BIOLOGICALLY ACTIVE COMPOUNDS IN ROASTED COFFEE

Viesturs Kreicbergs, Fredijs Dimins*, Velga Mikelsone, Ingmars Cinkmanis

Latvia University of Agriculture, Department of Chemistry, Liela street 2, Jelgava, LV-3001, Latvia,
**e-mail: fredisd@tvnet.lv*

Abstract

Coffee is one of the products rich in biologically active compounds. The aim of this research work was to investigate the content of total phenols, flavonoids and caffeine in coffee available in the local markets as well as its changes depending on the brand. Eighteen coffee brands used in the research were analysed. The content of total phenols and total flavonoids was determined spectrophotometrically. The content of caffeine and polyphenols (gallic acid, catehin, caffeic acid, vanillin, chlorogenic acid, epicatehin and ferulic acid) were determined by high performance liquid chromatography. Total phenols in analysed coffee samples ranged from 1300 to 3700 mg gallic acid eqivalents (GAE) 100 g^{-1}, total flavonoids – from 15 to 103 mg quercetin equivalents (QE) 100 g^{-1} coffee. The content of polyphenols varied in a wide range too: gallic acid from 2.5 mg to 80 mg 100 g^{-1}, catehin from 30 to 80 mg 100 g^{-1}, caffeic acid from 1200 to 2500 mg 100 g^{-1}, vanilin from 100 to 150 mg 100 g^{-1}, chlorogenic acid from 1.4 to 2.8 g 100 g^{-1}, epicatehin from 11 to 30 mg 100 g^{-1}, and ferulic acid from 23 till 120 mg 100 g^{-1} coffee. The content of caffeine ranged from 0.7 till 1.5 g 100 g^{-1} coffee. The highest content of caffeine, catehin, caffeic acid and ferulic acid was detected in coffee samples with a higher proportion of the Robusta coffee variety. Total phenols and total flavonoids content did not vary significantly between coffee varieties.

Key words: Coffee, polyphenols, coffee substitutes

Introduction

Phenols of plant origin are one of the most significant primary antioxidants. Coffee is a product rich in these compounds.

Evergreen coffee trees are grown in the equatorial region in more than 50 countries of Asia, Africa, America, and the Carribean region. From about 70 coffee tree types only two have commercial value. They are Arabica (*Coffea arabica*) – which accounts for 75% and Robusta (*Coffea canephora*) accounting for 25% of the world's consumption. The coffee bean is a pit of a berry; it is a seed from the coffee tree.

Coffee which is made from Robusta is more concentrated, (the content of caffeine in Robusta beans reaches 4–5%) characterised by its bitterness and is less aromatic. Coffee from Arabica has a much more sophisticated flavour and intense complex aroma resembling those of flowers, fruit, honey and chocolate. Arabica contains more aromatic oils, but it has two times less caffeine than Robusta variety coffee beans. The world's best coffees are produced only from 100% Arabica coffee beans and therefore are much more expensive than blends containing Robusta coffee beans (Jansen, 2006).

In order to produce the specific and desirable aroma, the consumer is offered not one pure coffee variety but a blend of different varieties. One of the most popular varieties − Arabica and Robusta, mixed in different proportions, offer a wide range of tastes and aromas of coffee beverages.

Coffee roasting is one of the most important stages in the process of turning the beans into a beverage. The chemical changes that take place during the process of roasting are very complex and they have not yet been sufficiently researched (Eggers et al., 2002). During the roasting process at temperatures of 200−300 ºC the most important heat and chemical reactions take place which create a pleasant flavour and aroma of the roasted coffee – the beans change colour and they increase in volume by 50−80%, simultaneously losing 13−20% from its mass at the expense of moisture. Green unroasted coffee beans contain about 250 different compounds, whereas roasted coffee beans have about 655−800 compounds influencing the flavour. Thus from the same coffee beans it is possible to obtain completely different flavour characteristics during the process of roasting.

The main component in coffee that creates a tonic effect is caffeine (Fig. 1.) – a stimulant of the central nervous system.

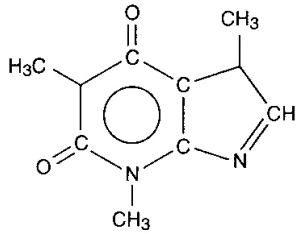
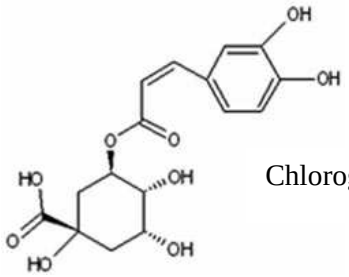


Figure 1. Structural formula for Caffeine

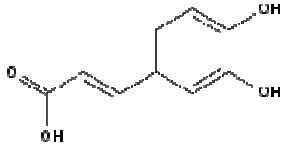
From the compounds present in coffee not only caffeine but also different compounds of phenols have a significant role. Phenolic compounds belong to different classes: phenolic acid and its derivatives, flavonoids (Belitz et al., 2008).

