ELSEVIER

Food Chemistry 53 (1995) 309–313

0308-8146/95/$9.50

Caffeoyl-tyrosine and Angola II as characteristic markers for Angolan robusta coffees

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(Received 21 July 1994; accepted 15 August 1994)

The composition of the chlorogenic acid-rich fractions of 10 commercial coffee samples from five countries and including the four major coffee-producing regions of Angola were analysed by HPLC. The presence of two chlorogenic acid-like components, previously reported as unique to and characteristic of Angolan robustas, was confirmed for three of the main Angolan coffee producing regions. Only one of these two components caffeoyl-tyrosine, was found in the sample from Cabinda. Neither could be detected in the coffee samples from Cameroon, Indonesia, Ivory Coast or Zaire. It was established that, if present in the green beans, both of these components survive a medium intensity roasting and are extracted into the coffee brew at concentrations in the range 1·2–6·7mg/100 ml.

INTRODUCTION

For many years the analysis of phenolic compounds, especially flavonoids, has been used for chemotaxonomic purposes. The development of HPLC, ideally suited for the analysis of these compounds, has facilitated such studies. Recently, analyses of chlorogenic acids (CGA), unidentified CGA-like components, caffeine and mozambioside have been applied successfully to a reclassification of the wild coffees, *Coffea* spp. and *Psilanthus* spp. (Anthony *et al.*, 1989; 1993, Clifford *et al.*, 1989*a*). An attempt to identify the geographic origin of commercial robusta coffees (seeds of *C. canephora*) was able to discriminate only those coffees from Angola (Clifford & Jarvis, 1988). Samples of green robusta coffee from two of the major Angolan coffee-producing regions (Amboin and Ambriz) were characterised by the presence of significant amounts of two CGA-like components present only as traces or not detectable in robustas from other origins. These components were not detectable in Angolan arabicas. One of these components, Angola I, was identified subsequently as *N*–*β*-caffeoyl-tyrosine (Clifford, 1989*b*); the identity of the second (Angola II), which spectrally is similar, is still under investigation. A specimen chromatogram has been published elsewhere (Clifford, 1986). It was never anticipated that Angolan robustas would have such a unique composition, and, while to date no explanation has been found it is presumed that the differences are more likely to be due to genetic factors rather than agricultural practices.

The CGA and CGA-like components form a quantitatively important fraction of green and roasted coffee beans, soluble coffee powders and coffee brews (Clifford, 1985*a*). It has been suggested that this fraction has a bearing on brew quality (Vilar & Ferreira, 1974; Ohiokpehai *et al.*, 1983; Clifford, 1985*b*; Maier, 1987). Since Angolan robustas have in the past been prized for the distinctive but neutral taste of their beverage (Smith, 1985) it was logical to enquire whether these two CGA-like components might survive roasting and transfer to the brew at concentrations which might be of organoleptic significance.

Accordingly, the objectives of the present study were:

(a) to confirm that current supplies of Angolan robustas do indeed contain these two components; and
(b) to ascertain whether these substances survived roasting and appeared in the brew.

MATERIALS AND METHODS

Materials

Samples were of commercial green robusta coffees from each of the four Angolan producing regions (Amboim, Ambriz, Cabinda and Cazengo) and from an arbitrary selection of other origins that were available on the

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Table 1. Composition of the chlorogenic acid fraction in green and roasted Angolan coffees (% dry mass basis)

