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3,4-Dimethoxycinnamic acid levels as a tool for differentiation of *Coffea canephora* var. *robusta* and *Coffea arabica*

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Thirteen green *Coffea canephora* var. *robusta* and seven green *Coffea arabica* coffee beans from different geographical origins were analysed by HPLC/diode-array detector for hydroxycinnamic acids (HCAs) discrimination and quantification to assess the possible markers for their botanical and geographical origins. While the HCAs, *p*-coumaric, *o*-coumaric and 3,4-dimethoxycinnamic acids were detected in the majority of samples, caffeic and ferulic acids were present in all of them. Sinapic and 4-methoxycinnamic acids were found in only one sample from Mexico and Honduras, respectively. In spite of the preliminary nature of this study, it seems that the relative amount of the HCAs could be related to the botanical origin of coffee. In fact, *Coffea canephora* var. *robusta* contains a higher level of 3,4-dimethoxycinnamic ($\bar{X} \pm$ SD:0.433 ± 0.15, ranging from 0.237 to 0.691 g kg^{-1}) than the *Coffea arabica* samples ($\bar{X} \pm$ SD:0.059 ± 0.03, ranging from 0.016 to 0.095 g kg^{-1}).

INTRODUCTION

The variety and origin of the beans, as well as storage conditions and time, roasting circumstances and the grading of coffee contribute to the quality of the brewing coffee. Adulteration of coffee is frequently practised which poses a big problem for quality control.

Guaranteeing the authenticity of coffee beans is an important task for the coffee trade. Lipids (Chassevent *et al.*, 1974; Ratnayake *et al.*, 1993), caffeine (Ferreira *et al.*, 1987; Clifford *et al.*, 1989, 1991; Clifford & Martinez, 1991; Rakotomalala *et al.*, 1992; Gennaro & Abrigo, 1992), trigonelline (Mazzafera, 1991; Stennert & Maier, 1994), terpenes (Chassevent *et al.*, 1967; IARC Working Group, 1991; Urgert *et al.*, 1995), chlorogenic acids (Clifford *et al.*, 1989; Clifford, 1985; Bennat *et al.*, 1994; Bicchi *et al.*, 1993, 1995; Menezes, 1994; Murata *et al.*, 1995) and caffeoyltyrosine (Correia *et al.*, 1995) evaluations have been the analyses most frequently carried out for differentiation of *Coffea* species. Recently, Rakotomalala *et al.* (1993*a*,*b*) also selected hydroxycinnamic acid (HCA) derivatives to distinguish African *Coffea* species.

The aim of the work herein, represents a contribution to HCAs discrimination and quantification in the two most representative commercial varieties, green *Coffea arabica* and green *Coffea canephora* var. *robusta*, to assess the authenticity of the commercial coffee varieties and eventually, their geographical origins.

The methodology applied, which was specifically developed for this purpose (Andrade *et al.*, 1997), was simple, precise, accurate and appropriate for routine analysis.

MATERIALS AND METHODS

Coffee samples and standards

Green coffee bean samples were supplied by the coffee industry. Green coffee beans were ground in a hammer mill to pass 0.8 mm. Caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, *o*-coumaric acid, 3,4-dimethoxycinnamic acid, 3,4,5-trimethoxycinnamic acid and 4-methoxycinnamic acid were obtained from Sigma Chemical Co.

Extraction of phenolic acids from coffee

The phenolic acids were extracted as described previously (Andrade *et al.*, 1997). The available powdered green coffee samples (≅5 g) were thoroughly mixed with

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methanol/water (40/60) (≅60 ml) for complete extraction of the phenolic fraction (24 h). The extract was then filtered and the filtrate concentrated under vacuum at 40°C to a final volume of 5 ml. This solution was hydrolysed with 5 ml of 2 N NaOH for 240 min. The pH of the mixture was adjusted to pH 7.00 with 2 N HCl and the phenolic acids were extracted by liquid/liquid extraction with ethyl acetate (20 ml×3). The extracts were then combined and the ethyl acetate removed under reduced pressure. The residue was dissolved in 7 ml of methanol and 20 μl were analysed by HPLC.

