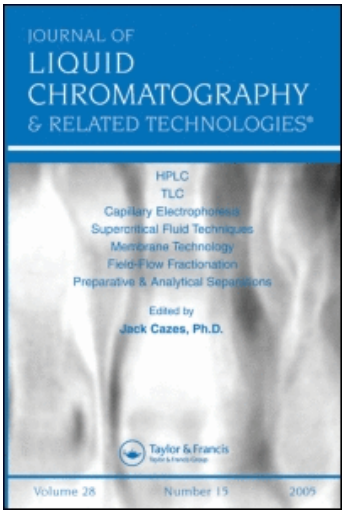

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ANALYSIS OF HYDROXYCINNAMIC ACIDS OF COFFEE: A COMPARISON OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND CAPILLARY ZONE ELECTROPHORESIS

S. Casal[a]; P. B. Andrade[a]; M. B. Oliveira[a]; F. Ferreres[b]; C. Garcia-Viguera[b]; M. A. Ferreira[a]
[a] Faculdade de Farmácia da Universidade do Porto, Porto, Portugal [b] Laboratório de Fitoquímica, Departamento de Ciencia y Tecnología de Alimentos, CEBAS (CSIC), Murcia, Spain

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ANALYSIS OF HYDROXYCINNAMIC ACIDS OF COFFEE: A COMPARISON OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND CAPILLARY ZONE ELECTROPHORESIS

S. Casal,[1] P. B. Andrade,[2] M. B. Oliveira,[1] F. Ferreres,[3]
C. Garcia-Viguera,[3] M. A. Ferreira[1]

[1] CEQUP/Laboratório de Bromatologia
[2] Laboratório de Farmacognosia
Faculdade de Farmácia da Universidade do Porto
4050 Porto, Portugal

[3] Laboratório de Fitoquímica
Departamento de Ciencia y Tecnología de Alimentos
CEBAS (CSIC)
P.O. Box 4195
Murcia 30080, Spain

ABSTRACT

The purpose of this work was to evaluate the heat degradation of hydroxycinnamic acids (HCAs) present in coffee by CZE and HPLC. The HCAs were obtained by alkaline hydrolysis of a coffee sample *Coffea canephora* var. *robusta*, heated at programmed temperatures and constant time, previously fixed with the limits of industrial roasting. The thermal degradation rate of each acid could apparently be explained by its specific sensitivity, which is correlated with its chemical structure. 3,4,5-Trimethoxycinnamic acid was the most resistant and caffeic acid the most sensitive of all analyzed HCAs.

This study suggests that CZE, while lacking the versatility of HPLC, may be regarded as a complementary analytical technique for the evaluation of heat degradation of these compounds.

INTRODUCTION

The decision of Rakotomalala et al.[1,2] to select hydroxycinnamic acids (HCAs) for chemiotaxonomical purposes in the discrimination of African *Coffea* species led Andrade et al.[3] to develop an accurate and reproducible HPLC methodology with diode-array detection for simultaneous determination of eight of these phenolic compounds in green coffee. The method developed was to be applied in the differentiation of *Coffea arabica* and *Coffea canephora* var. *robusta* varieties.

Owing to the highly efficient separation of CZE and its increased popularity in the analysis of natural organic compounds, in which its application is possible, we performed a comparative study between the HPLC initially developed and a CZE performance herein presented. The evolution of HCAs during heating, determined by both methodologies was evaluated.

EXPERIMENTAL

Coffee Samples and Standards

C*offea canephora* var. *robusta* green coffee bean samples from Ivory Coast were supplied by the coffee industry. Green coffee beans were ground in a hammer mill to pass 0.8 mm. Heat treatment was performed at 140, 160, 180, 200, 210, and 220ºC, during 14 minutes. Caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, *o*-coumaric acid, 3,4-dimethoxycinnamic acid, 3,4,5-trimethoxycinnamic acid, 4-methoxycinnamic acid were obtained from Sigma Chemical Co.

