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The Influence of Coffee Bean Maturity on the Content of Chlorogenic Acids, Caffeine and Trigonelline

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ABSTRACT

This paper reports changes, with coffee fruit maturity, in the coffee bean content of chlorogenic acids, caffeine and trigonelline. The major change was a sigmoidal increase in total caffeoylquinic acid essentially in parallel with the total dry matter gain, and representing between 5% and 12% thereof. The corresponding changes in the contents of several other chlorogenic acids, caffeine and trigonelline were slight on a mass per 100 beans basis.

INTRODUCTION

Immature green coffee beans are the processed seeds derived from immature fruit of coffee shrubs. Coffee fruit develop during a period of some 35 weeks after the shrubs blossom, as illustrated in Fig. 1 (Cannell, 1985). Maturity is indicated by the loss of chlorophyll from the pericarp and its replacement, by yellow flavone pigments in some cultivars, or more often by red flavonoid pigments (Poisson, 1977; Lopes *et al.*, 1984).

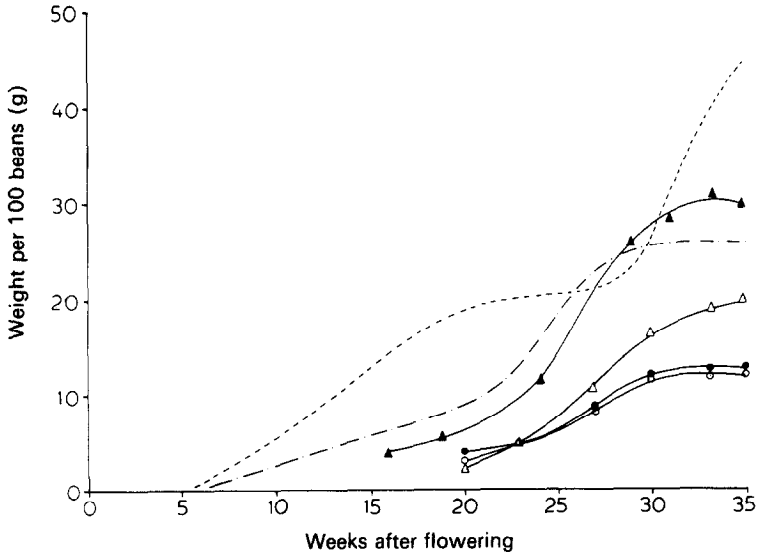

Fig. 1. Increase in weight of bean and pericarp as coffee fruit matures. ———, Pericarp; — · —, bean. Data reproduced from Cannell (1985, p. 125) with permission of Croom Helm Ltd Publishers. —▲—, Bean Arabica maragogipe; —△—, bean Liberica indenie; —●—, bean Robusta IC 503; —○—, bean Robusta IC 182.

The fruit are harvested either as they ripen, after a period of drying on the shrub, or after falling to the ground. Traditionally, the fruit have been hand-picked, selectively so, when ripe and unripe fruit are present simultaneously. However, mechanised harvesting is becoming established (Willson, 1985), increasing the likelihood of less selective harvesting and thus the risk of immature beans reaching commercial supplies.

It is generally said that the presence of immature beans in commercial roasts leads to a reduction in beverage quality. This effect is presumably due to the immature beans differing physically and/or chemically from fully mature beans, but published data on immature beans are extremely sparse and whether there are significant changes in composition during the final weeks of maturation is uncertain. As part of an investigation designed to screen for significant changes in bean composition as the fruit matures, this paper reports on the contents of chlorogenic acids, caffeine and trigonelline in beans of three species.

MATERIALS AND METHODS

Materials

Dry processed samples of robustas (*Coffea canephora var robusta*) IC 182 and IC 503, which typically yield some 2 to 3 tonnes per hectare, were kindly supplied by IFCC in the Ivory Coast. They also supplied similar samples of

indenie, a selected population of a native *C. liberica*. Samples of arabica (*C. arabica cv Maragogipe*) were obtained from a plant in cultivation in the Palm House of the Royal Botanic Gardens at Kew. These fruit were depulped immediately by hand, the beans frozen in liquid nitrogen and freeze-dried. With the exception of the last arabica maragogipe sample, which consisted of cherries that had fallen from the plant, all fruit were collected at defined periods after flowering.

