Food Chemistry **29** (1988) 291–298

The Chlorogenic Acids Content of Green Robusta Coffee Beans as a Possible Index of Geographic Origin

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(Received 26 October 1987; accepted 24 November 1987)

ABSTRACT

This paper reports a preliminary survey of variations in the content of chlorogenic acids and 17 quantitatively-minor chlorogenic acid-like substances in commercial green robusta coffee beans. It was found that ten of the chlorogenic acid-like components have a restricted occurrence in the 42 samples analysed; the distinctive nature of Angolan robustas was confirmed.

INTRODUCTION

Accumulated experience in this laboratory indicated that the composition of the chlorogenic acid-rich (CGA-rich) fraction of commercial green robusta coffee beans can be quite variable. For example, the contents of the major CGA subgroups (caffeoylquinic acids (CQA), feruloylquinic acids (FQA) and dicaffeoylquinic acids (diCQA)) are significantly affected by seed maturity (Clifford & Kazi, 1987). In contrast the contents of the quantitatively minor, generally unidentified, CGA-like components, seem to be influenced more by the geographic origin of the beans (Clifford, M. N., unpublished results). Whether such variations in composition are due to genetic peculiarities of the bean being cultivated, or to differences in agricultural practice and green bean processing, in different producing countries, is not clear.

Published information is sparse, but attention has previously been drawn to the distinctive nature of the CGA-rich fraction of Angolan robustas

Food Chemistry 0308-8146/88/$03·50

(Pictet & Brandenberger, 1960; Rees & Theaker, 1977). This peculiarity of Angolan robustas has recently been confirmed (Clifford, 1986) although the significance of the two additional CGA-like components that were detected was not discussed in the context of an indicator of geographic origin.

Some 99% of World coffee is produced on an internationally agreed quota system,† and marketed as having a specified origin (Marshall, 1985), and robusta coffee beans from 34 origins account for some 25% of this. Concern has been expressed regarding the possibility of fraud perpetrated by smuggling non-quota coffee into a quota area and its subsequent disposal under a falsely described origin (Marshall, 1985, p. 257). In cases of uncertainty or dispute an objective indicator of geographic origin, such as variations in the composition of the CGA-rich fraction, would be of value. To gain a preliminary appreciation of the extent to which the composition of the CGA-rich fraction varies, both within and between origins, a further seven samples of Angolan robusta have been analysed. The data so obtained have been compared with the corresponding data simultaneously obtained by the analysis of at least one sample of robusta from each other commercial origin except Vietnam.

MATERIALS

Samples of commercial green robusta coffee beans were kindly supplied, between October 1986 and February 1987, by Brooke Bond Oxo, Douwe Egberts, General Foods UK, Hag GF, Jacobs Suchard, Lyons Tetley and the International Coffee Organisation. Such details as were provided regarding bean quality, size, date of harvest, etc., are recorded in Table 1. In the case of the Angolan robustas, although seven samples were obtained, it eventually proved possible to cover with certainty only two of the major producing areas within the country. All other reagents and standard compounds were obtained from normal commercial sources, or were gifts obtained as previously acknowledged (Clifford *et al.*, 1985).

METHODS

Samples (25 g) were ground to pass 0·5 mm. The mass loss on drying, and the chlorogenic acid composition using reversed phase HPLC were determined essentially as previously described (Clifford *et al.*, 1985; Clifford, 1986). On this occasion a 15 cm × 4 mm analytical column packed (Hichrom) with

† At the time of writing (October 1987) the International Agreement has been suspended.

Spherisorb ODS1 3 μ stationary phase was used in conjunction with a 5 cm × 4 mm guard column packed with the corresponding 5 μ stationary phase. A linear gradient from 0·5% formic acid in 6% aqueous acetonitrile to 0·5% formic acid in 34% aqueous acetonitrile over 37 min at a flow rate of 1 ml min^{-1} was used to separate the CGA. Sample size was 20 μl introduced via a loop injector, with detector sensitivity as required but normally 0·2 AUFS.

RESULTS AND DISCUSSION

It should be noted that this publication uses the IUPAC (1976) numbering system for chlorogenic acids with the system of abbreviation proposed by Clifford (1985*a*,*b*).