Unprocessed coffee beans contain caffeine, chlorogenic acid, caffeic acid (Fig. 2), proteins, lipids, mineral salts and other substances. The content depends on the coffee variety, harvesting and processing methods and also from climatic conditions.

From acids, chlorogenic acid is most represented, accounting for 4.5−11.1% from the dry weight in unroasted coffee. In the medium roasting process chlorogenic acid content decreases by 30%, but after strong roasting it decreases by 70%. Chlorogenic acid exists in the form of several isomers and forms a hydrophobic complex of a proportion 1:1. Coffee substitutes, however, contain about 1% caffeine and about 65% chlorogenic acid (Jansen, 2006).



Chlorogenic acid



Caffeic acid

Figure 2. The main acids in coffee

The aim of this research work was to investigate the content of total phenols, flavonoids and caffeine in coffee available in the local markets as well as its changes depending on the brand.

Materials and Methods

Ten samples of coffee from the beans of known origin roasted in Latvia were selected for this research; the six most popular coffee brands available in retailing (two identical Merild coffee brands), in the processing of which the origin of the beans is unknown, and also two coffee substitutes. The names of the coffee brands used in the research are presented in Table 1.

The total phenolic compounds were determined by using the Folin-Ciocalteu method. Each coffee sample (0.5 g) was extracted in 50 ml distilled water 30 min. and filtered. This solution was then mixed with 0.2 N Folin- Ciocalteu reagent for 5 min and then a solution of sodium carbonate was added. After incubating at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank. Gallic acid was used as standard to produce a calibration curve. The total phenolic content was expressed in mg of gallic acid equivalents (GAE) 100 g^{-1} of coffee (Kaškoniene et al., 2009; Meda et al., 2005; Marinova et al., 2005).

Table 1

Coffee brands

Legends of samples	Coffee brands	Legends of samples	Coffee brands
1	Robusta Smile Tiger / Vietnam (Coffea canephora)	10	Exclusive (Coffee arabica) mixed selection
2	India Monsoon Robusta (Coffea canephora)	11	Merild–1
3	India Robusta Parchment (Coffea canephora)	12	Merild–2
4	Brazil Santos(Coffea canephora)	13	Double Coffee
5	Mexico Altura (Coffea canephora)	14	Tchibo
6	PNG A/X Plantation / Papua New Guinea (Coffea arabica)	15	Pauling
7	Nicaragua (Coffea arabica)	16	Jacobs espresso
8	Ethiopia Sidamo(Coffea arabica)	17	Chicory, barley coffee
9	Indonesia Sumatra lintong (Coffea arabica)	18	Barley – Chicory – acorn coffee

The total flavonoid content (expressed as quercetine equivalent, mg QE 100 g^{-1}) was determined using the Dowd method. 2% $AlCl_3$ solution in methanol was mixed with the coffee extract solution. Absorbance readings at 415 nm were taken after 10 min against a blank sample consisting of a 5 ml coffee solution with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin as a standard. The total flavonoid content was expressed in mg of quercetin equivalents (QE) 100 g^{-1} of coffee (Kaškoniene et al., 2009; Meda et al., 2005; Marinova et al., 2005).

The caffeine content in coffee was determined by HPLC using Shimadzu LC 20 Prominence chromatographer.

Determination parameters:

Detector: DAD SPD – M20A; Column: Perkin Elmer C18, 4.6x250 mm, 5µm; Temperature of the column and detector: 35 ^oC; Mobile phase: A – acetonitrile; B – 10 mM KH_2PO_4 (pH = 3.5). 15:85; Gradient regime; Capacity of the injection sample: 10 µl; Total time of the analysis: up to 10 minutes; Rate of the flow: 1.3 ml min^{-1}.

For determining chromatographic polyphenols the following determination parameters were used.

Detector: DAD SPD – M20A; Column: Perkin Elmer C18, 4.6x250 mm, 5µm; Temperature of the column and detector: 30^oC; Mobile phase: A – methanol un B – acetic acid (100%) : distilled water (2:98); Gradient regime: A:B (20:80); Capacity of the injection sample: 10 µl; Total time of the analysis: up to 30 minutes; Rate of the flow: 1.3 ml·min^{-1}; Wavelength: 278 nm.