Extraction of Coffee Hydroxycinnamic Acids

The HCAs were extracted as previously described.[3,4] The available powdered green coffee samples (≅5g) were thoroughly mixed with methanol/water (40/60) (≅60mL) until complete extraction of the phenolic fraction (24h). The extract was then filtered and the filtrate concentrated under vacuum at 40ºC to a final volume of 5 mL. This solution was hydrolyzed with 5 mL of 2N NaOH for 240 min. The pH of the mixture was adjusted to pH 7.00

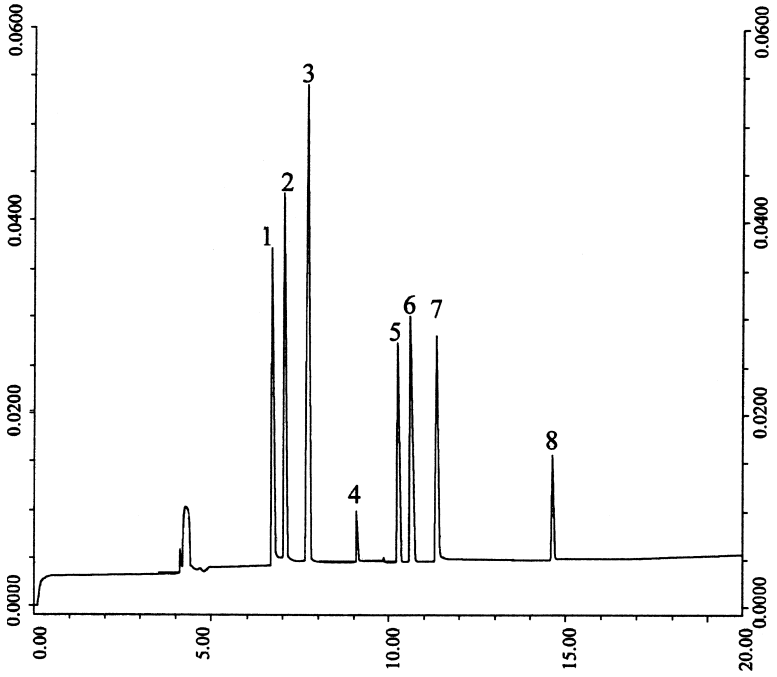

Figure 1. CZE profile of a standard solution of phenolic acids. Detection at 280 nm. (1) 3,4,5-trimethoxycinnamic acid; (2) 3,4-dimethoxycinnamic acid; (3) 4-methoxycinnamic acid; (4) Sinapic acid; (5) Ferulic acid; (6) *o*-coumaric acid; (7) *p*-coumaric acid; (8) Caffeic acid.

with 2N HCl and the phenolic acids were extracted by liquid/liquid extraction with ethyl acetate (20mLx3). 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 of the same extract was analyzed by CZE and HPLC.

CZE Analysis

CZE separations were carried out using a Beckman P/ACE System 2200 apparatus equipped with a fused silica column (50 cm X 75μm i.d.) at 30ºC using 0.1M sodium borate as buffer (pH 9.5) (prepared with H_3BO_3 – Sigma – and adjusted to pH 9.5 with NaOH), at 20 KV (average current of 38 μA). Samples were injected by hydrodynamic injection for 2 sec.[5]

