EVALUATION OF CHLOROGENIC ACID AND TOTAL PHENOLIC CONTENT OF GREEN COFFEE (*COFFEA CANEPHORA*) DRIED BEANS

EVALUAREA CONȚINUTULUI DE ACID CLOROGENIC ŞI TOTAL FENOLIC DIN BOABELE USCATE DE CAFEA VERDE (*COFFEA CANEPHORA*)

FILIMON V.R.[1], ROTARU Liliana[1], PATRAŞ Antoanela[1], FILIMON Roxana[1]
e-mail: razvan_f80@yahoo.com

Abstract. *Chlorogenic acids are a group of cinnamic acid derivatives with biological effects mostly related to their antioxidant and antiinflammatory activities. This paper aims to determine the chlorogenic acid (CGA) and total phenolic (TP) concentration in various solvent extracts of green coffee beans (Coffea canephora syn. Coffea robusta). Also, the influence of brewing time (water at 90 °C) on the extraction of CGA was analyzed. 70% methanol and 70% 2-propanol were the most effective solvents in extraction of CGA from dried grinded green coffee beans of C. canephora (34.80±0.21 mg CQAE/g d.w. and 31.20±0.32 mg CQAE/g d.w., respectively). The most important CGA concentration was registered after 10 to 15 minute of coffee brewing (38.20±0.24 mg CQAE/g d.w.), when CGA represents approximately 45% of total phenolic compounds. Data obtained are useful both for experts from the food (functional food) and pharmaceutical industry, and also for traders and consumers.*
Key words: *chlorogenic acid, phenolic compounds, solvent extraction, time of brewing, Coffea canephora*

Rezumat. *Acizii clorogenici reprezintă un grup de derivați ai acidului cinamic cu proprietăți biologic active datorate în principal activității lor antioxidante și antiinflamatoare. Lucrarea are ca scop determinarea concentrației de acid clorogenic (ACG) şi a totalului de compuşi fenolici (TF) a extractelor de cafea verde (Coffea canephora sin. Coffea robusta) obținute utilizând diferiți solvenți. De asemenea, a fost analizată influența timpului de infuzie (apă, 90 °C) asupra extracției ACG. Metanol 70% şi 2-propanol 70% au fost solvenții cei mai eficienți în extracția ACG din boabele uscate şi măcinate de cafea verde C. canephora (34,80±0,21 mg EACQ/g m.u., respectiv 31,20±0,32 mg EACQ/g m.u.). Cea mai importantă concentrație de ACG a fost înregistrată între 10 şi 15 minute de infuzare a materialului vegetal (38,20±0,24 mg EACQ/g m.u.), când ACG a reprezentat circa 45% din totalul compuşilor fenolici. Datele obținute sunt utile atât de specialiştilor din industria alimentară (alimente funcționale) şi farmaceutică, cât şi comercianților şi consumatorilor.*
Cuvinte cheie: *acid clorogenic, compuşi fenolici, extracție cu solvenți, timp de infuzare, Coffea canephora*

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

INTRODUCTION

Chlorogenic acids (CGA) are phenolic compounds, secondary metabolites in plants derived from phenylalanine, formed through the esterification of cinnamic acids, such as caffeic, ferulic, and *p*-coumaric acids, with quinic acid (Clifford, 1999; Vermerris and Nicholson, 2006).

A series of health benefits have been associated with the consumption of CGA in the last decade, such as reduction of the risk of cardiovascular disease, type II diabetes, Alzheimer, and also antibacterial and antiinflammatory activities (Almeida et al., 2006; Santos et al., 2006; Farah et al., 2008). Moreover, it was demonstrated that CGA have protective effects against liver, colon and tongue carcinogenesis (Glezer, 2003), and reducing of DNA damages (Bakuradze et al., 2011). On the other hand, the reducing of the absorption of different types of sugar from the gastrointestinal tract due to consumption of CGA was recently reported (Bakuradze et al., 2011; Vinson et al., 2012).

Coffee plant is an evergreen shrub belonging to the *Rubiaceae* family, genus *Coffea*. Among the 85 species of this genus only two are of commercial importance: *Coffea arabica* and *Coffea canephora*, commonly known as „*arabicas*" and „*robustas*" (Flament, 1995).