	Arabica of Angola		Amboim		Ambriz		Cazengo		Cabinda		Robusta of Angola		Cameroon		Ivory Coast		Indonesia		Zaire	
	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted
3-CQA	0·47	0·46	0·44	0·42	0·46	0·42	0·39	0·50	0·87	0·30	0·58	0·35	0·51	0·46	0·63	0·48	0·44	0·47	0·42	0·46
4-CQA	0·67	0·58	0·64	0·60	0·66	0·65	0·60	0·66	1·16	0·41	0·76	0·46	0·67	0·73	0·91	0·63	0·60	0·84	0·64	0·74
5-CQA	3·70	1·21	3·95	1·09	3·18	0·85	4·44	1·25	3·64	0·65	3·00	0·82	3·15	0·97	3·43	1·08	3·41	1·05	3·42	1·05
Subtotal CQA	4·84	2·25	5·03	2·11	4·30	1·92	5·43	2·41	5·67	1·36	3·43	1·63	4·33	2·16	4·97	2·19	4·45	2·36	4·48	2·25
3-FQA	0·03	0·03	0·03	0·05	0·05	0·07	0·04	0·07	0·05	0·06	0·05	0·05	0·06	0·08	0·08	0·07	0·05	0·09	0·04	0·11
4-FQA	0·04	—	0·04	—	0·06	—	0·08	—	0·09	—	0·07	—	0·07	—	0·12	—	0·06	—	0·08	—
5-FQA	0·21	0·10	0·36	0·13	0·46	0·15	0·60	0·25	0·65	0·12	0·42	0·14	0·48	0·17	0·55	0·17	0·67	0·21	0·63	0·23
Subtotal FQA	0·28	0·13	0·43	0·18	0·57	0·22	0·72	0·32	0·79	0·18	0·54	0·19	0·61	0·25	0·75	0·24	0·78	0·30	0·75	0·34
3,4-diCQA	0·15	0·06	0·40	0·10	0·42	0·10	0·44	0·12	0·50	0·08	0·38	0·09	0·47	0·12	0·46	0·12	0·33	0·09	0·36	0·11
3,5-diCQA	0·17	0·04	0·33	0·06	0·31	0·06	0·35	0·10	0·32	0·04	0·30	0·06	0·35	0·07	0·35	0·08	0·26	0·07	0·28	0·08
4,5-diCQA	0·21	0·05	0·52	0·13	0·50	0·13	0·49	0·16	0·57	0·11	0·52	0·13	0·55	0·19	0·65	0·16	0·32	0·11	0·41	0·13
Subtotal diCQA	0·53	0·15	1·25	0·29	1·23	0·29	1·28	0·38	1·39	0·23	1·20	0·28	1·37	0·38	1·46	0·36	0·91	0·27	1·05	0·32
Total CGA	5·65	2·53	6·71	2·58	6·10	2·43	7·43	3·11	7·85	1·77	6·08	2·10	6·31	2·79	7·18	2·79	6·14	2·93	6·28	2·91
Caffeoyl-tyrosine	—	—	0·28	0·16	0·36	0·21	0·24	0·14	0·13	0·05	0·31	0·16	—	—	—	—	—	—	—	—
Angola II	—	—	0·30	0·21	0·37	0·29	0·19	0·15	—	—	0·27	0·23	—	—	—	—	—	—	—	—

—, not detected.

Portuguese market (Cameroon, Indonesia, Ivory Coast and Zaire). Two other Angolan samples of arabica and robusta coffee were also obtained but it was not possible to ascertain precisely the growing area in which they had been produced.

Using a Probat pilot scale coffee roaster a sample of each green coffee (100 g) was subjected to a medium degree of roasting (11 min at 200°C).

Methods

Extraction of green and roasted coffee samples

The procedure described by Trugo and Macrae (1984), as adopted by Correia and Leitão (1992), was used for the preparation of green coffee extracts. Coffee liquor was prepared as follows. Boiling distilled water (80 ml) was added to ground roasted coffee (2 g) with stirring for 1 min and treated with 5 ml Carrez solution A and 5 ml Carrez solution B (Egan *et al.*, 1981). The mixture was allowed to stand 10 min after making up to 100 ml in a calibrated flask. The precipitate was removed by filtration (Whatman No. 1 filter-paper) and the extract was used directly for chromatography. Peak areas were interpreted against a calibration curve prepared with 5-CQA.

Chromatography

Separation of the CGA and related compounds was performed by using a Waters liquid chromatograph with gradient elution as described by Trugo and Macrae (1984) with peak area integration at 313 nm and 280 nm. A pre-column (30 × 3·9 mm i.d.) dry-packed with coarse silica (35–50 μm) and a Spherisorb 5 μm ODS column (Phase Separations Ltd, 250 × 5 mm i.d.) were used.