Methods

HPLC analysis of chlorogenic acids

Samples were ground, extracted and analysed by reversed phase HPLC as previously described (Clifford, 1986). Except where indicated otherwise, peak assignments were made by comparison with standards.

HPLC analysis of purine alkaloids and trigonelline

Ground green bean (1 g) was mixed in a beaker with 5 g heavy magnesium oxide and 15–20 ml hot distilled water. This mixture was heated, with occasional stirring, on a boiling water bath for 30 min. The hot extract (90°C) was filtered through a sintered glass funnel (porosity 1) packed with 5 g of a heavy magnesium oxide–dicalite mixture (1:1) using slight vacuum from a vacuum pump applied via a vacuum trap and a Büchner flask. The packing was washed with hot distilled water (90°C) using slight vacuum until an eluate of 150 ml was obtained. This solution was diluted as necessary prior to analysis.

Standards of caffeine, theophylline, theobromine and trigonelline (Sigma) were prepared in distilled water.

Separation was effected isocratically by using an Altex model 110A pump (Beckman), a Guard-pak pre-column module with a μBondapak C18 insert (Waters) and 15 cm × 3·9 mm stainless steel analytical column with fully end-capped reversed phase packing (Novapak C18, Waters). The mobile phase was 0·01 M phosphate buffer (pH7)/methanol (20:80 v/v) with a flow rate of 1·5 ml min^{-1}. The sample was injected via an Altex model 210 injection valve with 20 μl loop (Beckman) and detection was at 276 nm with Altex model 153 variable wavelength detector (Beckman).

Recoveries were in the range 95·8% to 102% for spikes of purine alkaloids (0·01 to 0·05 g 100 g^{-1}) and 98·5 to 100% for trigonelline (0·1 to 0·5 g 100 g^{-1}). The coefficient of variation for replicate analyses on typical samples was found to be not more than 1·5%. A specimen chromatogram has been published elsewhere (Kazi, 1985).

Moisture content

Duplicate samples (0·5 g) were dried to constant weight at 105°C.

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*).

The four types of bean examined showed typical sigmoid growth curves, which are illustrated in Fig. 1 alongside data from Cannell (1985). The almost identical behaviour of the two robustas (IC 182 and IC 503) is noteworthy, as is the relatively large bean weight reached by the arabica maragogipe, this characteristic being the reason for this cultivar's popular name of 'elephant bean'. (Clarke, 1985, p. 246).

TABLE 1
Composition of the Mature Samples Used in this Investigation Compared with Previously Published Data (% dry basis)[a]

	This investigation				*Previous publications*		
	Arabica maragogipe	*Robusta IC 182*	*Robusta IC 503*	*Liberica indenie*	*Arabica*	*Robusta*	*Liberica*
Total CQA	5·44	8·33	7·55	9·37	5·56 ± 0·56	6·70 ± 0·41	6·14–6·62
Total FQA	0·29	0·88	1·12	0·53	0·36	1·175	
Total diCQA	1·19	1·98	1·33	0·73	0·92 ± 0·09	1·83 ± 0·33	0·30–0·90
Trigonelline	1·31	0·69	0·57	0·29	0·97–1·15	0·88 ± 0·12	0·25
Caffeine	1·39	2·11	2·11	1·28	0·53–1·45	2·18–2·72	1·35
Theobromine	nd	nd	0·01	nd			
Theophylline	nd	tr	nd	0·01			

[a] Data from compilations by Clifford (1985*a*,*b*) and Macrae (1985).
nd = not detected, less than 0·005%.
tr = 0·005 to 0·01%.

