, 2008; Chu, Lin, Yu, & Ye, 2007; Stalmach, Mullen, Nagai, & Crozier, 2006). As it can be seen in Fig. 1, among the coffee varieties analysed in this study, Cherry coffee (*C. canephora*) generally exhibited the highest content of total phenols (42.37 mg GAE/g), followed by Minas, while Cioccolatato (*C. arabica*) contained the lowest TPC (21.01 mg GAE/g). Based on the results, it can be seen that the highest TPC was detected in coffees roasted at light and medium roasting conditions, which is in accordance with the fact that polyphenolic compounds are highly thermolabile compounds that are easily decomposed under the effect of high temperature (above 80 °C) (Katsube, Tsurunaga, Sugiyama, Furuno, & Yamasaki, 2009; Larrauri, Rupérez, & Saura-Calixto, 1997).

The content of total flavonoids mainly coincided with the content of total phenols, which was confirmed by a good correlation observed for these compounds ($r = 0.676$). The obtained results indicate that roasting affects the polyphenolic compounds of coffee, and confirmed that light and medium roasting are more favourable in terms of preserving these beneficial compounds during coffee roasting. Comparing the results of total phenols and flavonoids within the same variety, regularity in the results can be noticed, except for the Minas variety. For Cioccolatato, Vietnam and Cherry varieties, the highest content of total flavonoids (Cioccolatato – 17.29 mg GAE/g, Vietnam – 12.33 mg GAE/g, Cherry – 20.58 mg GAE/g) was determined in coffees roasted under the same conditions as the ones in which the highest TPC was determined. A deviation occurred in Minas variety, where light roasted coffee exhibited the highest TPC (39.75 mg GAE/g), while the highest TFC was determined in dark roasted coffee (15.42 mg GAE/g). Cioccolatato and Cherry coffees exhibited the highest values of total flavonoids at medium roasting degree (Cioccolatato – 17.29 mg

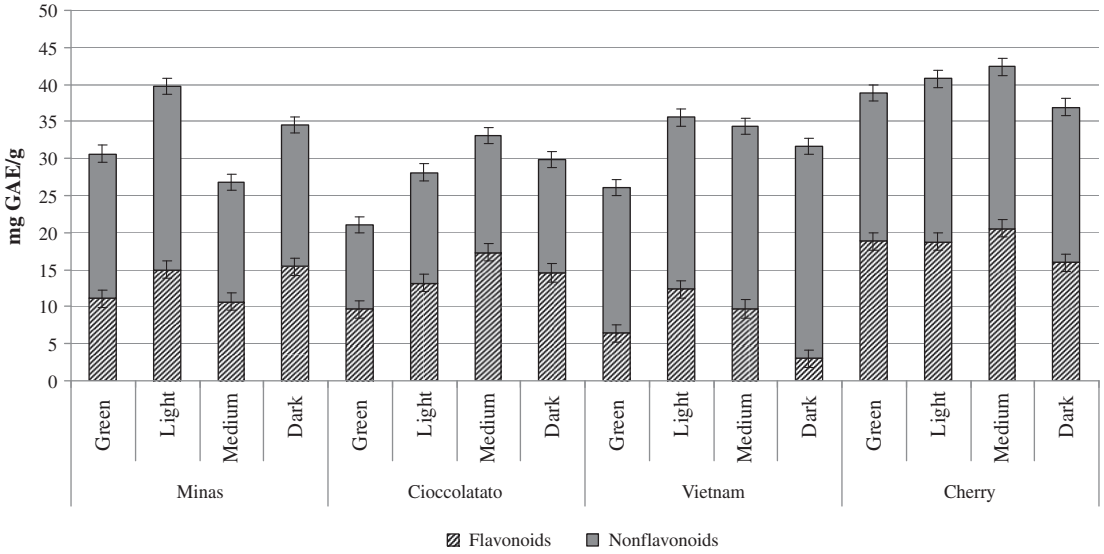

Fig. 1. Total flavonoids and non-flavonoids content (mg GAE/g) of coffees affected by different roasting degrees. The height of bars presents the total phenol content (TPC), obtained as the sum of flavonoids and nonflavonoids content. Both the content of flavonoids and nonflavonoids were significantly ($p<0.05$) affected by different coffee varieties, and not significantly ($p>0.05$) affected by the roasting degress.