Coffee is one of the most frequently consumed beverage worldwide. Since the 1960s and 1970s, epidemiological studies have shown that consumption of three or more cups of coffee per day may reduce several risk factors for obesity. Phenolic compounds other than caffeine that are found in coffee may contribute to these effects (Pimentel et al., 2014). The intake of green coffee (unroasted) has been associated with a lower risk of diseases of oxidative etiology apparently related to its high phenolic content (Baeza et al., 2014). At least five major groups of chlorogenic acid isomers were identified in green coffee beans: caffeoylquinic acids, dicaffeoylquinic acids, feruoylquinic acids, coumaroylquinic acids and caffeoylferuloylquinic acids (Shahidi and Naczk, 2004). Literature data indicate that „robustas" coffee has a substantially higher content of chlorogenic acid than „arabicas", varying from 7 to 10% of dry weight (d.w.) (Shahidi and Naczk, 2004).

In recent years, clinical studies conducted on humans and animals regarding the efficacy of green coffee beans and extracts in weight loss, suggest that CGA intake is an effective method in reducing weight, and might be considered as a less expensive means of preventing obesity (Vinson et al., 2012).

MATERIAL AND METHOD

Coffea canephora (syn. *Coffea robusta*) green beans were imported from India (under the name of „Indian Cherry") by local romanian traders. Coffee „dry processing" (also known as „unwashed" or „natural coffee") was used. The entire coffee cherry after harvest was first cleaned and then placed in the sun to dry in thin layers. The dried cherries were stored in bulk until they were sent to hulling, sorting and grading. Green coffee was transported in jute bags and milled 2 days before extraction.

Weight of 100 beans, moisture content (drying oven, 4 hours at 105 °C; ISO 1446:2001) and total mineral concentration (calcination oven, 4 hours at 525 °C; SR EN 1135:1997) of dried beans were determined.

The milled coffee beans were passed through a sieve with a 0.7 mm aperture. The ground material was extracted by shaking a 1 g of sample in a screw-capped tube with the following solvents: pure water, acetone (99.98%), 70% 2-propanol, 70% methanol and 70% ethanol, for a minimum of 30 minutes. The suspension was allowed to settle and decanted onto a Whatman No. 1 filter paper. The residue was re-extracted five times, the filtered extracts were bulked and diluted to 100 mL with solvent (Ohiokpehai, 1982).

Chlorogenic acid content was quantified using the molybdate assay proposed by Clifford and Wight, 1976 and presented by Chan et al., 2011. Molybdate reagent was prepared by dissolving 16.5 g sodium molybdate, 8.0 g dipotassium hydrogen phosphate, and 7.9 g potassium dihydrogen phosphate in 1 L distilled water (pH 6.5). An aliquot of each sample (0.2 mL) was added to 10 mL of molybdate reagent and mixed. This solution was examined spectrophotometrically (UV-vis Shimadzu 1700 Pharmaspec) against a blank (sample and buffer without sodium molybdate) at 370 nm. Different concentrations of caffeoylquinic acid solutions was used for expressing the results as caffeoylquinic acid equivalent (CQAE). The calibration equation used for CGA estimation was that proposed by Ohiokpehai (1982): $y = 0.003x + 0.0296$ ($R^2 = 0.9971$), where y represents absorbance while x is concentration of caffeoylquinic acid in mg/250 mL.

Total phenolic content was determined by Folin-Ciocâlteu colorimetric method, measuring the absorbance at 750 nm (Singleton and Rossi, 1965). A calibration curve of different concentrations of gallic acid solutions was used for expressing the results as gallic acid equivalent (GAE), with the equation $y = 0.8757x + 0.0438$ ($R^2 = 0.991$), where y represents concentration of gallic acid in mg/mL, while x is the absorbance. For obtaining the CGA percentage of total phenolic compounds, the results were also expressed as mg CQAE by equation mentioned.

All samples were examined in triplicate and standard deviation was mentioned.

The method used to discriminate among the means was Fischer's least significant difference procedure at 95% confidence level. P values lower than 0.05 ($p \leq 0.05$) were considered to be significant.

RESULTS AND DISCUSSIONS

Weight of 100 beans of *Coffea canephora* samples varied from 22 to 28 g, with a mean of 24.67±3.06 g, the values being within the range previously reported by Sureshkumar et al. (2013).

Moisture content of green beans influences the stability during storage and alters sensorial quality of end product, thus, a moisture content ranging between 8.0% and 12.5% is considered to be adequate (Reh et al., 2006). Moisture content of coffee beans analysed was about 11.42±0.94%, with a total mineral content, represented by ash, of 4.08±0.81%, in accordance to Bicho et al. (2012), which presented a mineral content in "robusta" green beans of about 4% (3.6%–4.8%).