Table 1 summarises the composition (% dry basis) of the mature samples and compares the current data with previously published values. These are similar, with the exception of a much higher total caffeoylquinic acid (CQA) content found for the Liberica indenie. This discrepancy cannot be explained with certainty, but may well be due to the analysis of different types of bean since the liberica–excelsoides group is thought to contain genetically diverse material that is difficult to distinguish morphologically (Charrier & Berthaud, 1985, p. 32).

The accumulations of total CQA (3-CQA, 4-CQA and 5-CQA), total diCQA (3,4-diCQA, 3,5-diCQA and 4,5-diCQA) and of 5-FQA (3-FQA and 4-FQA are very minor unresolved isomers) are shown in Figs 2A–D. The most striking change for each type of bean is the pronounced sigmoidal increase in the content of total CQA expressed on a weight per 100 bean basis. These increases account for between 5% and 12% of the total dry

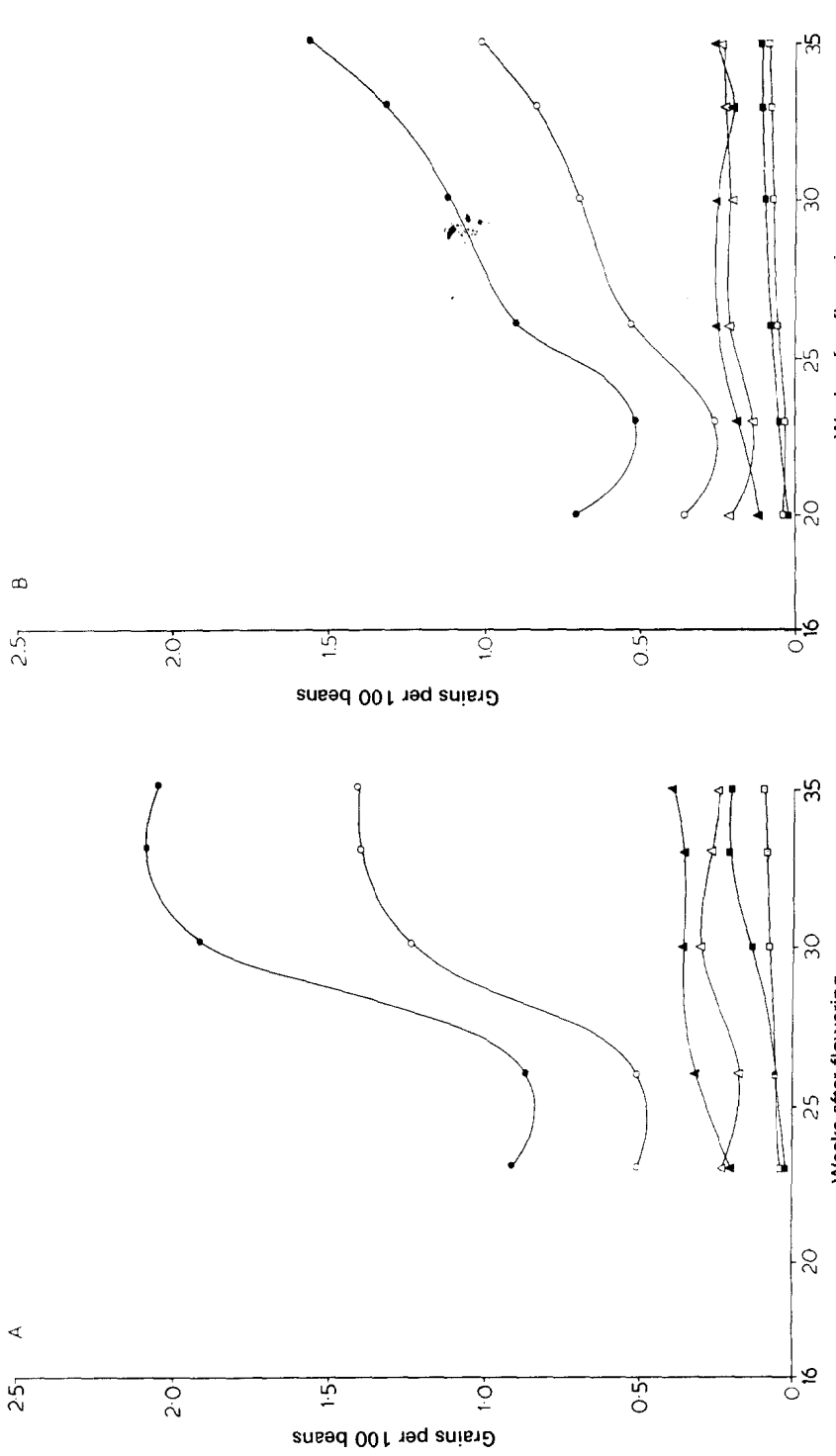

Fig. 2. Accumulation of chlorogenic acids, caffeine and trigonelline in beans as fruit matures. (A) Robusta IC 503; (B) Robusta IC 182; (C) Arabica maragogipe; (D) Liberica indenie. —●—, Total CGA; —○—, Total CQA; —■—, 5-FQA; —△—, Total diCQA; —▲—, Caffeine; —□—, Trigonelline.

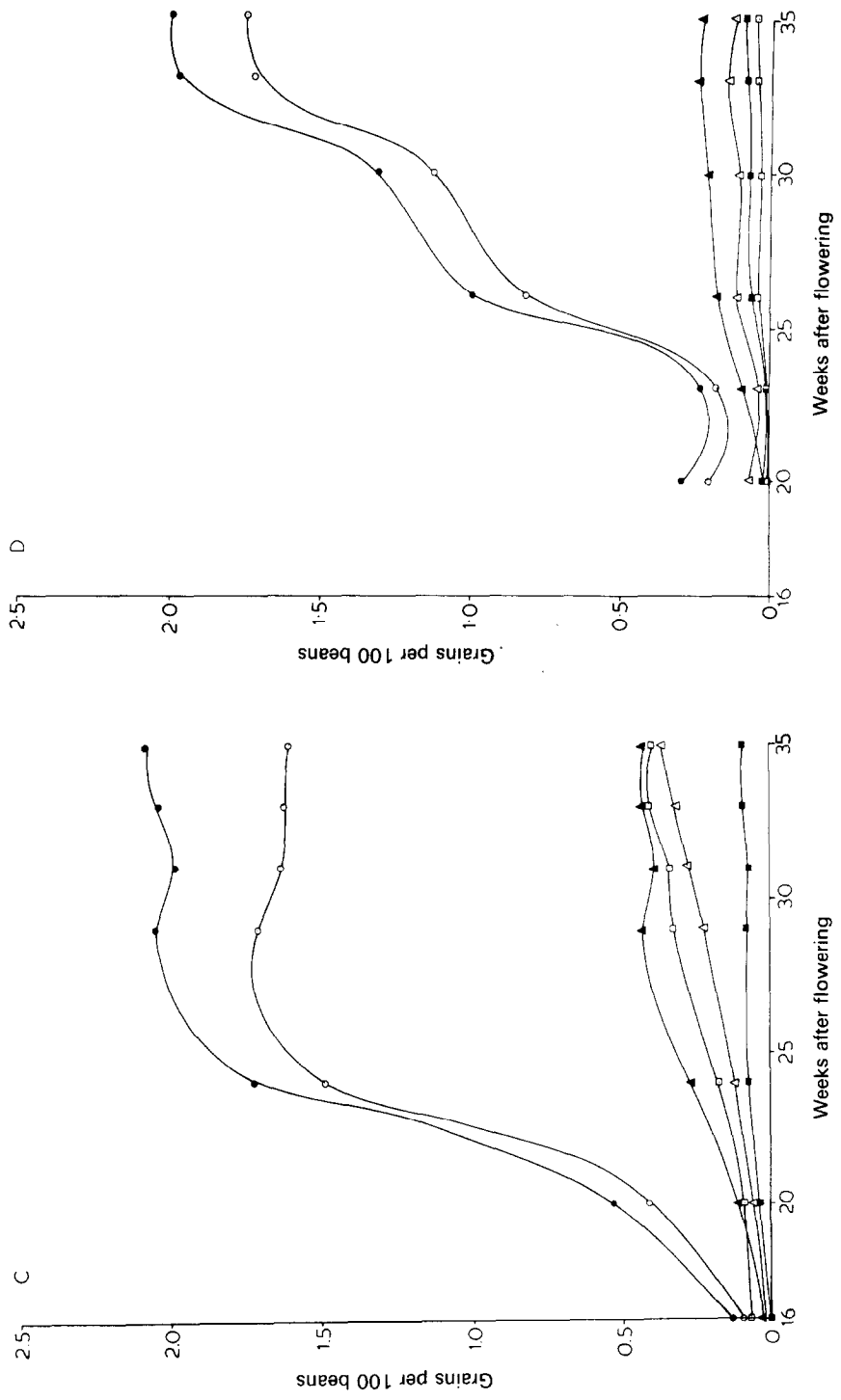
C
D
Grains per 100 beans
Weeks after flowering
0
0·5
1·0
1·5
2·0
2·5
16
20
25
30
35