GAE/g and Cherry – 20.58 mg GAE/g) followed by dark roasted Minas coffee (15.41 mg GAE/g), while the highest TFC of Vietnam variety was obtained at light roasting degree (12.33 mg GAE/g). As can be seen in Fig. 1, flavonoids constitute an average 44% of total polyphenols, which indicates that a larger portion of total polyphenols is attributed to non-flavonoid compounds, represented by phenolic acids.

In order to provide a better insight in the polyphenolic constituents of analysed coffees, the content of flavan-3-ols, proanthocyanidins and tannins was determined using several rapid and previously established assays, and the results are displayed in Table 3. Among the four analysed coffee varieties, Cherry coffee exhibited the highest content of three of the four evaluated polyphenolic classes, namely tannins (23.44 mg tannic acid/g) and flavan-3-ols (*p*-DAC assay) (0.31 mg (+)-catechin/g) at a dark roasting degree and proanthocyanidins (0.89 mg CyE/g) at a medium roasting degree.

The highest overall content of flavan-3-ols determined by the vanillin assay was observed in light roasted Minas coffee (2.38 mg (+)-catechin/g), while the other analysed coffee varieties displayed the highest flavan-3-ols in dark roasted coffees (1.60–2.28 mg (+)-catechin/g). As with the content of flavan-3-ols determined by the vanillin assay, the values obtained using the *p*-DAC assay decreased with the intensity of roasting in Minas coffee, while in the other three varieties the content of flavan-3-ols increased by prolonging the roasting procedure. According to these results, three coffee varieties, with the exception of Minas coffee, showed a regularity in the flavan-3-ols content, which increased with the prolongation of the roasting degree. Since the content of flavan-3-ols determined by the vanillin assay was very low in green coffees (0.17–0.57 mg/g (+)-catechin), and was not detected in green coffee using the *p*-DAC assay, the obtained results imply that coffee roasting contributes to the development of beneficial flavan-3-ol compounds. These compounds may be formed as a

Table 3
The content of flavan-3-ols, proanthocyanidins and tannins in green and roasted coffees.

Variety	Roasting degree	Vanillin (mg/g (+)-catechin)	*p*-DAC (mg/g (+)-catechin)	Proanthocyanidins (mg CyE/g)	Tannin (mg/g)
Minas	Green	0.20 ± 0.08	n.d.*	0.03 ± 0.01[a]	6.19 ± 1.90[e]
	Light	2.38 ± 0.16	0.19 ± 0.02	0.22 ± 0.01[a]	10.17 ± 4.50[e]
	Medium	2.31 ± 0.14	0.18 ± 0.05	0.24 ± 0.00[a]	8.33 ± 0.00[e]
	Dark	1.35 ± 0.02	0.09 ± 0.02	0.36 ± 0.00[a]	3.48 ± 0.50[e]
Cioccolatato	Green	0.17 ± 0.00	n.d.*	0.01 ± 0.00[b]	1.25 ± 0.40[f]
	Light	1.06 ± 2.06	0.07 ± 0.05	0.11 ± 0.50[b]	0.67 ± 1.80[f]
	Medium	1.28 ± 0.12	0.08 ± 0.02	0.23 ± 0.03[b]	1.74 ± 0.30[f]
	Dark	1.60 ± 0.04	0.20 ± 0.02	0.35 ± 0.08[b]	1.06 ± 2.10[f]
Vietnam	Green	0.39 ± 0.14	n.d.*	n.d.*[c]	7.36 ± 1.40[g]
	Light	1.41 ± 0.06	0.07 ± 0.00	0.18 ± 0.09[c]	10.26 ± 0.30[g]
	Medium	1.89 ± 0.04	0.13 ± 0.00	0.29 ± 0.00[c]	7.94 ± 4.70[g]
	Dark	2.28 ± 0.06	0.26 ± 0.02	0.67 ± 0.00[c]	4.16 ± 2.30[g]
Cherry	Green	0.57 ± 0.23	n.d.*	0.13 ± 0.03[d]	12.49 ± 3.20[h]
	Light	1.02 ± 0.04	0.14 ± 0.01	0.77 ± 0.00[d]	17.43 ± 3.60[h]
	Medium	1.32 ± 0.27	0.21 ± 0.03	0.89 ± 0.03[d]	19.66 ± 3.20[h]
	Dark	1.92 ± 0.29	0.31 ± 0.01	0.60 ± 0.05[d]	23.44 ± 1.40[h]

