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The Chlorogenic Acids Content of Coffee Substitutes

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ABSTRACT

This paper reports the chlorogenic acids content of unroasted chicory root and eleven coffee substitutes which contain either chicory or dandelion. Unroasted chicory root contained CQA and diCQA but at a much lower level than green coffee beans. In the roasted products only 5-CQA was found consistently and 4-CQA occasionally at levels approximately two orders of magnitude lower than in the corresponding roasted coffee products. The roasted substitutes were characterised chromatographically by very large amounts (some 80 to 95% of total chromatogram area) of rapidly eluting material. The chromatograms of most substitutes were characterised by a peak provisionally identified as 5-hydroxymethyl furfural.

INTRODUCTION

Green coffee beans and coffee products are characterised by significant chlorogenic acids contents. However, despite the extensive literature in that area (Clifford, 1985*a*,*b*) there are no recent comparable data for coffee substitutes based upon the roasted roots of chicory (*Cichorium intybus*) or dandelion (*Taraxacum* sp.).

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MATERIALS

A sample of raw chicory root (sample 1) and five samples of roasted chicory root (samples 2 to 6) were kindly donated by commercial sources. The other products were bought locally from a specialised retailer. Their declared contents were:

Sample 7: 100% roasted soluble chicory root.
Sample 8: Chicory, figs, wheat, malted barley, acorns.
Sample 9: Soluble solids of roasted malted barley, barley and chicory.
Sample 10: Barley, chicory, fig, soyabeans.
Sample 11: Lactose, soluble solids of roasted dandelion root.
Sample 12: Extract of roasted dandelion root, lactose, flavouring.

METHODS

Extraction

If necessary, samples were ground in a hammer mill to pass 0·7 mm. Extracts were prepared using a Tecator HT 1043 continuous extraction apparatus (chicory root 2·5 g, soluble chicory-containing powders 2·5 g, other substitutes 5·0 g) and five 30 min extractions into 50 ml aliquots of 70% methanol. The extracts were bulked, treated with Carrez Reagent (2 ml reagent A plus 2 ml reagent B) filtered, and reduced in volume to 100 ml.

Chromatographic analysis

A Waters Associates (Milford, Massachusetts, USA) liquid chromatograph consisting of two Model 6000 A solvent delivery pumps, Model 660A solvent programmer, Model U6K Injector (20 μl) and a Model 440 detector operating at 313 nm was used with a 25 cm × 5 mm stainless steel column containing Spherisorb 5 ODS. A flow rate of 2 ml min^{-1} and a linear gradient of 0·5% formic acid to 30% acetonitrile in 0·5% formic acid over 60 min. Solvents were degassed by sonication under vacuum. Peak area was determined using a LDC 308 computing integrator.

Peaks were assigned by reference to standards obtained as previously acknowledged. Quantification was achieved by reference to a 5-CQA (Sigma Chemical Co. Ltd) calibration curve (concentration μg/ml = 4·062 + 0·254 peak area mm^2, $r = 0{\cdot}999\,99$) with correction for relative molar absorbance as follows: 5-CQA = 1·00; 3-CQA and 4-

CQA × 1·07; 5-FQA × 1·04; diCQA × 0·90. These factors were calculated from a compilation of published molar absorbance values (Clifford, 1985*b*).

A sample of caffeoyltartaric acid was kindly supplied by Dr A. G. H. Lea of AFRC Long Ashton Research Station. Aesculin and aesculetin were obtained from Sigma Chemical Co. Ltd.

COLORIMETRIC ANALYSIS

Molybdate reagent

The reagent was prepared and used as described by Clifford & Wight (1976).

Periodate reagent

The reagent was prepared and used essentially as first reported (Clifford & Wight, 1976) but with the colour developing incubation performed at 27°C (Clifford & Staniforth, 1977).

