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Discrimination of Brazilian arabica green coffee samples by chlorogenic acid composition

R.F.A. Moreira, L.C. Trugo, C.A.B. de Maria, A.G.B. Matos, S.M. Santos & J.M.C. Leite

Universidade Federal do Rio de Janeiro - Rio de Janeiro-RJ, Brazil, Biomedical Institute, UNI-RIO

SUMMARY. Coffee is basically consumed for the pleasure given by its taste and aroma, that is, the quality and acceptance of the coffee beverage are directly related to its sensorial characteristics. Thus, nowadays coffee quality is basically evaluated by sensorial analysis. However, together with this kind of analysis, it should be important to have available more objective chemical methods to assess coffee quality. One possible approach could be based on the analysis of chlorogenic acids (CGA), since they are considered precursors of coffee flavour and pigments during roasting. In the present work, high performance liquid chromatography (HPLC) analysis of chlorogenic acids was applied to six different Brazilian arabica green coffee samples which were previously characterised by sensorial analysis. The results showed the potential to correlate the chemical data, evaluated by the Principal Components Analysis (PCA) statistical method, with sensorial analysis in order to discriminate the quality of the samples. It was observed that the 3,4-dicaffeoylquinic (3,4-diCQA) and 3,5-dicaffeoylquinic (3,5-diCQA) isomers are very important for grouping the coffees into good and bad samples.

Key words: Arabica green coffee, sensorial analysis, chlorogenic acids, principal components analysis.

RESUMO. Diferenciação de amostras brasileiras de café verde arabica com base na composição de seus ácidos clorogênicos. O café é consumido basicamente pelo prazer que seu sabor e aroma proporcionam, isto é, a qualidade e a aceitação da bebida do café estão diretamente relacionadas com suas características sensoriais. Por conseguinte, atualmente a avaliação da qualidade dessa bebida é realizada através de análise sensorial. Entretanto, é óbvia a importância do desenvolvimento de novas metodologias capazes de diferenciar, de forma prática e objetiva, os diferentes tipos de café. Nesse sentido, tem sido crescente os esforços a fim de correlacionar a qualidade do café com o conteúdo dos chamados ácidos clorogênicos (ACG) do café, uma vez que esse grupo de compostos apresenta importância reconhecida com relação à formação de pigmentos, sabor e aroma nesse produto.
No presente trabalho foram obtidos, por cromatografia líquida de alta eficiência (CLAE), os perfis dos diferentes isômeros dos ácidos clorogênicos de seis amostras de cafés verdes brasileiros da espécie *Coffea arabica,* previamente classificadas sensorialmente. Os resultados obtidos indicaram que existe um bom potencial para correlacionar os dados químicos, tratados pelo método estatístico de Análise dos Componentes Principais (ACP), com as análises sensoriais como forma de diferenciar a qualidade desses tipos de amostra. Foi observado que os isômeros 3,4-dicafeoilquínico (3,4-diCQA) e 3,5-dicafeoilquínico (3,5-diCQA) são muito importantes para o agrupamento das amostras boas e ruins mencionadas acima.
Palavras chave: Café verde arabica, análise sensorial, ácidos clorogênicos, análise dos componentes principais.

INTRODUCTION

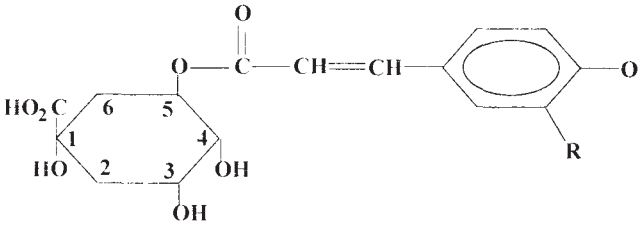
Green coffee beans represent an important item of international trade, believed second in importance only to oil, and contributes remarkably as an export commodity in Brazil. Besides, coffee growing and industrialisation provide many jobs with a considerable social impact in the country. An interesting characteristic of coffee brews is that they have been consumed mainly for pleasure, without concern about the nutritional value. The sensation of pleasure is intimately associated to the perception of the taste and aroma of the coffee, since coffee quality and acceptance are directly related to sensorial characteristics. The evaluation of coffee quality is achieved by sensorial analysis with trained panelists. However, many scientists are searching for new objective methodologies capable of discriminating the quality of coffee by chemical analysis of specific groups of compounds.