HPLC analysis of phenolic acids

This was achieved as recently reported (Andrade *et al.*, 1997) with an analytical HPLC unit (Gilson), using a reversed-phase Spherisorb ODS2 (5 μm, particle size; 25.0×0.46 cm) column. The solvent system used was the gradient of water-formic acid (19:1) (A) and methanol (B). The gradient was as follows: 0–15% B, 10–25% B, 25–30% B, 30–35% B, 34–50% B, 41–70% B, 43–75% B, 47–80% B. Elution was performed at a solvent flow rate of 0.9 ml min^{-1}. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 320 nm.

The different phenolic acids were identified by chromatographic comparisons with authentic standards and by their UV spectra. Quantification was based on the external standard method. Under the assay conditions described, a linear relationship between the concentration of phenolic acids and the UV absorbance at 320 nm was obtained. This linearity was maintained over the concentration range 4–400 μg ml^{-1}. The correlation coefficient for each standard curve invariably exceeded 0.99 for all phenolic acids. The calibration curves for phenolic acids were obtained by triplicate determinations of each of the calibration standards; the peak area values (arbitrary units) were plotted as average values. The relative percent average deviations of triplicates were less than 2% in all cases. The average regression equation for caffeic, *p*-coumaric, ferulic, *o*-coumaric, 3,4-dimethoxycinnamic, 3,4,5-trimethoxycinnamic and 4-methoxycinnamic acids were $y = 3.62 \times 10^8 x + 131114.3$, $y = 3.46 \times 10^8 x + 13614836$, $y = 4.10 \times 10^8 x + 151863.6$, $y = 2.23 \times 10^8 x + 60385.32$, $y = 2.67 \times 10^8 x + 172406.5$, $y = 1.87 \times 10^8 x + 68167.15$ and $y = 2.37 \times 10^8 x + 140739.3$, respectively. No regression equation for sinapic acid is shown because it could not be quantified. Given the similarity of the chemical structures of the acids analysed, and therefore their UV spectra and absorptivity, the detection limit and precision of the method were determined only for caffeic acid. The detection limit value was calculated for caffeic acid as the concentration corresponding to three times the standard deviation of the background noise and was 0.09 μg ml^{-1}. The precision of the analytical method was evaluated by measuring the peak chromatographic area of caffeic acid, 10 times on the same sample. The standard deviation was 0.006 and the coefficient of variation was 0.5%.

In order to study the recovery of the procedure, and bearing in mind that the method includes an alkaline hydrolysis, one coffee sample was added to known quantities of 5-caffeoylquinic acid and the percentage recovery calculated as caffeic acid. The sample was analysed in triplicate before and after the addition of 5-caffeoylquinic acid. Thus, this procedure demonstrated the effectiveness of the extraction and hydrolysis step and the accuracy of the proposed method. Recovery values were between 88.3 and 93.2% (Andrade *et al.*, 1997).

RESULTS AND DISCUSSION

The HCAs present in *Coffea canephora* var. *robusta* and *Coffea arabica* green coffee samples from different geographical areas were extracted and analysed by HPLC/diode-array detector and the results are summarized in Tables 1 and 2. It is remarkable that caffeic and ferulic acids were present in 100% of the samples analysed, which agrees with previous work of Rakotomalala *et al.*, (1993*a*,*b*), and *p*-coumaric, *o*-coumaric and 3,4-dimethoxycinnamic acids were detected in the majority of the samples analysed. The unidentified compounds presented in the chromatograms shown in Fig. 1 had identical UV spectra when recorded with a diode-array