Results and Discussion

The total phenols content in the analysed samples is characterised in Figure 3. It should be concluded from the data presented in Figure 3, that from the known coffee brands the coffee brand Brasil Santos has a comparatively lower content of polyphenols. Double Coffee has the highest content of polyphenols. Coffee substitutes (Chicory, barley coffee and Barley – Chicory – acorn coffee) have the lowest content of polyphenols.

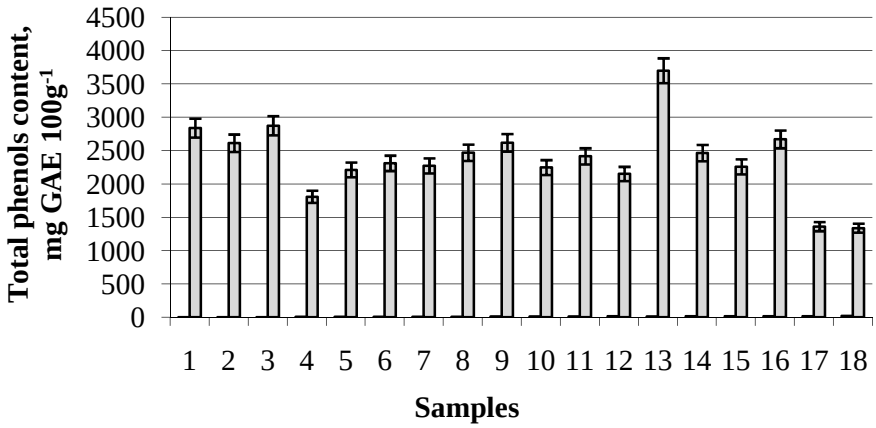


Figure 3. Total phenols content in coffee samples

To fully characterise total phenols content, the total flavonoids content was determined, which is shown in Figure 4.

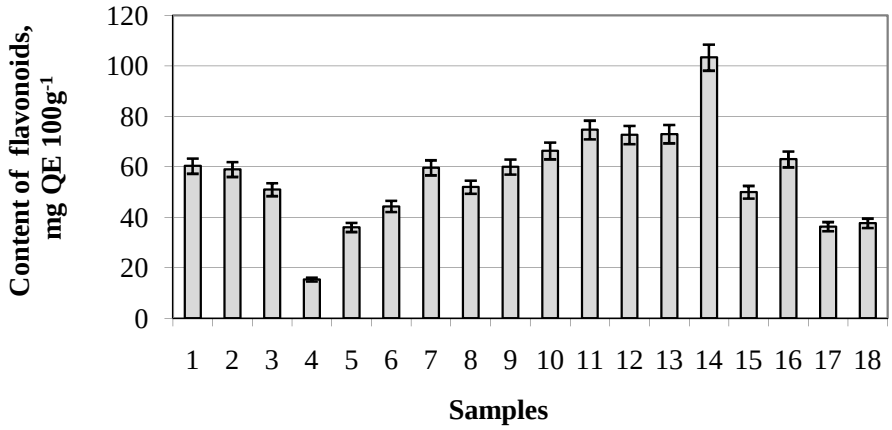


Figure 4. Total content of flavonoids in coffee samples

Tchibo has the highest content of flavonoids. Similarly to total phenols content the total flavonoids content is the lowest in Chicory, barley coffee and Barley - Chicory - acorn coffee. Brasil Santos coffee brand is an exception. The content of flavonoids is even lower there.
The content of several phenolic compounds in different coffee brands is presented in Table 2.

Table 2

Content of several polyphenolic compounds in roasted coffee

Sample No.	Gallic acid, mg $100g^{-1}$	Catechin, mg $100g^{-1}$	Caffeic acid, 10^3mg $100g^{-1}$	Vaniline, mg $100g^{-1}$	Chlorogenic acid, 10^3mg $100g^{-1}$	Epicatechin, mg $100g^{-1}$	Ferulic acid, mg $100g^{-1}$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	**78.97**	68.15	23.24	146.85	2.73	**28.55**	**120.72**
2	4.68	76.05	23.08	99.03	1.75	21.04	63.42
3	3.86	83.13	24.90	140.96	2.04	19.42	83.95