The CGA, which are in fact a group of natural phenolic compounds mainly formed by quinic acid esterification with either caffeic, ferulic or p-coumaric acids (Figure 1), perform an important role in the formation of pigments, taste and flavour of coffee. Some studies have indicated a relationship between the composition of the CGA fraction and the quality of the coffee bean or beverage (1–7). In one of these studies, a correlation between the coffee astringency and the content of the chlorogenic acids was observed (1). In another, the authors reported on the possibilities of discriminate either coffees of the same quality but different origins or plantations

or coffee blends of different compositions by the CGA fraction (7). The content of the CGA in the coffee beverage is dependent on the species, the variety and processing conditions of the coffee beans. A wide range of data related to the content of these acids in green beans are found in the literature. For example, values of 5.8, 0.87 and 0.25 g % for caffeoylquinic, dicaffeoylquinic and 5-feruloylquinic acids, respectively, were found in Arabica and of 6.8, 1.4 and 0.6 g %, respectively, in Robusta coffees (8). The 5-caffeoylquinic acid was always the major chlorogenic acid present, representing 66% of the total CGA in the Arabica and 56% in the Robusta coffee (8).

The purpose of the present work was to study a possible relation between the individual content of the CGA and the coffee quality of different Brazilian arabica green coffees, using coffee samples that were previously discriminated by sensorial analysis.

FIGURE 1
General formulas of chlorogenic acids



R=H, 5-p-coumaroylquinic acid (5-pCoQA)
R=OH, 5-caffeoylquinic acid (5-CQA)
R=OCH_3, 5-feruloylquinic acid (5-FQA)

MATERIALS AND METHODS

Materials

The study was carried out with five different varieties of Brazilian arabica green coffees (Table 1) totaling six samples which were previously classified and divided into two groups (good and bad samples) by sensorial analysis and then kindly supplied by ILLYCAFFÉ (Italy). The 5-caffeoylquinic acid standard (5-CQA) and the trifluoroacetic acid (TFA) were obtained from Sigma Chemical Company (U.S.A.). The dicaffeoylquinic acid isomers mixture (commercially termed isochlorogenic acid) was obtained from Carl Roth (Karlsruhe, Germany). The caffeoylquinic acid isomers mixture (3-CQA, 4-CQA and 5-CQA) was obtained following a previously described method (8). A feruloylquinic acid isomers mixture was prepared from a 5-feruloylquinic acid fraction (5-FQA) kindly supplied by Dr. M. Clifford (University of Surrey, UK) following the same procedure as for caffeoylquinic acids obtained. HPLC grade solvents were obtained from CARLO ERBA (Italy). All other reagents were of laboratory grade.

TABLE 1
Sensorial characterization of the Brazilian arabica coffees under analysis

Sample	Classification
A (Catuai amarelo)	Good sample
B (Icatu)	Good sample
C (Laurina - natural mutant of the Bourbon variety)	Good sample
D (Catuai vermelho)	Bad sample
E (Catuai vermelho)	Bad sample
F (Mundo Novo)	Bad sample

Methods

Extraction: The green coffee bean samples were frozen with liquid nitrogen and ground in a mill to pass 0.75 mm. Then, the lipid content was exhaustively removed from the samples by Soxhlet extraction. Defatted dry samples (500 mg) were extracted by shaking with bidistilled water (80°C) during 15 minutes. The extracts were treated with Carrez reagent (9) to precipitate coloidal material, diluted to 100 mL with bidistilled water and filtered through a Whatman N° 1 filter paper. These extracts were used directly for chromatography.

HPLC analysis: The HPLC-UV analysis was carried out following a previous method described in the literature (9) using a Pharmacia-LKB gradient system with a 20 (L loop injector and an UV detector at 315 nm. The separation of the CGA isomers was achieved using a 5 (m Lichrospher RT-18 column, 4.6 mm i.d. x 250 mm (Hibar, Merck, Germany). Samples or standards were analysed using a gradient from 100% solvent A (aqueous 0.5% TFA) to 98% solvent B (45% acetonitrile in 0.5% aqueous TFA, v/v) in 56 min. at a flow rate of 1 mL/min.