The different letters (a–h) denote the content of bioactive compounds, which are significantly ($p > 0.05$) affected by the coffee variety and roasting degree.
* n.d. – not detected.

consequence of Maillard reactions. Since polyphenolic compounds are known to undergo polymerisation and form complexes with proteins and sugars, specific reactions may have occurred during roasting, that have resulted with the formation of a wide variety of compounds, including flavan-3-ols complexes.

The content of proanthocyanidins, determined with the butanol–hydrochloric acid assay (Porter et al., 1986), displayed a similar pattern as the content of flavan-3-ols regarding the roasting degree. Total proanthocyanidins were again the highest at the dark roasting degree in three (0.35–0.67 mg (+)-catechin/g) of the four analysed coffee varieties. Only Cherry variety showed a deviation from this pattern.

The content of tannins exhibited higher variability concerning the different roasting degrees. Of all analysed coffee varieties, dark roasted Cherry coffee exhibited the highest overall content of tannins (23.44 mg TA/g). One variety of *C. arabica* (Minas) contained the highest amount of tannins at the light roasting degree (10.17 mg TA/g), while the other (Ciccolatato) exhibited the highest tannin content at the medium roasting degree (1.74 mg TA/g). The same pattern can be noticed in *C. canephora* , where the Vietnam variety exhibited the highest content for light roasting (10.26 mg/g), while the Cherry variety behaved similarly for dark roasting (23.44 mg TA/g).

Comparison of the obtained results indicates that the highest content of three of the four determined polyphenolic classes were determined at the same degree of roasting. Namely, in Minas coffee, the highest content of flavan-3-ols, determined by both vanillin and *p*-DAC assays, and the tannin content were detected in light roasted coffee, while in the other three analysed coffees the highest contents of flavan-3-ols, proanthocyanidins (Cioccolatato and Vietnam) and tannins (Cherry) were determined in dark roasted coffees.

Chemically, chlorogenic acid is an ester formed between caffeic acid and quinic acid. Chlorogenic acid is hydrolysed by intestinal microflora into various aromatic acid metabolites including caffeic acid and quinic acid (Gonthier, Verny, Besson, Rémésy, & Scalbert, 2003). Coffee is a major source of chlorogenic acid in the human diet. The daily intake of chlorogenic acid in coffee drinkers is 0.5–1.0 g, while coffee abstainers will usually ingest <100 mg/day (Olthof et al., 2001). Owing to vicinal hydroxyl groups on the aromatic residue, chlorogenic acid and caffeic acid act as scavengers of reactive oxygen species (ROS), and thus contribute to exhibiting antimutagenic, carcinogenic and antioxidant activities *in vitro* (Rice-Evans, Miller, & Paganga, 1996). The content of chlorogenic acid was therefore determined as the sum of all caffeoylquinic acid derivatives (3-CQA, 4-QA and 5-QA), in order to provide a detailed information about the content of chlorogenic acid in various coffees affected by the roasting degree. Fig. 2 displays the sum of caffeoylquinic acid derivatives (CQA) in the analysed coffees. The content of chlorogenic acid in the coffee beverage is dependent on the species, the variety, and the processing conditions of the coffee beans (Daglia et al., 2000; Moreira, Monteiro, Ribeiro-Alves, Donangelo, & Trugo, 2005).