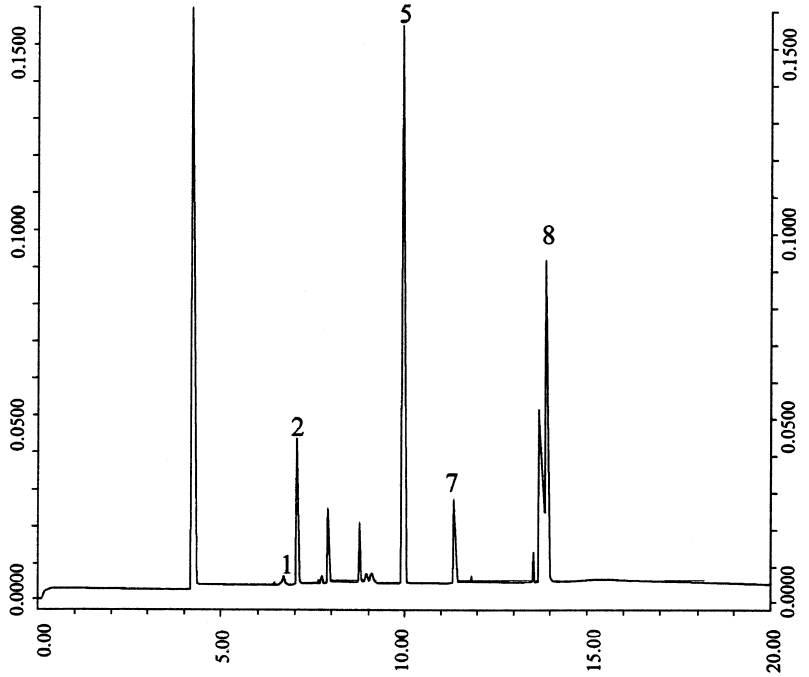

Figure 2. CZE phenolic acids profile of a green Robusta coffee sample from Ivory Coast. Detection at 280 nm. (1) 3,4,5-trimethoxycinnamic acid; (2) 3,4-dimethoxycinnamic acid; (5) ferulic acid; (7) *p*-coumaric acid; (8) caffeic acid.

Detection was achieved with a diode-array detector which allowed the recording of the UV spectra related with the different phenolic compounds in the 240-400 nm range. Electropherograms were recorded at 280 nm. All analyses were conducted in duplicate. In order to improve the reproducibility of the migration times, the capillaries were conditioned daily as previously reported.[6]

The different phenolic compounds were identified by their UV –vis spectra recorded with the diode-array detector and by electrophoretic comparisons (migration times) with authentic standards.

HPLC Analysis

This was achieved as recently reported[3,4] with an analytical HPLC unit (Gilson), using a reversed-phase Spherisorb ODS2 (5μm, particle size; 25.0 x 0.46 cm) column. The solvent system used was the gradient of water-formic

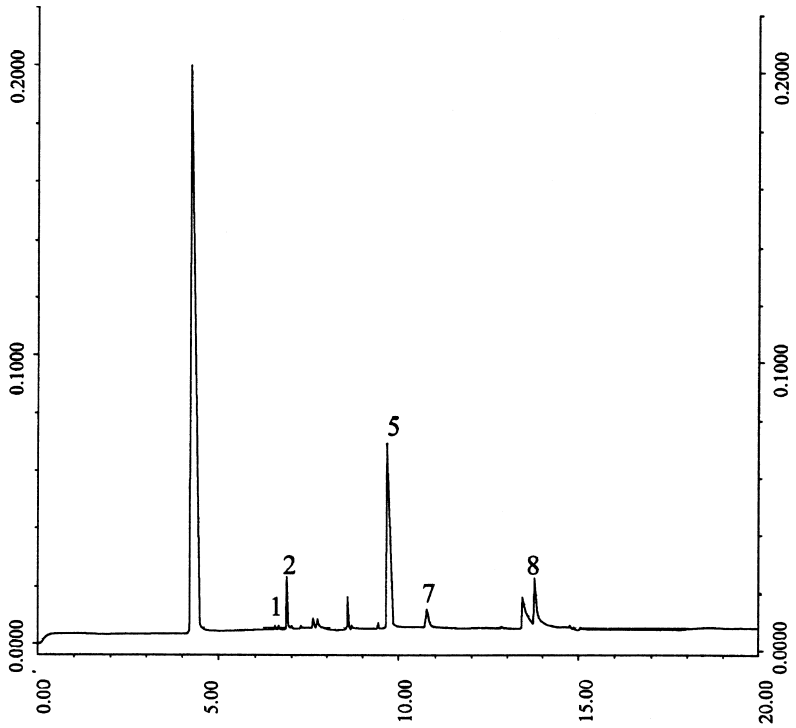

Figure 3. CZE phenolic acids profile of a roasted (T 220 ºC) Robusta coffee sample from Ivory Coast. Detection at 280 nm. (1) 3,4,5-trimethoxycinnamic acid; (2) 3,4-dimethoxycinnamic acid; (5) ferulic acid; (7) *p*-coumaric acid; (8) caffeic acid.