The extraction of CGA into the coffee beverage depends on a variety of factors like: the proportion of grinded coffee to water, coffee freshness, grinding size, method of coffee brewing (temperature and time) (Shahidi and Naczk, 2004).

Concentration of chlorogenic acids in dry green coffee infusions (water at 90 °C) has increased during first 5 to 15 minutes of brewing, with a maximum of 38.20±0.24 mg CQAE/g d.w. (after 15 minutes), followed by a decrease in concentration, the trend line forming a plateau after 20 to 30 minutes of infusion (fig. 1). According to Shahidi and Naczk (2004), holding the coffee brew at elevated temperatures results in a loss of CGA, depending on the temperature and time; about 15% of chlorogenic acid was lost after 24 h at 83°C.

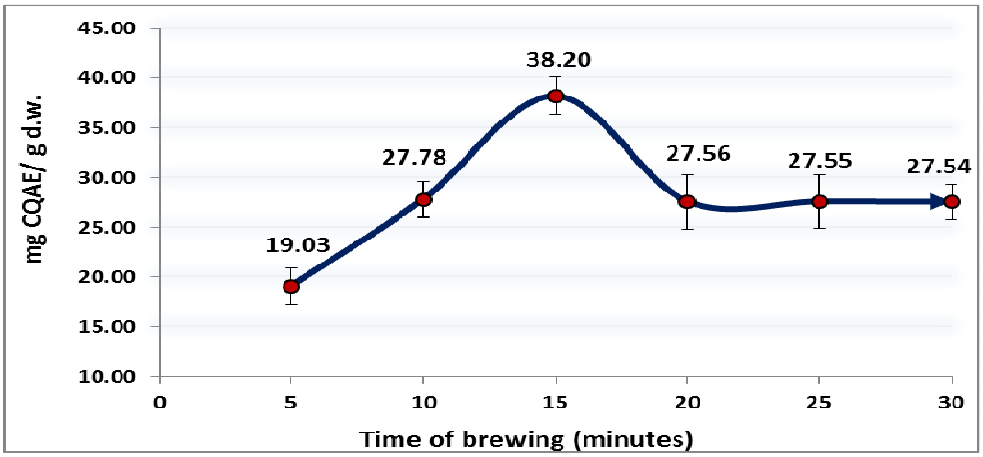

Fig. 1 - Concentration of chlorogenic acid (mg CQAE/g d.w.) in green coffee infusions (90°C) during brewing

Acetone does not seems to be specific for CGA extraction as long as the its concentration in acetone extract was only 4.48±1.02 mg CQAE/g d.w. 70% methanol was 7.76 times more effective than acetone, 1.33 times than water and only 1.12 times more effective than 70% 2-propanol (fig. 2).

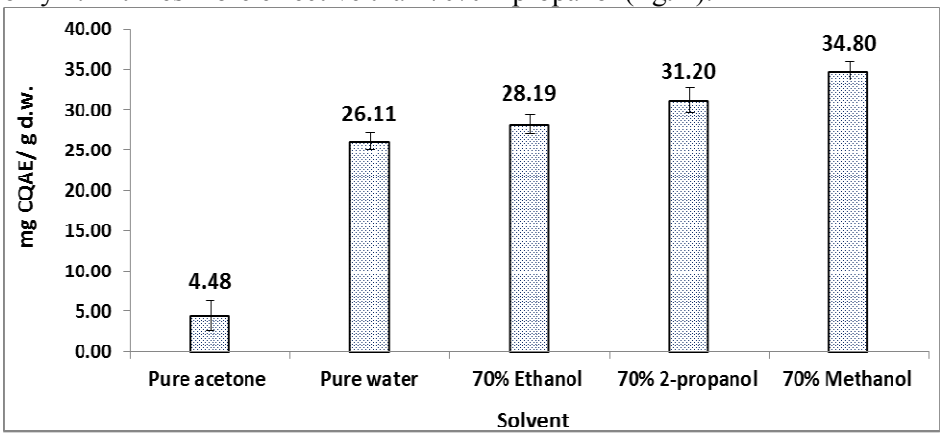

Fig. 2 - Concentration of chlorogenic acid (mg CQAE /g d.w.) in green coffee extracts obtained with different solvents

Regarding total phenolic content (TP) of green coffee infusions, positive statistically significant differences to the mean, were determined only for the values recorded after 10 and 15 minute of infusion, when the percentage of CGA from TP increased to a maximum of 45.18±0.68% (table 1).