The total content of CQA derivatives determined in our study was the highest in light roasted Minas and Cioccolatato coffees (*C. arabica*), while for *C. canephora* the same was observed in green coffee beans (Vietnam and Cherry). Comparing the highest overall CQA derivatives content of analysed coffees, it can be noticed that Cioccolatato coffee contains the highest content of total CQAs (50.17 mg CQA/g). Also, it was noticed that opposed to the content of flavan-3-ols, proanthocyanidins and tannins, CQA derivatives are more prevalent in light roasted samples and under milder processing conditions. At the same roasting conditions, and higher temperatures, dark roasting produced higher contents of flavonoid compounds. These observations are in agreement with previous findings, which confirmed the roasting results with the degradation of chlorogenic acid and its derivatives (Nicoli, Anese, & Parpinel, 1999).

The antioxidant capacity of coffee is attributed to the presence of polyphenolic compounds, and it is well established that roasting affects the antioxidant properties of coffee. The effect of coffee roasting on the antioxidant capacity of coffee brews was investigated in several earlier studies but discordant results were obtained: (i) an increase of antioxidant capacity in brews from medium roasted coffee and an antioxidant capacity decrease in those from dark roasted coffee (Nicoli, Anese, Parpinel et al., 1997; Nicoli, Anese, Manzocco, & Lerici, 1997; Steinhart et al., 2002; Del Castillo, Ames, & Gordon, 2002; Cämmerer & Kroh, 2006; da Silveira-Duarte, De Abreu, Castle de Menezes, Dos Santos, & Paiva-Gouvea, 2005) (ii) a decrease of antioxidant capacity in brews from light roasted coffee and an increase in those from dark roasted coffee (Anese & Nicoli, 2003; Daglia et al., 2000; Wen et al., 2005); (iii) an increase of antioxidant capacity of brews with roasting (Anese, De Pilli, Massini, & Lerici, 2000; Sanchez-Gonzales, Jimenez-Escrig, & Saura-Calixto, 2005); (iv) a decrease of antioxidant capacity of brews with roasting (Richelle, Tavazzi, & Offord, 2001).