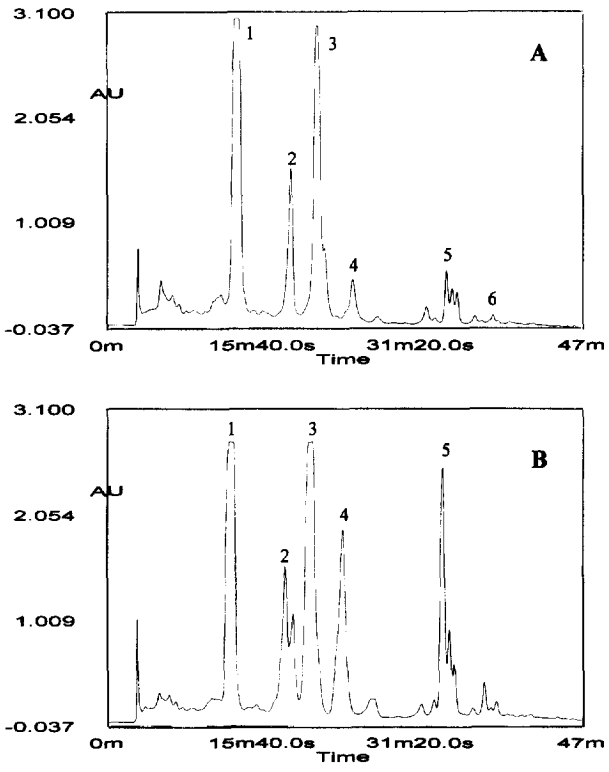

Fig. 1. HPLC phenolic acids profile of green coffee samples. (A) *Coffea arabica* (Honduras); (B) *Coffea canephora* var. *robusta* (Angola, Amboim). (1) caffeic; (2) *p*-coumaric; (3) ferulic; (4) *o*-coumaric; (5) 3,4-dimethoxycinnamic; and (6) 4-methoxycinnamic.

Table 1. Phenolic acids content of *Coffea canephora* var. *robusta* green coffee samples (g phenolic acid per kg coffee)[a]

	Caffeic	*p*-coumaric	Ferulic	Sinapic	*o*-coumaric	3,4-MeOcinnamic	3.4.5-MeOcinnamic	4-MeOcinnnamic
Angola[1]	1.19 ± 0.10	0.51 ± 0.10	1.02 ± 0.05	—	—	0.69 ± 0.02	—	—
Angola[2]	0.88 ± 0.05	0.28 ± 0.02	0.84 ± 0.00	—	nq	0.29 ± 0.02	—	—
A.Amboim[1+]	1.01 ± 0.05	0.35 ± 0.00	0.98 ± 0.00	—	nq	0.55 ± 0.005	—	—
A.Amboim[2+]	1.16 ± 0.05	0.40 ± 0.02	1.02 ± 0.05	—	0.22 ± 0.01	0.66 ± 0.005	—	—
Uganda[1]	1.13 ± 0.01	0.30 ± 0.007	1.15 ± 0.05	—	0.17 ± 0.005	0.49 ± 0.02	0.73 ± 0.05	—
Uganda[2]	1.15 ± 0.05	0.19 ± 0.005	1.08 ± 0.05	—	0.15 ± 0.00	0.24 ± 0.00	—	—
Cameroons[1]	1.19 ± 0.20	0.21 ± 0.005	0.88 ± 0.05	—	0.14 ± 0.03	—	—	—
Cameroons[2]	0.98 ± 0.00	0.20 ± 0.01	0.91 ± 0.1	—	0.20 ± 0.02	0.30 ± 0.004	—	—
Ivory Coast[1]	1.26 ± 0.10	0.20 ± 0.02	1.19 ± 0.10	—	0.18 ± 0.04	0.45 ± 0.04	—	—
Ivory Coast[2]	0.91 ± 0.00	0.29 ± 0.005	1.12 ± 0.00	—	0.28 ± 0.005	0.28 ± 0.00	—	—
Vietnam[1]	1.36 ± 0.05	0.28 ± 0.004	1.22 ± 0.05	—	0.16 ± 0.04	—	—	—
Vietnam[2]	1.51 ± 0.03	0.38 ± 0.02	1.23 ± 0.20	—	0.19 ± 0.03	0.38 ± 0.04	—	—
India	1.05 ± 0.10	0.20 ± 0.02	0.98 ± 0.00	—	0.20 ± 0.05	nq	—	—
$\overline{X}$ mean	1.14	0.29	1.05	—	0.19	0.43	—	—
SD	0.17	0.09	0.13	—	0.04	0.15	—	—
V_{min} (minimum value)	0.88	0.19	0.84	—	0.14	0.24	—	—
V_{max} (maximum value)	1.51	0.51	1.23	—	0.28	0.69	—	—

[a]Values are expressed as mean ± standard deviation of three determinations.
nq, Not quantified; 3,4-MeOcinnamic, 3,4,5-MeOcinnamic amd 4-MeOcinnamic are respectively: 3,4-dimethoxycinnamic acid, 3,4,5-trimethoxycinnamic acid and 4-methoxycinnamic acid. *A. Amboim-Angola (Amboim).