RESULTS AND DISCUSSION

This paper uses the IUPAC (1976) numbering system for CGA, with the system of abbreviation as proposed by Clifford (1985*b*). Table 1 summarises the composition (% dry mass basis) of the CGA-rich fraction of the green and roasted coffee samples analysed. The contents of the CQA, FQA and diCQA subgroups were similar to those previously reported (Clifford & Jarvis, 1988; Correia, 1990; Correia & Leitão, 1992), although with somewhat higher values for the Angolan robustas produced in Cazengo and Cabinda compared to those produced in Amboim and Ambriz.

A number of unidentified components were observed on the chromatograms. Most were minor but, as previously reported, larger quantities of certain characteristic peaks were detected only in extracts or brews prepared from Angolan robusta coffees (Fig. 1). Their retention time, relative to 5-CQA, and spectral properties, corresponded to those previously reported (Clifford & Jarvis, 1988; Clifford *et al.*, 1989*b*) and accordingly

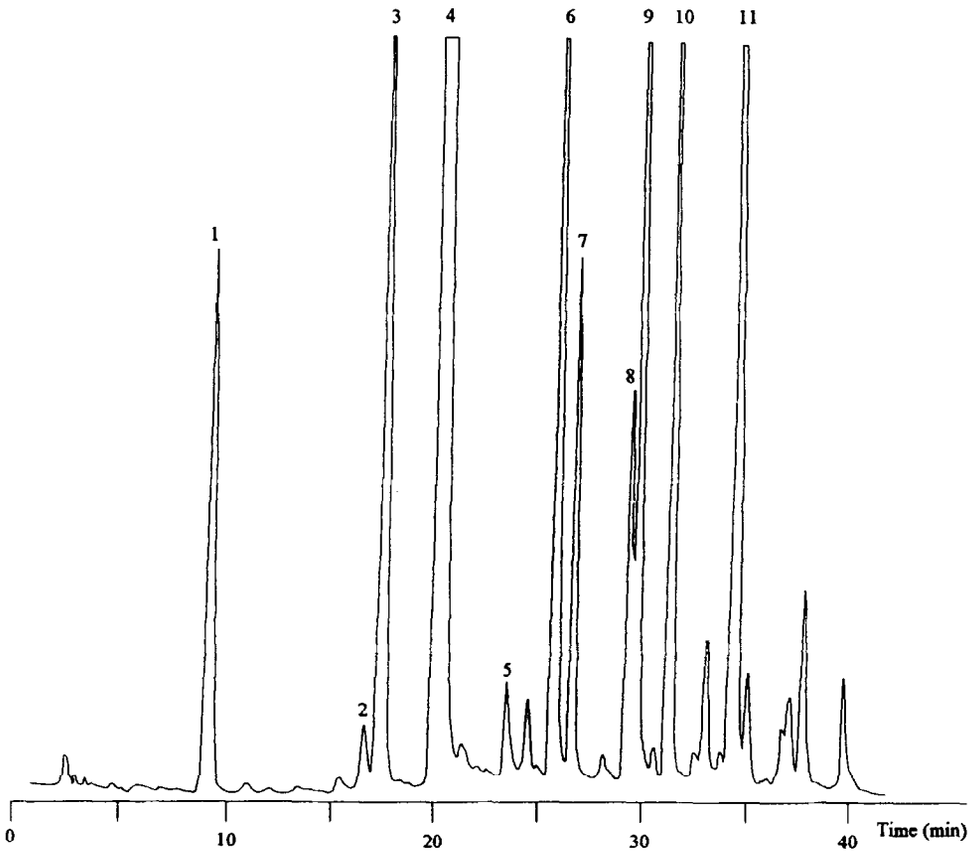

Fig. 1. Chromatogram of a 70% methanol extract of unroasted Angolan robusta coffee beans produced in Cazengo. Peak identification: 1 = 3-CQA; 2 = 3-FQA; 3 = 4-CQA; 4 = 5-CQA; 5 = 4-FQA; 6 = 5-FQA; 7 = caffeoyl-tyrosine; 8 = Angola II; 9 = 3,4-diCQA; 10 = 3,5-diCQA; 11 = 4,5-diCQA.

