Food Chemistry **40** (1991) 35–42

Phenols and Caffeine in Wet-Processed Coffee Beans and Coffee Pulp

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(Received 2 May 1990; accepted 21 May 1990)

ABSTRACT

The contents of low molecular mass phenols and caffeine have been analysed in five samples of beans and the associated pulp, derived from two species of coffee and two associated hybrids. The composition of the coffee beans was consistent with previous reports. The pulp contained smaller quantities of the same caffeoylquinic acids, feruloylquinic acids and dicaffeolyquinic acids as the beans, but caffeoylferuloylquinic acids were not found even in the pulp from a robusta coffee. Pulp from a robusta coffee had a lower caffeine content than the pulp from two arabica cultivars, the reverse of the situation existing in the beans. A significant component in all pulp samples was isolated and identified as protocatechuic acid.

INTRODUCTION

The coffee shrub produces a fleshy fruit commonly known as a cherry. The skin (exocarp) is red, or in some varieties, yellow when ripe. Enclosed within the exocarp are a pericarp (pulp), a mesocarp (mucilage), an endocarp (parchment) and one, or usually, two seeds. The seeds, which form the green

Food Chemistry 0308-8146/91/$03·50 © 1991 Elsevier Science Publishers Ltd, England. Printed in Great Britain

coffee beans of commerce, account for 55% of the fruit dry matter, and the pulp for a further 29%. It has been estimated that much of the 1·5 million metric tonnes of coffee pulp solids produced per annum in Latin America is dumped in water courses where it gives rise to serious pollution (Bressani & Braham, 1980).

For economic and environmental reasons, attempts have been made to utilise coffee pulp as feed for cattle, swine and poultry. Only limited success has been achieved due to the presence of anti-nutritional factors that reduce weight gain, unacceptably so when coffee pulp is used as a replacer at greater than 10% of the normal rations.

Various components in the dry pulp, including caffeine, potassium and various phenols, have been blamed for the reduction in weight gain, but the causative agent(s) and its mechanism of action have never been clearly identified. In general, the quantitative data for the phenols content of pulp have been obtained by methods current in the 1960s and since superseded, and in some cases identification of the phenols has been only tentative (Bressani, 1979; Bressani & Braham, 1980; Zuluaga-Vasco & Tabacchi, 1980; Ramirez-Martinez, 1988). Accordingly it was felt that further investigation was required.

This paper reports the contents of caffeine, chlorogenic acids (CGA) and protocatechuic acid in the pulp and beans from two species of coffee and two associated hybrids. The contents of condensed tannins will be reported separately.

MATERIALS AND METHODS

Materials

Five samples of wet-processed coffee beans and the associated coffee pulp (sun-dried), as listed below, were supplied by the Estación Experimental Agrícola de Bramon, Venezuela.

(1) *Coffea canephora*;
(2) Timor Hybrid (*C. arabica* × *C. robusta*);
(3) Catimor (Timor Hybrid × *C. arabica* var. *caturra*);
(4) *Coffea arabica* var. *caturra vermelho*;
(5) *Coffea arabica* var. *bourbon vermelho.*

Protocatechuic acid, caffeine and 5-caffeoylquinic acid (5-CQA) were obtained from Sigma Chemical Company Ltd, Poole, UK. Other chlorogenic acid (CGA) standards were prepared and characterised as described previously (Clifford *et al.*, 1989*a*,*b*). All other reagents were standard items from reputable commercial sources.

Methods

Extractions

The green coffee bean samples were frozen, ground and extracted (500 mg) with 70% methanol (5 × 50 ml, 30 min each) as previously described (Clifford, 1986). The bulked extracts were treated with Carrez Reagents (1 ml A plus 1 ml B) to precipitate colloidal material, diluted to 250 ml, and filtered. Carrez Reagent A was prepared by dissolving 21·9 g zinc acetate dihydrate in water containing 3 g glacial acetic acid and diluting to 100 ml with water. Carrez Reagent B was prepared by dissolving 10·6 g potassium ferrocyanide trihydrate in 100 ml water.

The coffee pulp samples (1 g) were extracted similarly. These methanolic solutions were used directly for analytical HPLC.

Timor Hybrid coffee pulp (6 × 2 g) was extracted also with 80% acetone, the extracts bulked (total ≈ 1·5 litre), concentrated to ≈ 25 ml under reduced pressure at 40°C, filtered through a Whatman No. 4 filter paper and this solution used directly for preparative HPLC.