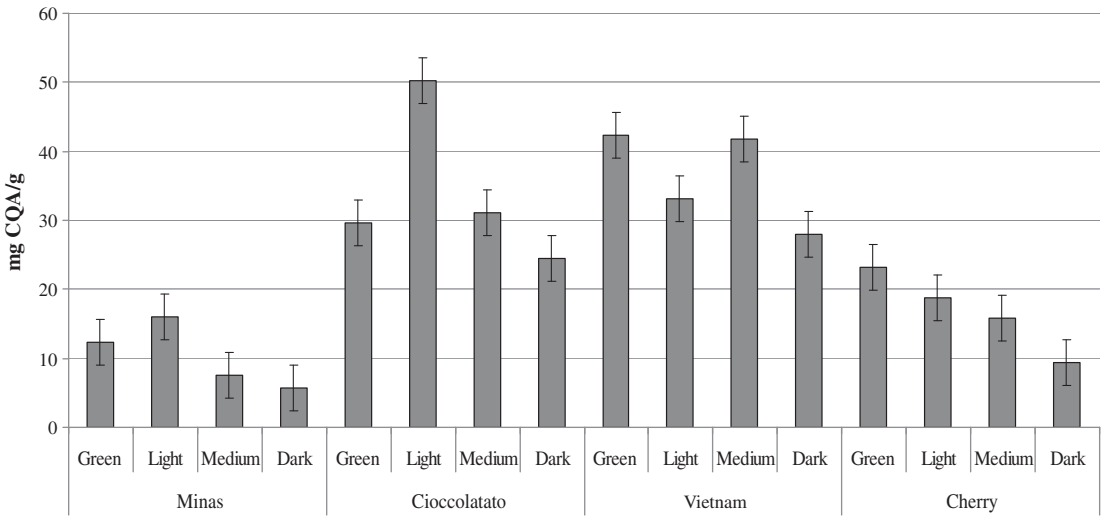

Fig. 2. The content of caffeoylquinic acid (QCA) derivatives (mg QCA)/g) of coffees affected by different roasting degrees. The content of chlorogenic acid derivatives was significantly ($p<0.05$) affected by different coffee varieties, and not significantly ($p>0.05$) affected by the roasting degress.

The antioxidant capacity of coffees roasted under different conditions was therefore also determined in this study. The obtained results are displayed in Fig. 3a and b. ABTS radical scavenging assay is frequently used by the food industry and agricultural researchers to measure the antioxidant capacity of foods (Huang, Ou, & Prior, 2005). This assay is often referred to as the Trolox equivalent antioxidant activity (TEAC) assay. According to the results of this assay (Fig. 3a), Vietnam coffee exhibited the highest antioxidant capacity (25.77 mmol/l Trolox), followed by Cherry (19.24 mmol/l Trolox) and Cioccolatato (15.64 mmol/l Trolox), while Minas coffee possessed the poorest radical scavenging properties (12.87 mmol/l Trolox). Regarding the effect of roasting on the antioxidant capacity determined with the ABTS assay, no regularity was determined. Namely, the highest antioxidant capacity for Vietnam variety (25.77 mmol/l Trolox) was determined for light roasted coffee, for Minas (12.87 mmol/l Trolox) and Cherry (19.24 mmol/l Trolox) it was at medium roasting, while for Cioccolatato variety dark roasted coffee exhibited the best antioxidant capacity (15.64 mmol/l Trolox). Also, considering the antioxidant capacity of green coffees in relation to roasted ones, a negative effect of intensified roasting degree can be noticed. The antioxidant capacity increased in Minas and Cherry coffees from green to medium roasted coffee, and decreased at dark roasting. Cioccolatato and Vietnam coffees showed a similar pattern, but the capacity increased from green to light roasted coffee and then decreased at medium and dark roasted coffees. Although, according to the results of statistical analysis, no significant differences ($p > 0.05$) were observed between antioxidant capacities of coffees affected by different roasting degrees, the fluctuations in the antioxidant capacity imply that prolonged roasting at higher temperatures causes a decrease of antioxidant capacity. Our results are in agreement with previous studies, which observed that the antioxidant capacity of roasted coffee exceeded that of green coffee beans, and an optimum of antioxidant action was found for medium-roasted samples (Del Castillo et al., 2005; Nicoli, Anese, Manzocco et al., 1997; Stalmach et al., 2006).

Unlike the ABTS method, where no general regularity could be established regarding the roasting degree effect on the antioxidant capacity of coffees, results of the FRAP assay (Fig. 3b) revealed that all analysed coffees exhibited the highest antioxidant capacity at light roasting degree (2.38–5.82 mmol/l Fe(II)). Comparing the antioxidant properties determined with the FRAP assay among each coffee variety, Vietnam coffee exhibited the highest reducing power (5.82 mmol/l Fe(II)), while Cherry resulted in the lowest reducing power (1.77 mmol/l Fe(II)). Both applied assays confirmed that roasted coffees possess better antioxidative properties in comparison to green coffee, and that intensified roasting negatively affects the antioxidant properties of coffees.