Thiobarbituric acid reagent

The reagents used are based upon those used by Pierpoint, Ireland & Carpenter (1977) modified by Ohiokpehai (1982) as described below. For free quinic acid 1·0 ml of extract in a stoppered test tube was buffered with 1·0 ml 1 M toluene sulphonic acid adjusted to pH 1·4. Periodic acid (0·5 ml, 0·25 M in 0·062 M sulphuric acid) was added, mixed thoroughly, and the mixture incubated 20 min at 27°C in a thermostatically controlled water bath. Excess periodic acid was destroyed by adding aqueous sodium arsenite (3·0 ml, 4% m/v) and mixing immediately until the colour due to iodine was fully discharged. Thiobarbituric acid solution (1·0 ml, 0·2% m/v aqueous) was added, the contents mixed and colour developed by heating the test tubes in a gently boiling water bath for 20 min. The red solution was diluted to 25·0 ml using distilled water and absorbance measured at 549 nm against a blank prepared similarly but from which the TBA was omitted. The free quinic acid content was obtained by reference to a calibration curve (concentration μg/ml = 7·29 + 270·05 A_{549}, $r = 0{\cdot}950$). Total quinic acid was determined similarly on aliquots (1·0 ml) which had been saponified overnight with 1·0 ml 2M NaOH and neutralised with 1·0 ml 2M HCl. The bound quinic acid content was obtained by correcting the absorbance due to total quinic acid for that due to free quinic acid and interpreting against a 5-CQA calibration curve (concentration μg/ml = 17·42 + 2567·48 A_{549}, $r = 0{\cdot}999$).

Moisture content

The moisture content was determined as the loss on drying to constant weight at 70°C in a vacuum oven.

RESULTS AND DISCUSSION

Table 1 summarises the precision of the analytical data obtained by each analytical method applied to five replicate extractions of unroasted chicory root and soluble chicory powder. Recovery, as judged by HPLC, of added 5-CQA was 99·7 and 94·5% respectively.

Table 2 summarises the results obtained for the free quinic acid and the chlorogenic acids (CGA) contents of unroasted chicory root and 11 coffee substitute products. In all cases the values obtained by a particular method of analysis were significantly lower, often by nearly two orders of magnitude, compared to the values typically obtained for a green coffee or roasted coffee product (for reviews see Clifford, 1985*a*,*b*).

For the unroasted chicory root similar results for CQA content were obtained by colorimetry (0·22% db) and chromatography (0·26% db). The chromatogram (see Fig. 1) is consistent with previous publications recording 5-CQA and 3,5-diCQA as the major CQA in chicory (Paulet & Mialoundama, 1976). Feruloylquinic acid was not detected. There was no trace of caffeoyl tartaric (caftaric) acid nor any unassigned peak that might correspond to dicaffeoyltartaric (chicoric) acid which have previously been reported in the leaves of chicory (*Cichorium intybus*) (Scarpati & Oriente, 1958; Scarpati & d'Amico, 1960). Similar checks for the presence of aesculin (also known as cichorin or chicorin) and its aglycone, aesculetin, were also negative.

TABLE 1
Precision of Analytical Methods

Method	*Sample and coefficient of variation* (%) ($N = 5$)	
	Unroasted chicory, sample 1	*Roasted product, sample 7*
Molybdate	3·6	4·2
Thiobarbituric acid	8·2	9·6
Chromatography		
Peak 1	not detected	4·3
Peak 2	not detected	8·5
Total CGA	0·9	2·7

TABLE 2
The Contents of Quinic Acid and Chlorogenic Acids in Unroasted Chicory Root and Roasted Coffee Substitutes (% db)[a]

Sample	*TBA Quinic acid*		*Molybdate*	*Chromatography*								
				CQA				*diCQA*				*Total CGA*
	Free	*Bound*		*3*	*4*	*5*	*Subtotal*	*3,4*	*3,5*	*4,5*	*Subtotal*	
1	0·01	0·20	0·22	0·01	0·02	0·16	0·19	0·01	0·04	0·02	0·07	0·26
2	nd	nd	0·37	—	—	0·02	0·02	—	—	—	—	0·02
3	nd	nd	0·70	—	—	0·02	0·02	—	—	—	—	0·02
4	nd	nd	0·29	tr	0·01	0·03	0·04	—	—	—	—	0·04
5	nd	nd	0·40	—	—	0·04	0·04	—	—	—	—	0·04
6	nd	nd	0·28	—	—	0·03	0·03	—	—	—	—	0·03
7	0·07	0·57	0·27	tr	0·03	0·02	0·05	—	—	—	—	0·05
8	0·04	0·44	0·24	tr	0·02	0·02	0·04	—	—	—	—	0·04
9	0·04	0·33	0·21	—	tr	0·01	0·01	—	—	—	—	0·01
10	0·04	0·76	0·18	—	tr	0·01	0·01	—	—	—	—	0·01
11	0·03	0·14	0·10	—	tr	tr	tr	—	—	—	—	tr
12	0·17	0·53	0·09	—	tr	tr	tr	—	—	—	—	tr