Table 2. Phenolic acids content of *Coffea arabica* green samples (g phenolic acid per kg coffee)[a]

	Caffeic	*p*-coumaric	Ferulic	Sinapic	*o*-coumaric	3,4-MeOcinnamic	3,4,5-MeOcinnamic	4-MeOcinnamic
Brasil[1]	0.86 ± 0.05	—	0.66 ± 0.05	—	0.12 ± 0.01	0.02 ± 0.004	0.07 ± 0.002	—
Brasil[2]	0.98 ± 0.10	0.31 ± 0.005	0.73 ± 0.06	—	0.12 ± 0.03	0.10 ± 0.005	—	—
Honduras	1.05 ± 0.0007	0.34 ± 0.03	0.84 ± 0.007	—	0.20 ± 0.005	0.06 ± 0.004	—	nq
Mexico	0.84 ± 0.00	—	0.55 ± 0.01	nq	—	0.02 ± 0.004	—	—
Guatemala	1.12 ± 0.00	0.26 ± 0.01	0.68 ± 0.03	—	0.13 ± 0.03	0.06 ± 0.02	—	—
Columbia	0.91 ± 0.00	0.30 ± 0.005	0.77 ± 0.00	—	0.19 ± 0.005	0.09 ± 0.005	—	—
Costa Rica	0.95 ± 0.05	0.31 ± 0.02	0.67 ± 0.02	—	0.21 ± 0.005	0.06 ± 0.005	—	—
$\overline{X}$ (mean)	0.96	0.30	0.70	—	0.16	0.06	—	—
SD	0.09	0.03	0.09	—	0.04	0.03	—	—
V_{min} (minimum value)	0.84	0.26	0.55	—	0.12	0.02	—	—
V_{max} (maximum value)	1.12	0.34	0.84	—	0.21	0.10	—	—

[a]Values are expressed as mean ± standard deviation of three determinations.
nq, Not quantified; 3,4-MeOcinnamic, 3,4,5-MeOcinnamic and 4-MeOcinnamic are respectively: 3,4-dimethoxycinnamic acid, 3,4,5-trimethoxycinnamic acid and 4-methoxycinnamic acid.

detector (identical shape and maximum at 320 nm) which suggests that they could be HCAs. In spite of the very different geographical origin of the green coffee samples, they show HCA patterns composed of only a reduced number of common compounds. On the other hand, some compounds were detected in only one green coffee sample from a specific geographical area, and could be considered as potential geographical markers. Thus, sinapic acid seems to be characteristic of a green coffee sample from Mexico and the phenolic acid, 4-methoxycinnamic acid of a green coffee sample from Honduras.

In other samples, it seems that the relative amount of the HCAs could be related to the botanical origin of coffee. Thus, *Coffea canephora* var. *robusta* green coffee samples contain higher amounts of 3,4-dimethoxycinnamic and ferulic acids than *Coffea arabica* green coffee samples (Fig. 1). Mean values of both acids of *Coffea canephora* var. *robusta* and *Coffea arabica* green coffee samples were significantly different ($P < 0.01$; determined by ANOVA methodology).

This preliminary study shows that HCAs analysis could be useful in botanical studies of green coffee beans, but further studies with a larger number of samples are necessary to confirm the differences observed. The HCA diagram may be used as a single source or in conjunction with some other chemical parameters, namely, caffeine, trigonelline, and chlorogenic acids, in the identification of the botanical origin of the two green coffee varieties. In conclusion, monitoring HCAs in coffee can be helpful: (i) for the definition of authenticity of the commercial varieties and eventually for the characterization of their geographical origins; (ii) in the coffee industry control, both for evaluation of their levels in raw materials and to follow the roasting process; and (iii) to follow hybridization assays.

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