Due to strong physiological effects of caffeine on human physiology, the content of caffeine is a very important quality parameter

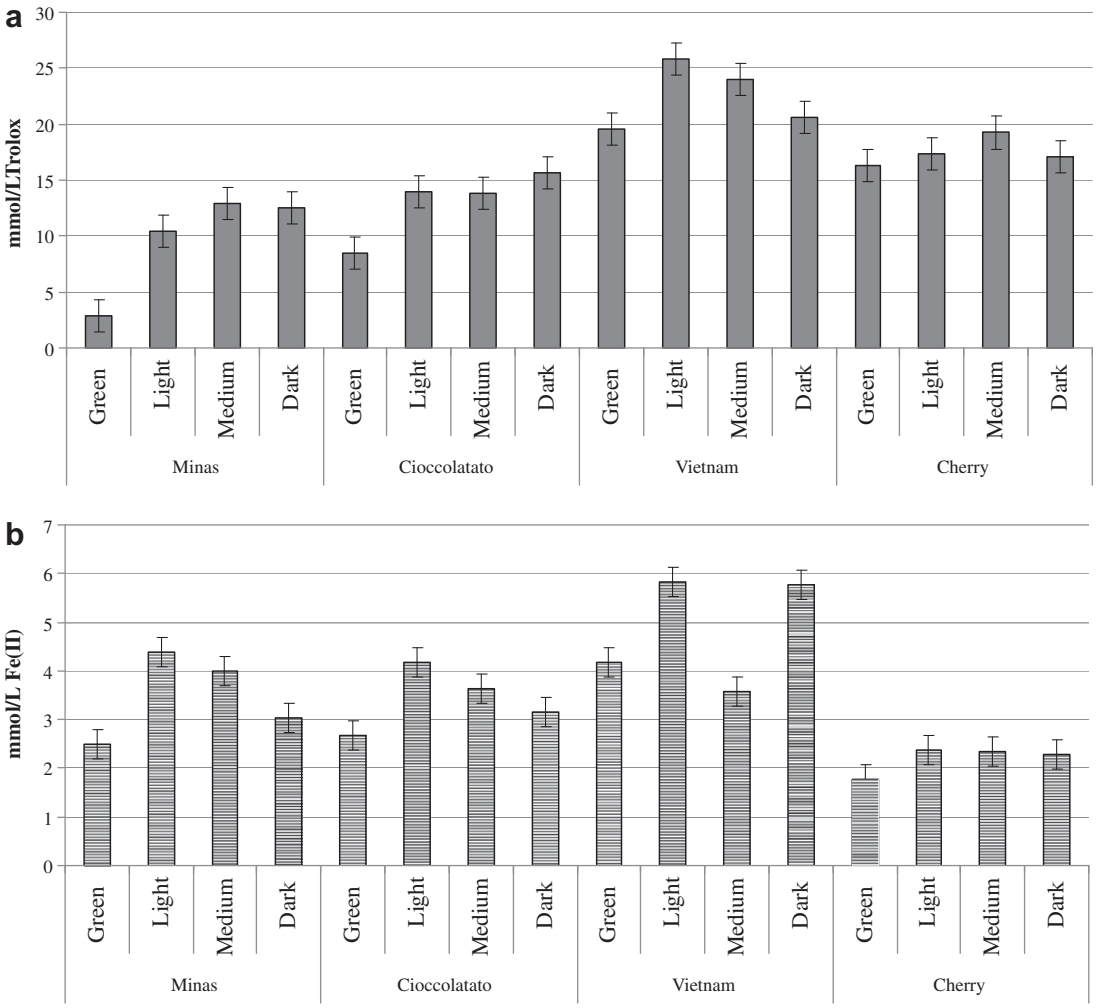

Fig. 3. Antioxidant capacity of green and roasted coffees determined by the (a) ABTS assay (mmol/l Trolox) and (b) FRAP assays (mmol/l Fe(II)). The antioxidant capacity determined by both the ABTS and FRAP assays was significantly ($p<0.05$) affected by different coffee varieties, and not significantly ($p>0.05$) affected by the roasting degress.

Table 4
The content of caffeine in coffees affected by different roasting degrees and determined by several different assays.