acid (19:1) (A) and methanol (B). 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. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 320 nm. All analyses were done in duplicate. The compounds in each sample were identified by comparing their retention times and UV-Vis spectra in the 240-400 nm range with the library of spectra previously compiled by the authors. Quantification was based on the external standard method.

RESULTS AND DISCUSSION

Both methods (CZE and HPLC) showed good resolutions for most of the detected compounds (Fig.1-6), but HPLC gave less separation efficiency, concerning ferulic/sinapic acids and 3,4,5-trimethoxycinnamic/3,4-dimethoxy-

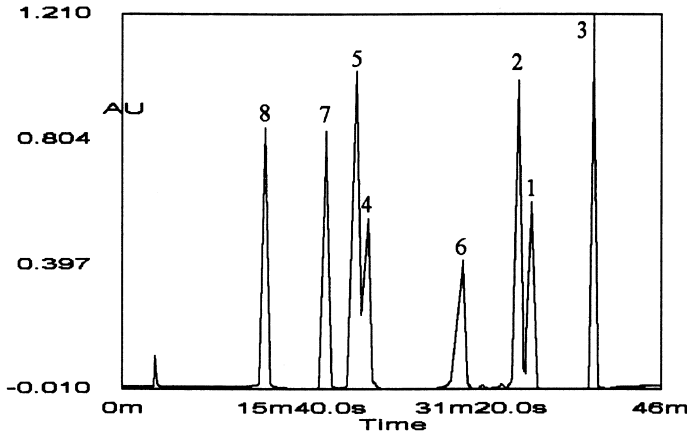

Figure 4. HPLC profile of a standard solution of phenolic acids. Detection at 320 nm. (1) 3,4,5-trimethoxycinnamic acid; (2) 3,4-dimethoxycinnamic acid; (3) 4-methoxycinnamic acid; (4) sinapic acid; (5) ferulic acid; (6) *o*-coumaric acid; (7) *p*-coumaric acid; (8) caffeic acid.

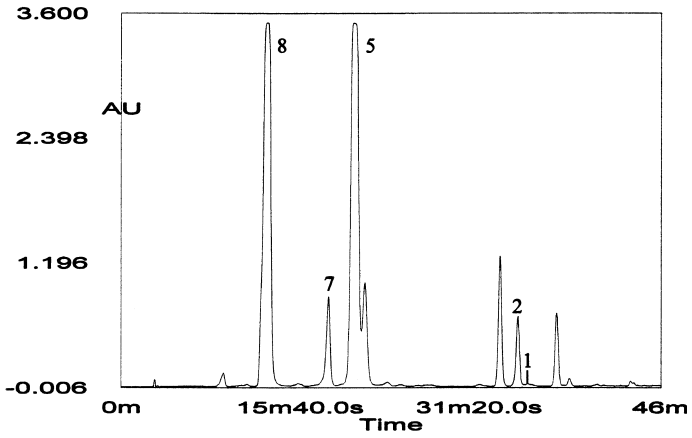

Figure 5. HPLC phenolic acids profile of a green Robusta coffee sample from Ivory Coast. Detection at 320 nm. (1) 3,4,5-trimethoxycinnamic acid; (2) 3,4-dimethoxycinnamic acid; (5) ferulic acid; (7) *p*-coumaric acid; (8) caffeic acid.