Chromatography

Analytical HPLC of caffeine, protocatechuic acid and CGA was performed using the system previously described (Clifford & Jarvis, 1988) consisting of a 3 μ reversed phase non-end capped C_{18} packing (Spherisorb ODS1) and an acidic (pH 2·5) acetonitrile gradient (solvent A = 0·5% formic acid in water; solvent B = 0·5% formic acid in 50% aqueous acetonitrile; gradient 12% solvent B to 70% solvent B in 30 min; 1 ml per min). The eluate was monitored sequentially at 276 nm (caffeine and protocatechuic acid) and 313 nm (CGA). CGA and caffeine were identified by comparison with authentic materials as previously prepared, and quantified against caffeine or 5-CQA peak area calibration curves as appropriate. Protocatechuic acid was identified as described in the discussion, and quantified against a peak area calibration curve.

Preparative HPLC was performed in a similar manner but using a 25 cm × 8 mm column, containing the equivalent 5 μ packing, and a non-linear gradient profile (15% solvent B 10 min isocratic, 15 to 70% B in 15 min, and reset, 2 ml per min).

Spectroscopy

Proton NMR spectra were obtained on a Brucker AC 300 NMR spectrometer operating at 300 MHz. Samples were dissolved in CD_3OD and examined at room temperature against a tetramethylsilane standard.

UV spectra were measured in 70% methanol, against a 70% methanol blank, using a Kontron Uvikon 860 recording spectrophotometer.

Spot tests

Aliquots (10 μl) of Component 1 were spotted onto Whatman No. 1 filter papers and treated individually with the molybdate reagent (Clifford & Wight, 1976), ninhydrin and 4-dimethylamino cinnamaldehyde reagent (Harborne, 1984). The ninhydrin treated sample was heated at 105°C for 10 min prior to inspection. Reagent blanks and positive controls (protocatechuic acid, tyrosine and tryptophan) were performed simultaneously.

Moisture content

Duplicate 1 g samples of pulp and ground beans were dried conventionally to constant weight at 105°C.

RESULTS AND DISCUSSION

Moisture contents fell in the range 14–15% fresh weight for the pulp, and in the range 9–13% for the green beans. All extracts were examined by reversed phase HPLC with sequential monitoring of the eluate at 313 nm for the CGA and 276 nm for caffeine and protocatechuic acid. Where these components were found in concentrations in excess of the lower limit of integration, the contents have been recorded in Table 1 as per cent dry basis

TABLE 1

The Contents (%db)[a] of Chlorogenic Acids, Protocatechuic Acid and Caffeine in Green Coffee Beans and the Associated Pulp

Sample number	*CQA*				*5-FQA*	*diCQA*				*Total CGA*	*Protocatechuic acid*	*Caffeine*
	3	*4*	*5*	*Sub total*		*3,4*	*3,5*	*4,5*	*Sub total*			
1 Bean	0·30	0·49	4·56	5·33	0·79	0·44	0·56	0·05	1·05	7·17	+	1·07
1 Pulp	0·13	0·14	0·83	1·10	0·02	0·10	0·34	0·06	0·49	1·61	0·10	0·54
2 Bean	0·26	0·48	3·97	4·71	0·33	0·09	0·26	0·23	0·58	5·62	+	0·65
2 Pulp	0·01	0·02	0·36	0·39	0·02	0·01	0·05	+	0·06	0·47	0·71	0·89
3 Bean	0·25	0·54	4·72	5·51	0·35	0·08	0·22	0·16	0·45	6·31	+	0·78
3 Pulp	0·01	0·01	0·34	0·36	+	0·01	0·05	+	0·06	0·42	0·51	1·65
4 Bean	0·23	0·48	3·92	4·63	0·33	0·10	0·30	0·26	0·66	5·62	+	0·73
4 Pulp	0·02	0·03	0·92	0·97	0·02	0·03	0·14	+	0·17	1·16	0·17	1·67
5 Bean	0·24	0·52	4·00	4·77	0·34	0·09	0·25	0·21	0·56	5·67	+	0·82
5 Pulp	0·02	0·03	0·82	0·87	+	0·02	0·12	+	0·14	1·01	0·47	1·38

[a] Mean of duplicates.

(%db). Components detected visually on the chromatograms at concentrations below this level have been recorded in the table by the symbol (+). The chlorogenic acids (CGA) are referred to by the IUPAC (1976) numbering system and the system of abbreviations proposed by Clifford (1985*a*,*b*).

The CGA and caffeine contents of the coffee bean samples were consistent with previously published data (Clifford, 1985*a*,*b*; Clifford & Jarvis, 1988; Clifford *et al.*, 1989*c*). Each species contained the seven major CGA. The seeds of *C. canephora* also contained several CGA-like components (absorbance at 313 nm significantly greater than at 276 nm) previously observed in commercial robustas (Clifford & Jarvis, 1988). Those originally referred to as Components 10, 13 and 15 and since characterised as the six CFQA isomers (Clifford *et al.*, 1989*b*) totalled ≈0·15%. Components 14 and 17 (0·30% and 0·04%, respectively, in the *C. canephora* beans) were also observed in the Timor Hybrid beans (0·04% and trace, respectively). None of these CGA-like components were observed in the beans of the back-crossed Catimor.