Statistical analysis: The chromatographic data were transferred to a personal computer and analysed with a PCA program (10).

RESULTS AND DISCUSSION

Different extraction systems have been described in the literature, using either organic solvents or hot water (11). We adopted the hot water (80°C) extraction system because it is cheaper, safer and also because the heating inhibit the action of the poliphenoloxidase reducing the possibility of CGA oxidation. In addition, water is the solvent used for coffee beverage preparation and we assumed that the chemical results could then be more comparable to those of sensory data.

The six Brazilian arabica green coffee samples that were used in this study were previously characterised by standard conditions in the industry by sensory tests as A, B and C (samples of good quality) and D, E and F (samples of poor quality) as can be observed in Table 1.

For universal comparison chemical analyses were carried out in the dried samples and their moisture contents are showed in Table 2. From the HPLC-UV chromatograms of the coffee extracts, six peaks with the lowest variation coefficients for their areas (less than 5%) were considered (Figure 2). Peak identification was achieved by comparing the retention time in the sample chromatograms with the available standards. The 4-CQA and 5-CQA isomers were coeluted. However, 4-CQA was found in much lower amounts and for the purpose of this work we considered both compounds together.

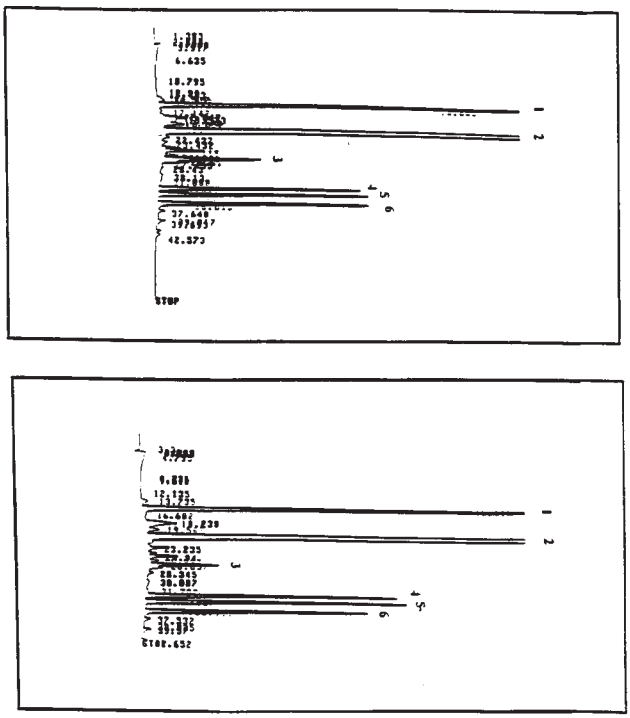
TABLE 2
Moisture content (%) of the six sensorial characterised coffee samples

Sample	Percentage of moisture (g/100 g)
A	12,43
B	11,86
C	11,82
D	11,95
E	11,22
F	12,01

Results are the average of duplicate determinations following the method of direct heating at 105°C.

The mean areas of the selected peaks (Table 3) were statistically evaluated by the multivariate method, Principal Component Analysis (PCA) (10). The principal components related to the variability of the system (i. e., the components that retain most of the variability between the samples) are listed in Table 4. Misunderstanding must not be done between these principal components, numbered from 1 to 6, and the chromatogram peaks, numbered in the same way. Actually, the principal components are linear combinations of the original variables (peak areas showed in Table 3). The principal component 1 and the principal component 2 showed in Table 4 accounted for 41.36% and 34.93% of the variability of the system, respectively. Together they were responsible for 76.29% of the variability between the samples. Using these two principal components it was possible to make a biplot (Figure 3) to obtain a graphical perspective of the distribution of the samples. The six lines presented in the biplot graphic are intersecting at (0,0) and represent the original variables. The length of each vector is proportional to its contribution to the principal components and the angle between any pair is inversely proportional to the correlation between them. Based on these last informations, it is apparent that 3,4-diCQA and 3,5-diCQA isomers are very important for the grouping of the samples into good and bad. There is a strong correlation between these variables and their influence is well marked in the samples classified as having poor quality. These results are in agreement with a previous publication (1), wherein a correlation between the coffee astringency and the content of the chlorogenic acids was noticed. In this work, evidences of a metallic taste related to the diCQA isomers that may negatively influence coffee acceptability were established. The PCA biplot also clearly shows a high dispersion of the samples, however, they still may be arranged in agreement with the sensory evaluation. The grouping of the samples can be better visualized by the hand outline provided in Figure 3.