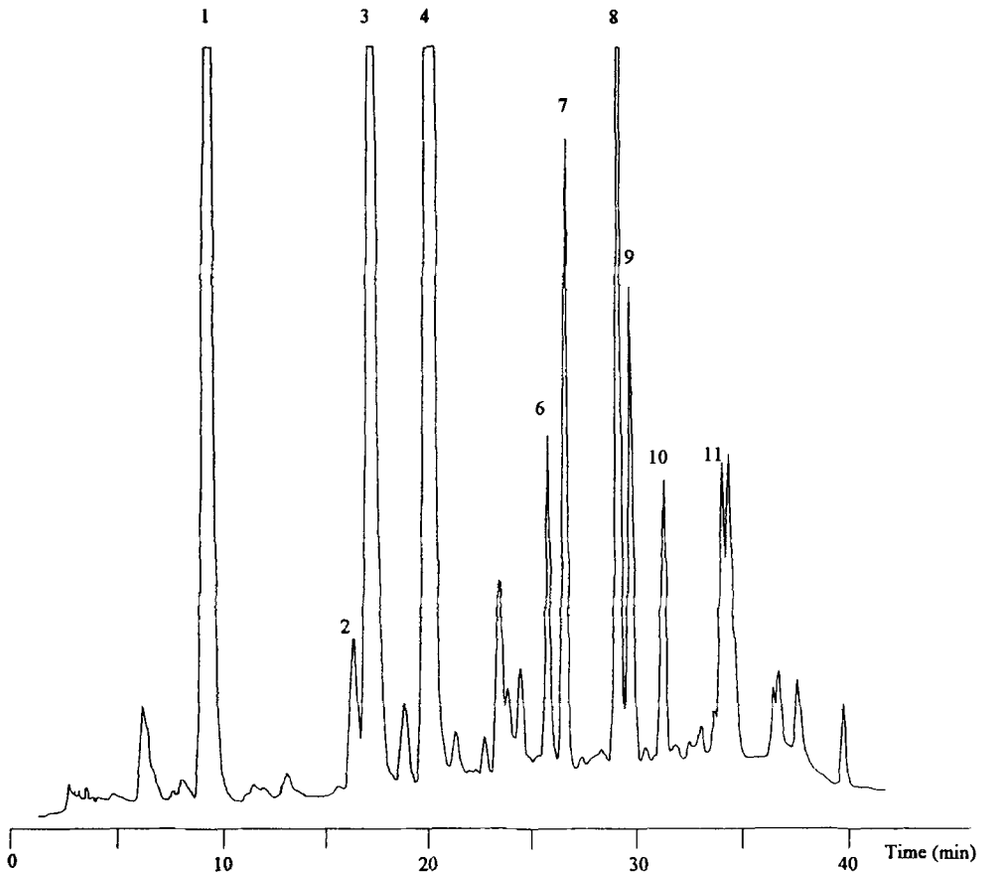

Fig. 2. Chromatogram of a 2% aqueous extract of roasted robusta coffee produced in Amboim, Angola. Peak assignments as in Fig. 1.

the earlier eluting component was identified as caffeoyl-tyrosine. The identity of the second (Angola II), which has a similar spectrum, is still under investigation. In contrast to the other Angolan robustas the Cabinda sample appeared to contain only the caffeoyl-tyrosine.

Table 1 and Fig. 2 both show that these CGA-like components survived a medium roast. Indeed, both were more stable than total CQA with average retentions in the Angolan robustas of 38·5%, 52·6% and 73·6%, respectively, and were transferred efficiently to the brew as illustrated by the data in Table 2. It has not been possible to investigate the sensory properties of these two substances, but since the concentrations found in coffee brew are, in most cases, well in excess of the recognition threshold for 5-CQA in water (0·05–0·10 mg/ml, Kellard *et al.*, (1988) it is not unreasonable to suggest that these substances might influence the character of the brew. However, whether there are other significant chemical or physical differences between these Angolan robustas and those from other geographic origins is not known and it would be premature to suggest that these CGA-like components are a quality-determining factor.

