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Tannins in Wet-Processed Coffee Beans and Coffee Pulp

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ABSTRACT

The tannins contents have been investigated in five samples of beans and the associated pulp, derived from two species of coffee and two associated hybrids. Tannins were not found in any bean sample, and in contrast to previous reports, hydrolysable tannins sensu strictu *were not detected in pulp. The presence of soluble condensed tannins in* Coffea arabica *pulp was confirmed at approximately 1% db. Similar levels were found in pulp from Timor hybrid and Catimor, but* Coffea canephora *pulp yielded* $\approx 2{\cdot}7\%$ *db. In all cases prodelphinidins exceeded procyanidins, but the ratio varied from 2·2 to 6·0:1. Evidence was obtained also for significant amounts of insoluble condensed tannins, but reliable quantification was not possible with the analytical methods currently available.*

INTRODUCTION

Coffee pulp is a by-product from the wet processing of coffee beans. Much of this pulp is currently dumped in water courses where it causes serious pollution (Bressani & Braham, 1980). For economic and environmental reasons attempts have been made to utilise coffee pulp as a feed for cattle,

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swine and poultry, but with little success. When used at levels in excess of 10% of normal rations feed utilisation and growth rate are impaired due to the presence of ill-defined anti-nutritive or toxic factors.

Various components, including caffeine, low molecular mass phenols and tannins, have been blamed for these undesirable effects. Recently, it has been confirmed (Clifford & Ramirez-Martinez, 1990) that arabica pulp used at 12–15% of normal rations would provide a level of caffeine previously shown in controlled studies to impair the growth of 100-day old calves (Bressani & Braham, 1980), whereas robusta pulp, due to a lower caffeine content, would be tolerated at such a proportion of the diet.

The low molecular mass phenols of coffee pulp have been characterised and quantified (Ramirez-Martinez, 1988; Ramirez-Martinez & Clifford, 1990; Clifford & Ramirez-Martinez, 1991), although their effect on acceptability is still unclear. In contrast, although tannins are known to influence the acceptability of food and feed (Mehansho *et al.*, 1987), data for coffee pulp are restricted to estimates of both condensed and hydrolysable tannins obtained by empirical methods which have since been superseded (Bressani, 1979; Bressani & Braham, 1980; Zuluago-Vasco & Tabacchi, 1980), or by a method which also detects the flavan-3-ols (Garcia *et al.*, 1985). Moreover, these data are restricted to pulp derived from *Coffea arabica*. In order to improve our knowledge of these environmentally important constituents, more modern methods of tannin analysis have been applied to the pulp and beans from two species of coffee and two associated hybrids.

MATERIALS AND METHODS

Materials

Five samples of wet processed coffee beans and the associated sun-dried coffee pulp were supplied by the Estación Experimental Agrícola de Bramon, Venezuela. The species were:

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

Pelargonidin, cyanidin and delphinidin hydrochlorides were obtained from Apin Chemicals Ltd, Abingdon, UK. All other reagents were standard items from reputable commercial sources.

Methods

Qualitative spot tests

The tests for ellagitannins and gallotannins (collectively known as hydrolysable tannins) were based upon procedures recommended by Harborne (1984), but modified as shown below to accommodate a solid sample rather than an extract. For ellagitannins 2 ml 0·1M nitrous acid (from freshly prepared sodium nitrite and hydrochloric acid) were added to 200 mg coffee pulp or ground green bean in a screw-capped test tube and the headspace flushed with nitrogen. The contents were mixed and inspected at intervals for the development of a blue colour which is considered positive. No positive control was available.

For gallotannins, 200 mg coffee pulp or ground green bean was reacted at approximately 15°C in a screw-capped test tube with 2 ml potassium iodate (12% in 33% methanol). The contents were mixed and inspected at intervals for the development of a red-brown colour. Commercial tannic acid was used as a positive control.

The test for condensed tannins was based upon Porter's improved autoxidative procedure (Porter *et al.*, 1986). Coffee pulp or ground green bean (200 mg) was weighed into screw-capped (Teflon liner) test tubes and Porter's Reagents added (6 ml butanol–concentrated hydrochloric acid 95:5 v/v, followed by 0·2 ml 2% ferric ammonium sulphate). The contents were mixed thoroughly and the tubes placed in a boiling water bath for 40 min. An agitated but unheated control was prepared on a mixing wheel to check for the presence of pre-existing anthocyanins and/or anthocyanidins. In the absence of an authentic positive control, a more intense red colour in the sample compared to the unheated control was taken to indicate the presence of condensed tannins.