Total phenolic content expresed as mg GAE, is useful in order to allow comparison with other species and to compare data reported for similar species. Thus, total phenolic content of analysed beans (23 mg GAE/g d.w.) was higher than that reported for *C. arabica* (21.00 mg GAE/g d.w.), but lower than that for *C. canephora* green beans (26.08 mg GAE/g d.w.) (Komes and Voivodic, 2014).

Table 1

Total phenolic content (TP) of green coffee infusions (water at 90 °C) during brewing and the percentage of chlorogenic acid (CGA) from TP

Time (min.)	TP (mg GAE/g d.w.)	St. error.	TP (mg CQAE/g d.w.)	St. error.	% of CGA from TP	St. error.
5	15.81^{000}	1.02	80.31^{000}	5.18	23.70^{000}	1.20
10	16.61***	0.84	85.02***	4.27	32.67^{000}	0.98
15	16.53***	0.65	84.56***	3.30	45.18***	0.68
20	15.83^{000}	0.87	80.46^{000}	4.42	34.26***	1.04
25	15.82^{000}	1.10	80.41^{000}	5.59	34.26***	1.02
30	15.83^{000}	0.55	80.43^{000}	2.79	34.24***	0.43
Mean	16.07	0.39	81.87	2.27	34.05	6.83
CV%	2.41	-	2.77	-	20.06	-

Note: Data expressed as mean values with standard error (n = 3). $^{NS, *, **, ***}$ - indicate nonsignificant and positive significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean; $^{o, oo, ooo}$ - indicate negative significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean. CV% - coefficient of variability (ratio of standard error to the mean (%)).

In the case of extraction with various solvents, total phenolic content of green coffee extracts followed the same trend as for CGA, the percentage of CGA from TP being maximum (24.30±1.19%) in 70% 2-propanol extracts (table 2).

Table 2

Total phenolic content (TP) of green coffee extracts obtained with different solvents and the percentage of chlorogenic acid (CGA) from TP

Solvent	TP (mg GAE/g d.w.)	St. error.	TP (mg CQAE/g d.w.)	St. error.	% of CGA from TP	St. error.
Pure acetone	9.07^{000}	1.02	37.22^{000}	4.18	12.05^{000}	1.12
Pure water	18.60**	0.98	109.74***	5.82	25.69***	1.01
70% ethanol	19.06***	1.23	113.20***	7.26	23.06***	1.64
70% 2-propanol	21.05***	1.11	128.40***	6.77	24.30***	1.19
70% methanol	23.26***	0.65	145.20***	4.06	23.97***	1.01
Mean	18.21	5.43	106.75	41.33	21.81	5.54
CV%	29.82	-	38.71	-	25.39	-

Note: Data expressed as mean values with standard error (n = 3). $^{NS, *, **, ***}$ - indicate nonsignificant and positive significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean; $^{o, oo, ooo}$ - indicate negative significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean. CV% - coefficient of variability (ratio of standard error to the mean (%)).

Statistical analysis of the data revealed a negative significant difference to the mean only for acetone extraction of phenolic compounds.

CONCLUSIONS

1. The most important concentration of chlorogenic acids from dried green coffee beans of *C. canephora* was registered after 15 minute of coffee brewing (38.20±0.24 mg CQAE/g d.w.), when CGA represents approximately 45% of total phenolic compounds ($p<0.05$).

2. 70% methanol and 70% 2-propanol were more effective in extraction of chlorogenic acids from green coffee beans in comparison to acetone, pure water and 70% ethanol; total phenolic content of extracts following a similar trend.

3. Temperature appears to be a decisive factor in the extraction of CGA from green coffee; hot water (90-93 °C) extracted, at the maximum point (15 min), an additional CGA percentage of 32% comparing to cold water (25 °C).

4. High amounts of chlorogenic acid in green coffee *C. canephora* beans, justify their consumption as *functional food* in preventing diseases and obesity.