Table 2. Concentration (mg/100 ml) of caffeoyl-tyrosine and Angola II in coffee brew (2 g roasted coffee per 100 ml)

Angolan robusta samples	Caffeoyl-tyrosine	Angola II
Amboim	3·53	5·16
Ambriz	4·41	6.69
Cazengo	3·26	3·31
Cabinda	1·19	—
Robusta of Angola	3·81	4.15

CONCLUSION

The results reported in this study confirm the presence of caffeoyl-tyrosine in current supplies of Angolan robustas and of the related Angola II in robustas from three of the four major growing areas. Neither compound was detected in Angolan arabica or robustas from Cameroon, Indonesia, Ivory Coast or Zaíre. These two components survive medium roasting better than CQA and enter the brew at levels which might be sufficient to influence the brew character.

REFERENCES

Anthony, F., Clifford, M. N. & Noirot, M. (1989). La diversité biochimique dans les genres *Coffea* et *Psilanthus*. In *Treizième Colloque Scientifique International sur le Café*. Association Scientifique International du Café, Paris, pp. 474–84.

Anthony, F., Clifford, M. N. & Noirot, M. (1993). Biochemical diversity of the genus *Coffea* L.: chlorogenic acids, caffeine and mozambioside. *Genetic Resource Crop Evolut.* **40**, 61–70.

Clifford, M. N. (1985*a*). Chemical and physical aspects of green coffee and coffee products. In *Coffee: Botany, Biochemistry and Production of Beans and Beverage*, eds M. N. Clifford, & K. C. Willson). Croom Helm Ltd, London, UK, pp. 305–74.

Clifford, M. N. (1985*b*). Chlorogenic acids. In *Coffee 1 — Chemistry*, eds (R. J. Clarke & R Macrae) Elsevier Applied Science Publishers, London, UK, pp. 153–202.

Clifford, M. N. (1986). Coffee bean dicaffeoylquinic acids. *Phytochemistry*, **25**, 1767–9.

Clifford, M. N. & Jarvis, T. (1988). The chlorogenic acids content of green robusta coffee beans as a possible index of geographic origin. *Food Chem.*, **29**, 291–8.

Clifford, M. N., Williams, T. & Bridson, D. (1989*a*). Chlorogenic acids and caffeine as possible taxonomic criteria in *Coffea* and *Psilanthus*. *Phytochemistry*, **28**, 829–38.

Clifford, M. N., Kellard, B. & Ah-Sing, E. (1989*b*). Caffeoyltyrosine from green robusta coffee beans. *Phytochemistry*, **28**, 1989–90.

Correia, A. M. G. (1990). Influência da torra na evolução dos acidos clorogénicos do café. PhD thesis, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Portugal.

Correia, A. M. G. & Leitão, M. C. A. (1992). Phenolic acids in green and roasted coffee. In *Proceedings of the XVIth International Conference of the Groupe Polyphenols*. JIEP, Narbonne, France, pp. 117–20.

Egan, H., Kirk, R. S. & Sawyer, R. (1981). *Pearson's Chemical Analysis of Foods* 8th edn. Churchill Livingstone, London, UK, p. 234.

IUPAC (1976). Nomenclature of cyclitols. *Biochemical J.*, **153**, 23–31.

Kellard, B., Clifford, M. N. & Birch, G. G. (1988) Progress in the chemical synthesis and organoleptic evaluation of chlorogenic acids. In *Douzième Colloque Scientifique Internationale sur le Café*. ASIC, Paris, pp. 254–9.

Maier, H. G. (1987). Les acides du café. *Cafe Cacao Thé*, **31**, 49–58.

Ohiokpehai, O., Brumen. G. & Clifford, M. N. (1983). The chlorogenic acids content of some peculiar green coffee beans and the implications for beverage quality. In *Dixième Colloque Scientifique Internationale sur le Café*. ASIC, Paris, France, pp. 177–86.

Smith, A. W. (1985). Introduction. In *Coffee 1 — Chemistry*, (eds R. J. Clarke & R. Macrae). Elsevier Applied Science Publishers, London, UK, pp. 1–41.

Trugo, L. C. & Macrae, R. (1984). Chlorogenic acid composition of instant coffees. *Analyst*, **109**, 263–6.

Vilar H. & Ferreira, L. A. B. (1974). Acide chlorogénique dans des varietés régionales d'Angola. In *Sixième Colloque Scientifique Internationale sur le Café*. ASIC, Paris, France, pp. 135–41.