Extraction and quantification of soluble condensed tannins

In a preliminary study, extracts of coffee pulp were prepared using 70% methanol, 80% acetone, and 50% acetone containing 0·5% formic acid (acidified acetone), both at room temperature (mixing wheel) and under reflux (Tecator HT 1043, Tecator Ltd, Bristol, UK). These preliminary trials indicated that the greatest yields of condensed tannins, as judged by the subsequent yield of pigment with Porter's Reagents, were obtained with acidified acetone extracts prepared at room temperature. The quantitative data for soluble condensed tannins reported in this paper were obtained as follows:

Coffee pulp (200 mg) was weighed into the test tube and 4 ml acidified acetone added. The tubes were capped and placed on a mixing wheel. The contents were mixed by rotation for 20 min, separated by centrifugation at

4000 rpm for 5 min, and the supernatant decanted into 25 ml volumetric flasks. The insoluble residues were re-extracted five times, the supernatants bulked and diluted to volume with acidified acetone. The insoluble residues were retained and examined for residual condensed tannins.

Aliquots (1·00 ml) of the acidified acetone extracts were transferred to screw-capped (Teflon liner) test tubes and treated with Porter's Reagents exactly as described above in Qualitative Spot Tests. The red pigment was diluted to a convenient volume with the butanolic Porter's Reagent and the absorbance at 550 nm converted to $E^{1\%}_{1cm}$ values after correction for the absorbance of the unheated control.

The red pigments were stored chilled and in the dark, and analysed as soon as possible (within 24 h) by HPLC as described under Chromatography.

Estimation of total condensed tannins content

When the pellets remaining after centrifugation were resuspended in Porter's Reagents and treated as described above in Qualitative Spot Tests it was apparent that acidified acetone had not completely extracted the condensed tannins. Accordingly an in-situ assay was developed.

Duplicate samples of coffee pulp (200 mg) were weighed into screw-capped (Teflon liner) test tubes and Porter's Reagents added as described above under Qualitative Spot Tests. The contents were mixed thoroughly and the tubes placed in a boiling water bath. At ten minute intervals each tube was removed and the contents remixed to keep the pulp particles in intimate contact with the reagents. After 40 min the pigment so produced was decanted into a volumetric flask, and the insoluble coffee pulp residue treated again with Porter's Reagents. The absorbance (550 nm) of the extracted pigment was measured (without dilution) against a reagent blank on a Cecil Series 2 spectrophotometer using cells of an appropriate pathlength.

The pigment production and monitoring were continued until the pigment obtained in a particular 40 min extraction period had declined to less than 2% of the total pigment so far obtained. Usually four extraction cycles were sufficient, and the accumulated pigment was diluted to volume (25 ml, or 50 ml if more than four extractions were required) with the butanol–HCl reagent.

Unheated, but agitated (mixing wheel) controls on preformed pigments were performed simultaneously, and used as blanks when quantifying the total pigment produced.

Chromatography

Analytical HPLC of anthocyanins and anthocyanidins was performed using the system previously described for chlorogenic acids (Clifford & Jarvis,

1988) consisting of a 3 μm reversed phase non-end capped C_{18} packing 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; linear gradient from 12% solvent B to 65% solvent B in 20 min; 1 ml per min). The eluate was monitored sequentially at 276 nm and 550 nm for anthocyanins and anthocyanidins.

Moisture content

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

RESULTS AND DISCUSSION

Qualitative spot tests

Three spot tests for the detection of different classes of tannins were applied to particulate coffee pulp and coffee beans. Tests for ellagitannins with sodium nitrite/nitrous acid were negative with all samples.

All tests for gallotannins produced a yellow-orange colour rather than the typical red-brown colour reported by Harborne (1984), and seen with the tannic acid control. It was found that 5-caffeoylquinic acid and protocatechuic acid, known pulp constituents (Clifford & Ramirez-Martinez, 1991) produced yellow-orange pigments in this test, and it was therefore concluded that gallotannins *sensu strictu* were absent.