The present data, obtained using authentic standards (Clifford *et al.*, 1989*a*,*b*), constitute the first report of 5-FQA in coffee pulp and confirm the presence of three CQA isomers and three diCQA isomers. Neither the CFQA isomers, nor the CGA-like Components 14 and 17, could be detected in any pulp sample.

The pulp CGA contents reported here (0·42–1·61%) are smaller than literature values (2·6%) for a Guatemalan (presumably arabica) coffee pulp obtained by an unspecified analytical method (Bressani & Braham, 1980), but essentially identical to HPLC data (0·40–1·42%) previously reported for Venezuelan arabica coffee pulp (Ramirez-Martinez, 1988).

Bressani and Braham's value for pulp caffeine (1·3%) is within the current range (0·54–1·67%) and close to the current arabica values (1·38–1·67%).

For a given species, the pulp consistently had a much smaller CGA content than the beans, a feature which was most pronounced in the case of 5-FQA, but the orders of ranking were not identical. Beans from *C. canephora* (commercial robustas) almost invariably have larger CGA and caffeine contents than beans of *C. arabica* (commercial arabicas). In contrast, although the *C. canephora* pulp had the largest CGA content, the *C. arabica* pulp had the largest caffeine content. While subsequent investigations have confirmed this unexpected reversal (Ramirez-Martinez, pers. comm.) it is now apparent that on a fresh weight basis, the pulp caffeine contents are similar.

CGA-like Component 1 was found at trace levels in all bean samples, and in larger concentration in each pulp sample. The much greater content in Timor Hybrid pulp facilitated its isolation by preparative HPLC, and its

identification as protocatechuic acid (3,4-dihydroxybenzoic acid) as described below.

The chromatographically homogeneous isolate was examined with several structure-specific reagents and proton NMR. Proton NMR (300 MHz) indicated three aromatic protons. The two one-proton doublets ($\delta = 6{\cdot}79$ ppm and 7·42 ppm) showing ortho coupling ($J = 8$ Hz) were assigned to H5 and H6, and the one proton singlet ($\delta = 7{\cdot}44$ ppm) to H2 of a 1,3,4-tri-substituted aromatic ring, such as is found in protocatechuic acid. A positive (yellow) response to the molybdate reagent (Clifford & Wight, 1976), indicating a 3,4-dihydroxy compound, negative responses to ninhydrin and 4-dimethylaminocinnamaldehyde, and failure to resolve from authentic protocatechuic acid, were taken as confirming the NMR assignment.

Traces (0·01–0·03%) of a component thought to be protocatechuic acid, have previously been observed in fresh arabica coffee pulp (Ramirez-Martinez, 1988) and irregularly in seeds and pulp during chemotaxonomic studies on *Coffeeae* (Lopes *et al.*, 1984; Clifford *et al.*, 1989*c*). In view of its irregular detection, it is interesting to note that Lydon and colleagues (Lydon & Duke, 1988; Becceril *et al.*, 1989) have shown that exposure of several species of plants (*Coffea* was not included) to the broad spectrum herbicide glyphosate, induced protocatechuic acid synthesis and accumulation by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase.

It has been reported that using coffee pulp solids at greater than 10% of the diet of cattle, swine and poultry has led to reduced feed intake, impaired weight gain and impaired feed conversion (Bressani & Braham, 1980). Pulp phenols and caffeine have both been blamed for these effects but very rarely in these studies was the diet composition controlled sufficiently to demonstrate the active principle unequivocally.

Singleton (1981) has reported low mammalian toxicity for orally administered 5-CQA and protocatechuic acid, and Lietti (1977) has reported, for rats, an oral LD_{50} in excess of 2 g 5-CQA per kg body weight. However, it has not been possible to locate data defining the tolerance of cattle, swine and poultry for chronic dietary exposure to these phenols, and therefore it is not possible to estimate whether the currently reported levels could exert an antinutritive effect.

In contrast, Bressani & Braham (1980) have established that the addition of 0·24% caffeine to the control diet fed to 100-day old calves (equivalent to 170 mg caffeine per kg body weight per day) reduced the feed intake and significantly ($p < 0{\cdot}05$) impaired the growth rate. The arabica coffee pulps currently under investigation would provide such intakes of caffeine if used at 12–15% of the diet, whereas the robusta pulp would have to be used at 37%. If caffeine were the sole or major antinutritive factor in coffee pulp, these data imply that robusta pulp would be better tolerated. Unfortunately,

little, if any, commercial robusta is wet-processed, and therefore this is not a practicable approach to improve pulp utilisation.

ACKNOWLEDGEMENTS

CONICIT (Venezuela) and the Royal Society (London) are thanked for financial support. Frauleins Jutta Blank and Andrea Henne are thanked for their technical assistance.

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