Up to 16 minor CGA-like components were readily separated from the major CGA. The present chromatograms differed from the original (Clifford, 1986) in that it was not possible to completely resolve component 9 (designated component 6 in the original study) from 3,4-diCQA, but the presence of component 9 was detectable by measuring the absorbance ratio $A_{313}:A_{276}$. This ratio is 2·0 ± 0·1 to 1 for pure CQA and diCQA, but fell to between 1·24 and 1·50 in samples previously shown to contain this component, and in the seven Angolan samples analysed in the present study. Although previous analyses have suggested contents in the region of 0·2 to 0·4%, during the present study it was not possible to quantify component 9 *per se* since the spectral characteristics of the pure compound are not known. Component 9 has perforce been interpreted as 3,4-diCQA and recorded along with the total diCQA content, but its presence has been recorded in Table 1 also by the use of the symbol (*).

The quantitative data (dmb) are presented in Table 1. Previous experience indicates that the between-replicate coefficients of variation are 2·76% for total CQA, 13·34% for 5-FQA and 5·55% for total diCQA (Clifford *et al.*, 1985). When the minor CGA-like components were visually detectable on the chromatograms, but were present at levels below the limit of integration (equivalent to 0·04% of 5-CQA) their presence has been recorded in Table 1 by the symbol (+). The between-replicates coefficients of variation for the contents of these CGA-like components have not been determined, but are likely to be relatively large.

The identities of these CGA-like components are not known, but there is good reason to believe that they will include 5-CoQA; 4-FQA; 4-C,5-FQA; 4-F,5-CQA; and caffeoyltryptophan (Clifford, 1985*a*; Iwahashi *et al.*, 1985; Morishita *et al.*, 1986; 1987). Pictet & Brandenberger (1960) considered *p*-coumaric acid to be present in Angolan robustas, but this was not

TABLE 1
Composition of the Chlorogenic Acid-Rich Fraction

Sample origin and identity	*Component identity, content (% dmb) and retention time relative to 5-CQA*																			
	Total CQA	*FQA*	*Total diCQA*	*0·46 1*	*0·70 2*	*0·75 3*	*0·83 4*	*1·25 5*	*1·28 6*	*1·57 7*	*1·72 8*	*1·91 9*	*2·16 10*	*2·23 11*	*2·28 12*	*2·31 13*	*2·37 14*	*2·48 15*	*2·62 16*	*2·67 17*	*Total CGA*
North and Central America																					
Guatemala	5·66	0·91	1·21	+					+				0·14			0·06	0·29	+		0·10	8·37
Mexico	5·98	1·11	1·20						+				0·18			+	0·18	+			8·65
Trinidad FAQ 1986	6·21	0·93	1·12	0·07	+	+			+	+			0·17	+		+	0·17	+	+		8·67
South America																					
Brazil (Conillon)	6·80	0·82	0·91	0·07	+	+			+	0·14	+		0·15	+	+	0·04	0·31	+	+		8·89
Brazil	6·94	1·04	1·24	+					+	+			0·12			0·08	0·16	+			9·58
Equador	6·54	1·08	1·23	+	+	+			+	0·07			0·16	+		0·07	0·21	+		0·05	9·41
Africa																					
Angola (Ambriz) 1a Qualidade Corrente	4·78	0·67	0·95*	+	+			+	+	0·32	+	+	0·15	+	+	+	0·19	+	+	+	7·06
Angola (Amboim) 2a Qualidade Grado	5·30	0·72	0·95*	+	+			+	+	0·31	+	+	0·17	+	+	0·04	0·21	+	+	0·06	7·76
Angola (Amboim) 1a Qualidade Grado	4·98	0·58	1·06*	+	+	+		+	+	0·26	+	+	0·14	+	+	+	0·17	+	+	0·06	7·25
Angola (Ambriz) 2a Qualidade Grado	4·90	0·74	1·04*	+	+			+	+	0·48	+	+	0·15	+	+	0·05	0·20	+	+	+	7·56
Angola	5·24	0·61	0·88*	0·04	+	+		+	+	0·32	+	+	0·13	+	+	+	0·18	+	+	+	7·40
Angola 3DD 1986	5·05	0·67	0·86*	0·05	+	+	+	+	+	0·25	+	+	0·12	+	+	0·04	0·15	+	+	+	7·19
Angola	4·86	0·67	1·14*	+	+			+	+	0·40	+	+	0·13			0·04	0·15	+	+	0·04	7·43
Benin Courant	6·66	0·75	0·93	+	+				+	0·15			0·13		+	0·04	0·31	+			8·97
Burundi	6·51	1·19	1·13	+	+	+			+				0·16	+		0·07	0·24	+		0·07	9·37
Cameroun Grade 1	6·28	0·88	1·73	+		+		+	+				0·14			+	0·23	+		0·06	9·32
Central African Republic Grade 2	6·07	0·74	1·11	+					+	+			0·19			+	0·26	+		0·07	8·44