Sun-dried coffee pulp has previously been reported (Zuluago-Vasco & Tabacchi, 1980) to contain 0·43% hydrolysable tannins. These investigators used the Folin–Ciocalteu method as reported by Kramling & Singleton (1969) and defined total tannins as the difference between total phenols and the phenols not precipitated with gelatin. Similarly, hydrolysable tannins were defined as the difference between total tannins (precipitated with gelatin) and condensed tannins (precipitated with formaldehyde), with all values expressed as gallic acid equivalents.

No data were presented to define the precision of either the fractionation or quantification procedures when used to analyse mixtures of chlorogenic acid with hydrolysable and condensed tannins. It is now known that chlorogenic acids (and probably protocatechuic acid) can bind to gelatin (Naish *et al.*, 1988, 1989) and it is suggested therefore that these observations by Zuluago-Vasco & Tabacchi may have been misinterpreted.

Porter's Reagents (Porter *et al.*, 1986) produced positive results with the pulp samples, but negative results with the bean samples. The chemistry of these reagents is now well understood, and the test is known to be very specific. Anthocyanidin pigment(s) is produced by hydrolysis of

anthocyanins and by autoxidation of condensed tannins and their 3,4-flavandiol precursors. Thus, if allowance is made for the presence of anthocyanins, and/or preformed anthocyanidins, the formation of a red colour with this procedure may be taken as a reliable indication that the sample contained condensed tannins and/or 3,4-flavandiols. Accordingly the positive response to Porter's Reagents obtained with the pulp samples can be accepted as confirming the more empirical observations previously made by Zuluago-Vasco & Tabacchi (1980).

Soluble and total condensed tannins content

In view of the results obtained with the spot tests, further investigation was restricted to the analysis of condensed tannins in coffee pulp. It has been shown that condensed tannins are difficult to extract from plant material. In the current study, acidified 50% acetone gave greater yields than either 70% methanol or 80% acetone, as judged by pigment intensity when the extracts were analysed by Porter's improved method (Porter *et al.*, 1986). Further analysis of the insoluble residues (as described above in Qualitative Spot Tests) produced more red pigment and indicated clearly that condensed tannins extraction had not been exhaustive.

In view of the difficulties of optimising condensed tannins extraction reported by Hagerman (1988), exacerbated by limited sample availability, an in-situ hydrolysis procedure was instituted in an attempt to obtain a better estimate of total condensed tannins contents. The contents of acetone-soluble and 'total' condensed tannins so obtained are presented in Table 1 calculated as cyanidin equivalents assuming $E^{1\%}_{550} = 470$ (Porter *et al.*, 1986).

Unexpectedly the 'total' tannins contents were apparently smaller than the soluble tannins contents. It was therefore concluded that the in-situ hydrolysis method caused significant degradation of the pigments during autoxidation of the polymer. Indeed, it is known that anthocyanidins are susceptible to attack by electrophilic carbonyls (e.g. 5-hydroxymethyl-furfural), and that such reactions may result in pigment loss (Debicki-Pospisil *et al.*, 1983). Since such carbonyls are certain to be produced from carbohydrate by the action of hot acid, some loss of anthocyanidins by this mechanism is assured.

Since it is known also that the yield of anthocyanidin pigments declines as the tannin ages (Porter *et al.*, 1986), the analyses for 'total' tannins were repeated after an interval of 12 months. The values obtained averaged 54% less than those originally observed, and it must therefore be possible that even larger values for 'total' and soluble condensed tannins would have been obtained if the analyses had been performed at the time the cherries were harvested. Certainly, one must conclude that the true total condensed

TABLE 1
Acetone-Soluble and 'Total' Condensed Tannins in Coffee Pulp

Pulp sample	*Condensed tannins*					
	Total 8/88[a]		*Total 8/89*[b]		*Acetone-soluble*[a]	
	$E^{1\%}_{550}$	*% db*	$E^{1\%}_{550}$	*% db*	$E^{1\%}_{550}$	*% db*
C. canephora var. *robusta*	2·70	0·68	1·48	0·37	10·91	2·71
Timor hybrid (*C. arabica* × *C. canephora*)	1·63	0·41	0·93	0·23	3·47	0·86
Catimor (Timor hybrid × *C. arabica* var. *caturra vermelho*)	1·52	0·38	0·88	0·22	4·21	1·04
C. arabica var. *caturra vermelho*	1·40	0·35	0·65	0·16	3·97	0·96
C. arabica var. *bourbon vermelho*	1·47	0·37	0·75	0·19	3·81	0·95

[a] Analysis performed August 1988.
[b] Analysis performed August 1989.

tannins contents are greater than the largest estimates (soluble condensed tannins contents) reported here.