FIGURE 2



(G) Profile of a sample characterised as having good quality
(P) Profile of sample characterised as having poor quality.
Key: Peak 1 = 3-CQA; peak 2 = 5-CQA + 4-CQA; peak 3 = 5-FQA; peak 4 = 3,4-diCQA; peak 5 = 3,5-diCQA; peak 6 = 4,5-diCQA.

TABLE 3
Mean area values of the peaks obtained from the chromatograms of the six analysed samples[1]

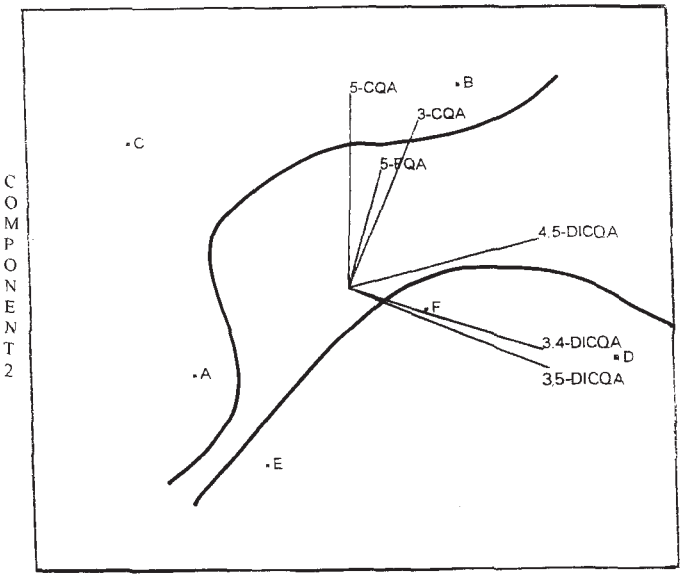
Peak	A	B	C	D	E	F	Variation coefficient (%)
1	2.36×10^6	3.09×10^6	2.75×10^6	2.58×10^6	2.53×10^6	2.85×10^6	1.6
2	1.78×10^7	2.28×10^7	2.27×10^7	1.98×10^7	1.84×10^7	1.87×10^7	3.4
3	6.09×10^5	5.98×10^5	6.17×10^5	6.17×10^5	4.74×10^5	5.81×10^5	0.8
4	8.58×10^5	1.02×10^6	8.09×10^5	1.76×10^6	1.03×10^6	1.16×10^6	3.9
5	9.20×10^5	1.13×10^6	8.14×10^5	1.46×10^6	1.19×10^6	1.26×10^6	1.8
6	9.90×10^5	1.27×10^6	8.19×10^5	1.18×10^6	9.26×10^5	1.09×10^6	1.8

[1]Results are the average of quadruplicate determinations of the areas of the peaks obtained by HPLC analysis.

TABLE 4
Principal component analysis

Component number	Percent of variance (%)	Cumulative percentage (%)
1	41.36032	41.36032
2	34.92779	76.28812
3	15.15491	91.44303
4	6.62347	98.06650
5	1.93350	100.00000
6	.00000	100.00000

FIGURE 3
Biplot of the two principal components



Despite the clear potential of the propose approach for coffee sample discrimination, the results presented have to be considered with care. In spite of the relation found between the content of some chlorogenic acid isomers and the quality of the Brazilian arabica green coffee samples evaluated by sensorial analysis, consubstantiation is still needed which could be achieved by applying this approach to a wider group of sensorialy characterised samples.

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