Continue of Table 2

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
4	4.09	29.70	12.76	124.12	2.19	12.75	37.49
5	11.16	36.11	12.79	125.87	2.18	13.12	37.55
6	2.71	32.15	13.63	105.86	2.35	12.41	31.97
7	5.01	41.46	12.28	117.83	1.76	11.32	23.93
8	2.95	46.44	12.59	137.52	1.72	14.05	24.12
9	6.06	37.52	11.62	141.79	2.13	13.20	29.04
10	2.82	51.90	12.72	118.45	1.47	13.75	22.77
11	4.14	25.29	11.93	116.97	2.46	10.80	22.74
12	1.80	28.14	21.25	123.72	2.33	20.14	49.75
13	5.00	41.17	12.19	130.15	2.45	14.26	23.38
14	8.60	10.94	12.53	123.68	3.10	12.48	33.25
15	3.96	29.11	12.12	102.45	2.67	14.17	21.22
16	2.86	39.98	13.96	98.27	1.45	15.19	64.80
17	**33.71**	**3.66**	**0.65**	**8.48**	**0.11**	**5.04**	32.24
18	**77.46**	**6.37**	**0.06**	**2.13**	**0.08**	**1.79**	**1.16**

As shown in Table 2, the content of different polyphenols in the analysed coffee samples fluctuates within quite a large range. An increased content of gallic acid, epicatechin un ferulic acid is observed in the coffee brand Robusta Smile Tiger. The results of chromatographic analyses show that the coffee brand Brazil Santos has a reduced content of polyphenols catechin and Ferulic acid. In untraditional coffee brands a reduced amount of almost all phenols has been observed, which coincides with the results of the previous analyses.

The results of caffeine determination are shown in Figure 5.

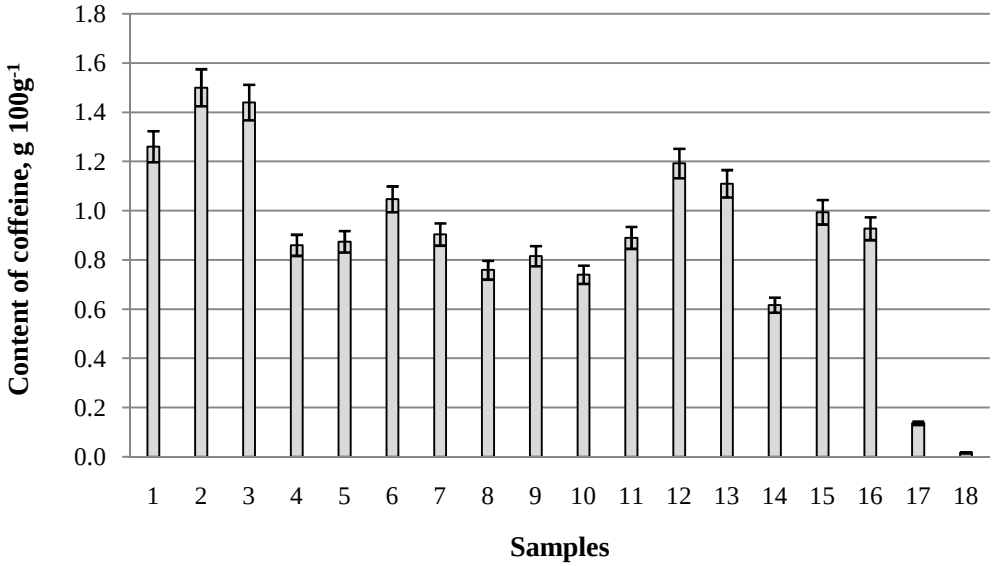


Figure 5. Content of caffeine in coffee samples

Increased content of caffeine has been found in the coffee brands Monsooned and India Robusta Parchment Robusta. In coffee substitute's chicory – barley and chicory – barley – acorn coffee the content of caffeine is rather low.

Conclusions

1. In analysed coffee brands the content of polyphenols varies over a large range.
2. Double Coffee contains the most of total phenols, whereas Tchibo has the highest content of total flavonoids. Robusta Smile Tiger coffee has the highest content of gallic acid, epicatechin and ferulic acid, while Brazil Santos coffee brand has the lowest content of polyphenols.
3. From Robusta variety coffees the highest content of caffeine was found in the brand India Monsooned, but from Arabica variety coffees the highest content of caffeine was found in Merild–2 and Double Coffee samples
4. Merild–1 un Merild–2 samples differ more than two times in the content of gallic acid, epicatechin and ferulic acid, which indicates that different raw materials were used in the processing of different Merild coffee batches.
5. Coffee substitutes have both a very low content of caffeine and a lower content of antioxidants compared to regular coffee.

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