Congo	5·89	0·66	1·04	+				+	+		0·19			+	0·25	+		+	8·03
Gabon	5·58	0·70	1·11	+				+	+		0·19			+	0·25	+		0·05	7·88
Ghana FAQ 1986	5·73	0·78	1·00	0·07	+			+	0·05	+	0·13	0·04		0·04	0·24	+	+	0·05	8·13
Guinea	5·48	0·72	1·05	+				+	+		0·18			+	0·27	+		0·09	7·79
Equatorial Guinea 83/84	6·11	0·90	1·05	0·07	+	+	+	+	0·09	+	0·16	+		0·06	0·19	+	+	+	8·63
Ivory Coast	7·55	1·12	1·33					+			0·12	+		+	0·12	+	+	+	10·24
Ivory Coast	8·33	0·88	1·98					+			0·18	+		0·17	0·16	+	+	0·12	11·32
Liberia FAQ	6·38	0·71	1·08	+				+	+		0·19			0·04	0·21	+		0·08	8·59
Madagascar	5·52	0·96	1·03	+	+	+	+	+	0·05	+	0·15	+		0·05	0·22	+	+	0·05	8·03
Nigeria	5·83	0·75	1·04	+				+	0·13		0·14			0·06	0·29	+		+	7·74
Rwanda	5·94	0·89	1·13					+	0·15		0·14		+	0·04	0·31	+		0·12	8·72
Sierra Leone FAQ	6·35	0·84	1·13	+	+	+		+	+	+	0·16			0·06	0·23	+		0·11	8·88
Tanzania	6·28	0·78	1·05	+				+	0·18		0·15		+	0·04	0·34	+		0·12	8·94
Togo	6·50	1·01	1·38	+				+			0·15			0·05	0·24	+		+	9·33
Uganda FAQ	5·62	0·83	1·07	+				+	0·10		0·18			0·04	0·30	+	+	+	8·10
Uganda	6·60	1·05	1·25	+				+	0·12		0·16			0·04	0·30	+		0·11	9·63
Uganda	6·09	0·65	1·01	0·04				+	0·07		0·19	+		0·05	0·26	+	+	0·07	8·56
Zaire HTCM 1986	6·11	0·66	0·83	+				+	+		0·13	+		0·04	0·20	+		0·05	8·02
Asia																			
India Cherry AB 1986	5·81	0·69	1·38	+				+	+	+	0·16	0·04		0·05	0·23	+		0·08	8·54
Indonesia	7·57	1·06	1·72					+			0·12			0·05	0·35	+		0·09	10·96
Malaysia	5·63	0·80	1·07	+				+	+		0·17			+	0·24	+		+	7·93
Philippines M1	5·90	0·92	1·04	0·04	+	+		+			0·17	+		+	0·21	+		0·08	8·36
Sri Lanka FAQ	5·72	0·77	1·12	0·05				+	0·10		0·19			0·05	0·13	+		+	8·13
Thailand Washome 1	5·72	0·88	0·92	0·05	+	+	+	+	+	+	0·13	+		0·04	0·19	+		0·06	7·99
Oceania																			
Papua New Guinea	7·64	0·94	1·19					+	0·08		0·21			0·06	0·29	+		+	10·41

* Value also includes content of Component 9.

confirmed in the present study. This apparent discrepancy can probably be explained by the greater resolution achievable with modern HPLC compared to paper chromatography. Comparison with authentic standards has also excluded 1,3-diCQA (Clifford, 1986), caffeic acid, ferulic acid, 3-CoQA, 4-CoQA, aesculin, scopolin and scopoletin.

Table 2 presents a statistical comparison of the current data with a compilation (Clifford, 1985*a*) of previously published data which were originally obtained by three separate groups of workers who used similar, but not identical, methods of analysis, on commercial robustas from seven (non-Angolan) origins. The present data differ from the compilation in terms of a significantly lower ($p < 0{\cdot}001$) diCQA content, for which no explanation can be offered at present.