Key: nd = not determined; tr = trace, i.e. less than 0·01%; — = not detectable.
[a] Mean of five determinations for samples 1 and 7, otherwise mean of two determinations.

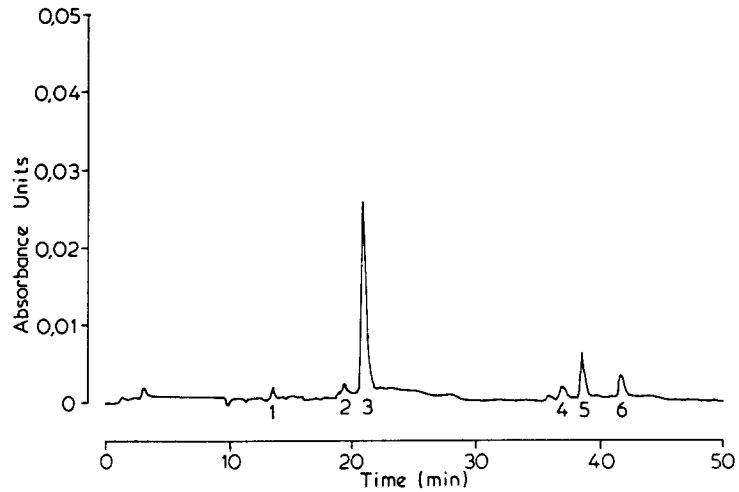

Fig. 1. Chromatogram of 70% methanol extract of unroasted chicory (sample 1). 1 = 3-CQA; 2 = 4-CQA; 3 = 5-CQA; 4 = 3,4-diCQA; 5 = 3,5-diCQA; 6 = 4,5-diCQA; other peaks unknown.

The colorimetric methods of analysis for CGA, particularly TBA, indicated much larger CQA contents in roasted coffee substitutes than were indicated by the chromatographic method. A similar effect has been observed previously with roasted coffee products (Clifford, 1985*a*,*b*; Kazi & Clifford, 1985) and has been attributed to interference by ill-defined

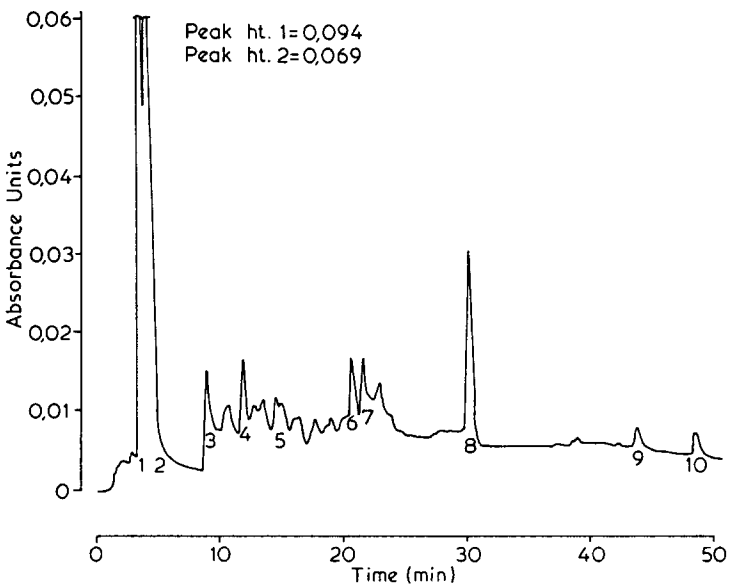

Fig. 2. Chromatogram of 70% methanol extract of soluble chicory powder (sample 7). Peaks 1 to 4, 8 to 10 = unknowns shown in Table 2. 5 = 3-CQA; 6 = 4-CQA; 7 = 5-CQA.

products of roasting. In the case of the molybdate reagent such products may well be derived from CQA since a 1,2-dihydroxyphenyl structure is necessary to produce the chromophore. However, in the case of the TBA reagent interference might also be expected from roasting-transformation products of the heat labile fructan. In view of this interference samples 2 to 6 were not examined by this method.