Variety	Roasting degree	HPLC (%)	Chloroform extraction (%)	Micro-method (%)	Lead-acetate method (%)
Minas	Green	0.66 ± 0.04[a]	0.56 ± 0.02	0.16 ± 0.00[e]	0.11 ± 0.01[i]
	Light	1.07 ± 0.03[a]	0.69 ± 0.02	0.25 ± 0.02[e]	0.13 ± 0.00[i]
	Medium	0.82 ± 0.03[a]	0.78 ± 0.00	0.24 ± 0.01[e]	0.13 ± 0.00[i]
	Dark	0.86 ± 0.01[a]	0.79 ± 0.05	0.23 ± 0.00[e]	0.15 ± 0.01[i]
Cioccolatato	Green	1.21 ± 0.11[b]	0.61 ± 0.03	0.08 ± 0.00[f]	0.17 ± 0.01[i]
	Light	2.24 ± 0.21[b]	0.64 ± 0.03	0.13 ± 0.00[f]	0.13 ± 0.00[i]
	Medium	1.59 ± 0.14[b]	0.68 ± 0.03	0.14 ± 0.01[f]	0.13 ± 0.00[i]
	Dark	1.53 ± 0.14[b]	0.73 ± 0.04	0.12 ± 0.01[f]	0.12 ± 0.01[i]
Vietnam	Green	1.92 ± 0.15[c]	1.07 ± 0.08	0.12 ± 0.00[g]	0.09 ± 0.00[i]
	Light	1.81 ± 0.14[c]	1.33 ± 0.10	0.16 ± 0.01[g]	0.11 ± 0.01[i]
	Medium	2.47 ± 0.23[c]	1.39 ± 0.10	0.19 ± 0.01[g]	0.11 ± 0.01[i]
	Dark	1.96 ± 0.16[c]	1.43 ± 0.12	0.16 ± 0.01[g]	0.11 ± 0.01[i]
Cherry	Green	2.07 ± 0.17[d]	1.07 ± 0.04	0.17 ± 0.01[h]	0.19 ± 0.01[i]
	Light	2.55 ± 0.21[d]	1.24 ± 0.09	0.28 ± 0.01[h]	0.11 ± 0.01[i]
	Medium	2.52 ± 0.19[d]	1.27 ± 0.10	0.27 ± 0.01[h]	0.13 ± 0.00[i]
	Dark	2.37 ± 0.13[d]	1.37 ± 0.12	0.30 ± 0.02[h]	0.15 ± 0.01[i]

The same letters (a–i) denote the content of caffeine, which is not significantly ($p > 0.05$) affected by the roasting degree and coffee varieties.

of processed coffee. Routine analysis of caffeine in food industry may be facilitated using fast and reliable assays. In this study, the authors aimed to compare different assays for the determination of caffeine in order to find the most effective alternative assay, which can be used in smaller coffee processing/roasting facilities that often do not possess the HPLC equipment required for these analyses. Determination of caffeine content in the coffees was carried out by four methods: HPLC analysis (Horžić et al., 2009), isolation with chloroform (Rapić, 1995), a method using lead-acetate solution (Yao et al., 1992, 1993) and micro-method (Groisser, 1978), in order to develop and employ a set of rapid and reliable assays for the determination of caffeine. The results of caffeine content determination are displayed in Table 4. The results were expressed as percentages due to easier comparison of the obtained results. HPLC method was used as a reference method, considering that this is the most reliable and accurate method of analysis. The results obtained by the HPLC analysis, were significantly higher in comparison to the other applied methods. According to the results of HPLC analysis, Minas, Cioccolatato and Cherry coffees yielded the highest caffeine content in light roasted coffees (0.66–2.55%), while the lowest caffeine content was determined in green Minas coffee beans (0.66%). Light roasted Cherry coffee contained 2.55% of caffeine, which was the highest overall value in all coffees. Our results are in agreement with the ones obtained by Wanyika, Gatebe, Gitu, Ngumba, and Maritim (2010), who also established that dark roasted coffee has less caffeine than lighter roasts, because the roasting process reduces the bean's caffeine content.