No previous data could be found for condensed tannins in robusta pulp. Zuluago-Vasco & Tabacchi (1980) extracted sun-dried Guatemalan coffee pulp (presumably arabica) with ethyl acetate–ethanol (1:1) and 80% aqueous ethanol. They reported 1·64% formaldehyde-precipitable condensed tannins using the Folin–Ciocalteu reagent with a gallic acid standard. For sun-dried arabica pulp Garcia *et al.* (1985) reported 2·56% alkali-soluble condensed tannins, a value inflated by flavan-3-ols, which may range from 0·19 to 0·86% (Ramirez-Martinez, 1988). The current values for arabica pulp, 0·95–0·96% db, are somewhat lower but not inconsistent.

HPLC analysis of the pigments produced by autoxidation of the soluble condensed tannins with Porter's Reagents showed two major components that were not detectable prior to this treatment. The slower eluting component is tentatively identified as cyanidin, and the faster eluting as delphinidin. The commercially available standards were too impure to permit unequivocal identification by spiking, but such assignments are consistent also with the known chromatographic behaviour of the anthocyanidins (Lea, 1988) and with the known structure of other condensed tannins (Haslam, 1989). In all cases, the faster eluting component

TABLE 2
Ratio of Delphinidin to Cyanidin Produced by Autoxidation of Acetone-Soluble Condensed Tannins from Coffee Pulp

Pulp sample	*Ratio of prodelphinidin to procyanidin monomers*[a]
C. canephora var. *robusta*	2·2
Timor hybrid (*C. arabica* × *C. canephora*)	6·0
Catimor (Timor hybrid × *C. arabica* var. *caturra vermelho*)	3·9
C. arabica var. *caturra vermelho*	2·7
C. arabica var. *bourbon vermelho*	2·8

[a] Ratio of peak areas without correction for relative molar absorptivities.

dominated the chromatogram, but the ratio varied as indicated in Table 2.

Although up to seven 550 nm-absorbing components (presumably anthocyanins) were present in the soluble tannin extracts prior to treatment with Porter's Reagents, it was concluded that the yield of cyanidin and delphinidin from this source would be small, even after making allowance for the relatively weak absorbance of the glycosides compared to the aglycones (Ribéreau-Gayon, 1972). Under these circumstances, and in view of the certainty that tannins contents are being under-estimated, no attempt has been made to correct the data presented in Tables 1 and 2 for this interference.

Singleton (1981) has reviewed the published evidence concerning the toxicity of tannins and flavonoids. Flavonoids have very low mammalian toxicity when given orally, and it seems unlikely that the preformed anthocyanin pigments of coffee pulp can be blamed for its adverse dietary effects.

In contrast, tannins have been reported to depress growth and reduce the utilisation of feed, particularly protein, increasing the secretion of sacrificial, tannin-binding salivary protein and thus faecal nitrogen levels. Such effects have been observed with swine and poultry, but to a lesser extent with ruminants (e.g. see review by Mehansho *et al.*, 1987).

Due to the difficulty of extracting and quantifying condensed tannins,

there seems never to have been any precisely controlled feeding trials using cattle, swine or poultry that would define the level at which condensed tannins became intolerable. In view of the relative widespread occurrence of low concentrations of condensed tannins in forage plants, it seems unlikely that the 0·1% soluble condensed tannins that would occur when arabica pulp was incorporated at 10% of normal rations would be sufficient to cause adverse effects. While an additive or synergistic effect with caffeine cannot be ruled out (Clifford & Ramirez-Martinez, 1991), one is led inevitably to the conclusion that some other agent is responsible and/or that a much greater amount of insoluble condensed tannin is present in the pulp. Before more definite conclusions may be drawn regarding the anti-nutritive agent(s) of coffee pulp there is a need for improved methods for quantifying the insoluble condensed tannins. Further studies are in progress.

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