Samples 2 to 6 (commercial roasted chicory) produced clean chromatograms from which the very low 5-CQA contents (see Table 1) could easily be determined. Traces of 3-CQA and 4-CQA were found only in sample 4. Samples 2 to 6 also contained two fast eluting peaks which varied in their relative intensities (see Table 3). Scanning of Peaks 1 and 2 while in the detector (pumps stopped) showed λ_{max} at 292 and 282 nm respectively. Spiking indicated that 5-hydroxymethylfurfural (λ_{max} 282 nm) and a known component of roasted chicory (Smith, 1981) co-eluted with Peak 2. Samples 7 to 9 yielded complex chromatograms (Fig. 2), characterised by at least one slow moving peak and at least five fast moving peaks relative to 5-CQA) of significant size with the fast moving peaks superimposed upon a distorted baseline in the region where the CQA eluted. This distortion may well have led to the CQA contents of these samples being overestimated. If this were so the discrepancy between the colorimetric

TABLE 3
Uncharacterised Components in Roasted Coffee Substitutes (Peak Area $mm^2 g^{-1}$ db)[a]

Sample	*Relative retention time (5-CQA = 1·000) and peak number*[b]									
	1	*2*[c]	*3*			*4*	*8*		*9*	*10*
	0·16	*0·19*	*0·42*	*0·45*	*0·46*	*0·52*	*1·40*	*1·64*	*2·00*	*2·20*
2		52				28				
3		74				8				
4		12				14				
5		34				83				
6		104				20				
7	71	94	12			9	77		+	+
8	67	32	38	25	30	+	+			5
9	60	14	31	16	40	+		9		
10	3	+	5	+	+	+				
11	+									
12	6	10	+	+	+	+				

Key: + less than 5 $mm^2 g^{-1}$.
[a] Mean of five determinations for sample 7, otherwise mean of two determinations.
[b] see Fig. 2.
[c] Tentatively identified as 5-hydroxymethylfurfural.

estimates and the chromatographic estimates referred to above would be even larger than is implied by the data in Table 2.

In an attempt to avoid this distortion, extracts were also prepared in boiling water and in boiling methanol (rather than 70% methanol), and alumina cream was examined as an alternative clearing agent to Carrez, but the chromatograms were not significantly different. An anion exchange solid phase sample clean-up column retained the chlorogenic acids and the interfering substances implying that they also are acidic. Use of a 3 μm reversed phase packing (Spherisorb 3-ODS) in the analytical column gave improved resolution in this area of the chromatogram but irreversibly bound Peaks 1 and 2. Smith (1981) has reported that 5-HMF is irreversibly bound to the Hypersil ODS reversed phase packing, suggesting that the furans may be very sensitive to minor chemical and physical differences in the solid phases. Work is in progress to further characterise these products and to eliminate the interference. Since this occurs in products based upon soluble chicory powders but not in roasted chicory root it would appear that these substances are formed during the commercial extraction of the chicory solubles.

The chromatograms obtained from sample 10 (chicory-containing) and samples 11 and 12 (containing soluble solids of roasted dandelion root) were qualitatively similar to those obtained from samples 7 to 9. However, peak areas were much less, especially sample 12, suggesting lower contents of chicory or dandelion in these products.

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NOTE

H. Haffke & U. H. Engelhardt (Chlorogensäuren in Kaffee-Ersatzstoffen. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, **183** (1986), 45–6) have reported 0·19% and 0·03% CGA in single samples, respectively, of dried and roasted chicory root. The relative amounts of individual CGA were also very similar to the data reported in this paper.