The seven Angolan samples differ slightly among themselves in the pattern of CGA-like components. All seven contain components 1, 2, 5 to 10, and 13 to 17, but components 3, 4, 11 and 12 occur irregularly. The contents of the major CGA subgroups are very similar. If the mean values for the Angolan samples are compared (Table 2) with the corresponding mean values for the 35 samples from other origins, then the Angolan samples are

TABLE 2
Statistical Comparison of Current and Previously Published Data

Component	*Previous studies* ($N = 7$)[a] (% dmb)	*Present investigation*	
		Non-Angolan samples ($N = 35$)	*Angolan samples* ($N = 7$)
Total CQA	6·70 ± 0·61	6·27 ± 0·67	5·02 ± 0·19
		$t = 1{\cdot}15$ NS	$t = 4{\cdot}34$***
5-FQA	0·84 ± 0·17	0·87 ± 0·15	0·67 ± 0·06
		$t = 0{\cdot}32$ NS	$t = 2{\cdot}99$**
Total diCQA	1·82 ± 0·33	1·17 ± 0·24	0·98 ± 0·10[b]
		$t = 3{\cdot}85$***	$t = 1{\cdot}76$*
10		0·16 ± 0·024	0·14 ± 0·010
			$t = 1{\cdot}80$*
14		0·24 ± 0·057	0·15 ± 0·023
			$t = 3{\cdot}54$***
Total CGA	9·04 ± 0·58	8·83 ± 0·93	7·39 ± 0·24
		$t = 0{\cdot}46$ NS	$t = 3{\cdot}62$***

[a] Data taken from Clifford (1985*a*).
[b] Including component 9.
NS = not significant.
* Significant at 0·1%.
** Significant at 0·01%.
*** Significant at 0·001%.

found to have significantly ($p < 0{\cdot}01$) lower contents of total CQA, FQA and total CGA. The total diCQA content is also lower but with an apparently lesser significance ($p < 0{\cdot}1$) due to the incorporation of the content of component 9 in the Angolan total diCQA value. Of the CGA-like components only two (10 and 14) occurred consistently at levels above the limit of integration. The contents of these two also were significantly lower in the Angolan beans.

However, if the individual values for the other 35 samples are compared individually with the corresponding mean values for the Angolan samples, it is clear that the single samples from eleven other origins (Congo, Gabon, Ghana, Guinea, Madagascar, Nigeria, Uganda (FAQ), Zaire, Malaysia, Sri Lanka and Thailand) have compositions that fall within, or near to, the corresponding upper (three standard deviation) limit for the Angolan sample distributions. Provisionally assuming similar distributions for the composition of samples from these other origins, and normality of distribution in each case, one must conclude that there would be difficulty in distinguishing unequivocally, on these compositional criteria, from which particular origin among these twelve, any particular sample was derived.

However, the Angolan samples used in this investigation are characterised also by the presence of CGA-like components 5, 7, 9 and 16, and this combination of characters was not observed in any other sample. If these distinctions prove to be real and consistent, then the use of these additional criteria would greatly increase the discrimination that could be achieved, but it would be premature to conclude that even this combination of characters would reliably and consistently distinguish an Angolan robusta.

Why these Angolan robustas are so distinctive is not known but it is said (Smith, 1985, p. 13) once to have been much sought after for its clean neutral taste. In a separate study to be reported elsewhere (Clifford, M. N., Williams, T. & Bridson, D., unpublished results) it was noticed that the Angolan pattern of CGA-like components more closely resembled the pattern of a *C. canephora* var. *Ugandae* from the living collection at Lyamungu in Tanzania, rather than *C. canephora* var. *robusta* or var. *Kouilou*. The var. *Ugandae* (also known as var. *nganda*) is known to have originated in Uganda and other parts of equatorial Africa.

Finally, in judging the potential of this chromatographic method for identifying the origin of green robustas it should be noted that seven other CGA-like components apparently show restricted occurrence among the non-Angolan samples (component 2 in 12 origins; component 3 in 10 origins; component 4 in 3 origins; component 8 in 11 origins; component 11 in 14 origins; component 12 in 5 origins and component 10 in 18 origins). If these peculiarities prove to be consistent they may manifest themselves as unique patterns that would discriminate at least some of the non-Angolan

origins. On this basis it is suggested that an extension of this study might provide a means whereby green robusta coffee from some origins could be identified with a low risk of error.

ACKNOWLEDGEMENTS

The technical assistance of Mr Derek Smith and Mr Gerald Rimbach is gratefully acknowledged.

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