cinnamic acids (fig. 1 and 4). CZE gave better peak shapes and shorter times than HPLC, but HPLC was superior in terms of accuracy. Generally, when applied to coffee samples HCAs gave higher values with HPLC analysis than with CZE. This discrepancy is not clear at present, but it may possibly be due to

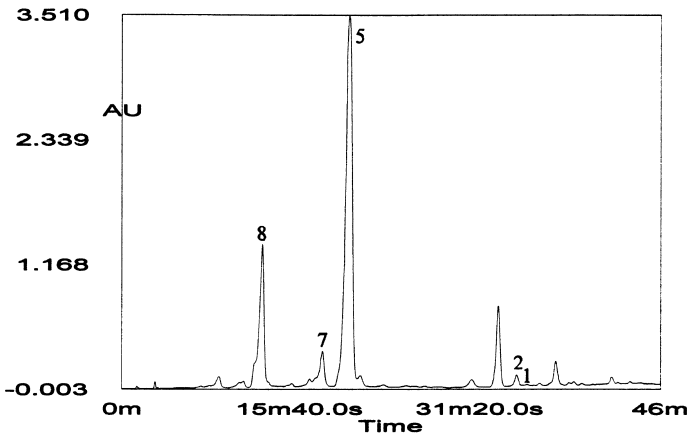

Figure 6. HPLC phenolic acids profile of a roasted (T 220 ºC) Robusta coffee sample from Ivory Coast. Detection at 320 nm. (1) 3,4,5-trimethoxycinnamic acid; (2) 3,4-dimethoxycinnamic acid; (5) ferulic acid; (7) *p*-coumaric acid; (8) caffeic acid.

the high pH (9.5) used in CZE analysis, which may have specific implications in the stability of HCAs molecules. The thermal degradation rate (Table 1) of each acid could apparently be explained by its specific sensitivity inherent to the molecular structure.

Caffeic acid, with one -OH group, was the most sensitive and 3,4,5-trimethoxycinnamic acid, with three -OCH3 was the most resistant. This last compound showed an increase from T=160ºC to 220ºC which suggests that this compound may be a degradation product.

From the results obtained, it can be concluded that HPLC is a more sensitive method for the analysis of phenolic compounds extracted from coffee samples. In CZE the data are poorer than in HPLC, especially since there is some compound degradation at pH 9.5.

In spite of this, CZE may be regarded as a complementary analytical technique for these compounds since it possesses several advantages over HPLC in terms of simplicity, running cost, and shorter analysis time.

These methodologies could be useful for industrial roasting optimization, due to the recognized importance of these compounds in the aromatic and in the tasting properties of drinking coffee.

Table 1

Thermal Degradation of HCAs Performed over a Temperature Range of 140-220°C*

Compounds		Samples: Green Coffee	Roasted Coffee: 140°C	160°C	180°C	200°C	220°C
Caffeic	HPLC	81.0	57.0	31.1	20.9	3.1	4.6
	CZE	100.0	48.6	16.8	9.6	0.8	1.5
p-Coumaric	HPLC	100.0	94.0	40.8	29.9	8.7	4.4
	CZE	75.2	36.8	12.8	9.2	2.0	1.7
Ferulic	HPLC	89.0	75.0	53.8	49.1	15.9	10.8
	CZE	100.0	85.3	29.0	25.0	5.5	4.7
3,4-Dimet.[a]	HPLC	100.0	72.6	28.6	24.1	4.9	5.3
	CZE	53.5	55.0	29.4	25.5	5.2	4.8
3,4,5-Trimet.[b]	HPLC	100.0	52.2	8.7	15.2	30.4	52.2
	CZE	56.7	43.5	20.0	17.4	20.2	50.2

* For each individual phenolic acid the relative percentage of 100 was considered for the higher concentration obtained with the two techniques.
[a] 3,4-Dimet.= 3,4-Dimethoxycinnamic acid.
[b] 3,4,5-Trimet. = 3,4,5-Trimethoxycinnamic acid.

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