***Acknowledgments**: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. **Almeida A.A., Farah A., Silva D.A.M., Nunam E.A., Gloria M.B.A., 2006** - *Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria*. J. Agric. Food Chem. Vol. 54, pp. 8738–8743.
2. **Baeza G., Amigo-Benavent M., Sarriá B., Goya L., Mateos R., Bravo L., 2014** - *Green coffee hydroxycinnamic acids but not caffeine protect human HepG$_2$ cells against oxidative stress*. Food Research Int. Vol. 62, pp. 1038–1046.
3. **Bakuradze T., Boehm N., Janzowski C., Lang R., ..., Baum M., 2011** - *Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: results from the intervention study*. Mol. Nu. Food Res. Vol. 55, pp. 793–797.
4. **Bicho N.C., Leitão A.E., Ramalho J.C., Lidon F.C., 2012** - *Use of colour parameters for roasted coffee assessment.* Ciênc. Tecnol. Aliment. Vol. 32, no.3, pp. 436-442.
5. **Chan W.C., Lim Y.Y., Shiau P.T., 2011** - *Standardised herbal extract of chlorogenic acid from leaves of Etlingera elatior Eric.* Pharmacog. Res. Vol. 3 (3), pp. 178–184.
6. **Clifford M.N., 1999** - *Chlorogenic acids and other cinnamates - nature, occurrence, and dietary burden*. J. Sci. Food Agric. Vol. 79, pp. 362–372.
7. **Clifford M.N., Wight J., 1976** - *The measurement of feruloylquinic acids and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans*. J. Sci. Food Agr. Vol. 27(1), p. 73-84.
8. **Farah A., Monteiro M., Donangelo C.M., Lafay S., 2008** - *Chlorogenic acids from green coffee extract are highly bioavailable in humans*. The J. of Nutrition. Vol. 138, no. 12, pp. 2309-2315.
9. **Flament I., 1995** - *Coffee, cacao, and tea*. In Volatile Compounds in Foods and Beverages (Ed. Maarse H.). Marcel Dekker, New York, pp. 617–669.
10. **Glezer V., 2003** - *Environmental effects of substituted phenols*. In The Chemistry of Phenols (Ed. Rappoport Z.). John Wiley & Sons, Ltd. p. 1347–1368.
11. **International Organization for Standardization, 2001** - *Green coffee - Determination of water content*. Basic reference method: ISO 1446:2001.
12. **Komes D., Voivodic A., 2014** - *Effects of varieties and growing conditions on antioxidant capacity of coffee*. In Processing and Impact on Antioxidants in Beverages (Ed. Preedy V.). Academic Press, USA. pp. 77–87.
13. **Ohiokpehai O., 1982** - *Chlorogenic acid content of green coffee beans*. Doctoral thesis, University of Surrey.
14. **Pimentel G.D., Micheletti T.O., Nehlig A., 2014** - *Coffee Intake and Obesity*. In Nutrition in the Prevention and Treatment of Abdominal Obesity (Ed. Watson R.R.). Academic Press, Elsevier. pp. 245–259.
15. **Reh C.T., Gerber A., Prodolliet J., Vuataz G., 2006** - *Water content determination in green coffee – Method comparison to study specificity and accuracy.* Food Chemistry. Vol. 96 (3), pp. 423–430.
16. **Santos M.D., Almeida M.C., Lopes N.P., Souza G.P., 2006** - *Evaluation of the antiinflamatory, analgesic and antypiretic activity of the natural polyphenol chlorogenic acid.* Biol. Pharm. Bull. Vol. 29, pp. 2236–2240.
17. **Shahidi F., Naczk M., 2004** - *Phenolics in Food and Nutraceuticals.* CRC Press, USA.
18. **Singleton V.L., Rossi J.A., 1965** - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid*. Am. J. Enol. Vitic. Vol. 16,p p. 144-158.
19. **Sureshkumar V.B., Nikhila K.R., Prakash N.S., Mohanan K.V., 2013** - *Interrelationship and association of characters in Robusta coffee (Coffea canephora var. robusta)*. Agriculture, Forestry and Fisheries. Vol. 2, No. 2, pp. 98–104.
20. **Vermerris W. and Nicholson R., 2006** - *Phenolic compound biochemistry*. Springer, Dordrecht, The Netherlands.
21. **Vinson J. A., Burnham B. R., Nagendran M. V., 2012** - *Randomized, double-blind, placebo-controlled, linear dose, crossover study to evaluate the efficacy and safety of a green coffee bean extract in overweight subjects.* Diabetes Metab. Syndr. Obes. Vol. 5, pp. 21–27.