The chloroform extraction method resulted with a specific uniformity of results, but with a notably lower content of caffeine (0.56–1.43%), compared to the one determined with the HPLC analysis. All four coffee varieties exhibited the highest caffeine content in dark roasted coffees. Unlike this method, micro- and lead-acetate methods yielded the highest caffeine content at different roasting degrees for each coffee variety. Medium roasting appeared to be the best for Cioccolatato (0.14%) and Vietnam (0.19%) coffee, while for Minas (0.25%) light was best and dark roasting for Cherry (0.30%).

The overview of the results reveals that Cherry coffee generally exhibited the highest caffeine content determined with the HPLC, micro- and lead-acetate methods. The chloroform extraction yielded the highest caffeine content in Vietnam coffee (1.43%). Comparing the caffeine content obtained by different methods within one coffee variety, it is obvious that the content of caffeine decreases as in the following sequence: HPLC analysis > chloroform extraction > micro-method > lead-acetate method. This pattern cannot be applied for Cioccolatato coffee, where the lead-acetate method yielded a higher content than the micro-method.

Considering the consumption pattern of Arabica and Robusta, the obtained results may indicate which coffee provides a higher intake of polyphenolic compounds or caffeine. Among consuming countries, Scandinavian countries (which have the highest level of consumption per capita in the world) and Germany prefer mild *C. arabica* in their blends. *C. canephora* is the key component in espresso blends and darker roasts, and is therefore important in Southern Europe. The US and UK markets prefer lighter roasts in general, but require a wide spectrum of qualities (McClumpha, 1988). Due to high variability in the consumer preferences of coffee, the results of this study contribute to highlighting the effect of roasting on the most significant bioactive compounds of coffee, polyphenols and caffeine. Although there have been papers evaluating the impact of roasting on the polyphenolic compounds and antioxidant capacity of coffee, there is no paper evaluating several coffee varieties and the influence of roasting conditions on caffeine content. By knowing the exact impact of roasting on the bioactive compounds of coffee, coffee processing techniques can be modified and conducted in a way to achieve the desired (increased or decreased) content of bioactive compounds. Due to the beneficial potential of polyphenolic compounds, light or medium roasting is recommended for coffee processing, in order for these compounds to remain preserved. Based on the results of our study it can be observed that the consumption of *C. canephora* (Cherry, Vietnam) can provide a higher caffeine intake, and that intensified roasting conditions decrease the caffeine content. This means that by using a specific blend of coffees of known caffeine content, commercial coffee blends can be produced containing lower or higher caffeine content. This could also facilitate the decaffeination process of coffee, since it would economically benefit the overall production costs to use coffee blends roasted in a way to contain less caffeine.

4. Conclusions

The results obtained in this study provide a detailed overview of the effect of different roasting conditions on the polyphenolic composition, as well as on the content of caffeine. The results revealed that the content of bioactive compounds and antioxidant properties of different coffees vary depending on the coffee variety and are affected by the roasting conditions. Cherry coffee (*C. canephora*) exhibited the highest overall content of total phenols and individual classes of polyphenols (flavan-3-ols, procyanidins, tan-

nins). The total content of CQA derivatives was the highest in light roasted Minas and Cioccolatato coffees (*C. arabica*), while for *C. canephora* the same was observed in green coffee beans (Vietnam and Cherry). The highest content of polyphenolic compounds was achieved in coffees roasted at light and medium roasting conditions, which was also observed for the content of CQA derivatives. Although roasted coffees exhibited higher antioxidant capacity than green coffees, intensified roasting resulted in a decrease of antioxidant potential of coffees. Light roasted Cherry coffee contained the highest overall content of caffeine in all coffees, which exhibited a decrease with intensified roasting.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.05.059.

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