Fig. 2.—*contd.*

matter increase, indicating that a significant photosynthetic effort is put into this synthesis. In contrast comparatively little effort (some 1% or 2%) is put into the synthesis of the other CGA subgroups. These either increase in an essentially linear manner; for example, total diCQA in the arabica maragogipe and robusta IC 503, or change hardly at all on a content per 100 beans basis. The reasons for such syntheses and the functions, if any, of the CGA are still a matter for debate (Haslam & Lilley, 1985).

Although the data are not shown graphically, it was observed that both robusta cultivars contained two groups of chlorogenic acid-like components which differed in their behaviour during seed development. The first group were detected only in the most immature samples (e.g. see Fig. 3). Nothing is known at present about the identity of these compounds, which have retention times relative to 5-CQA, of 1·59, 1·71 and 1·82 (IC 182) and 1·84 and 2·09 (IC 503).

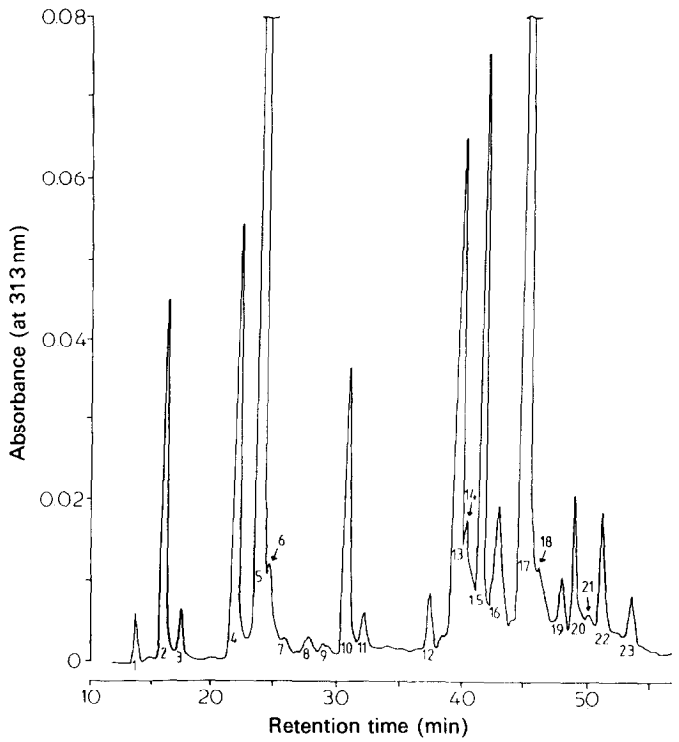

Fig. 3. Chromatogram of a 70% methanol extract from the most immature sample of Robusta IC 182. Peak 1 = unknown; peak 2 = 3-CQA; peak 3 = unknown; peak 4 = 4-CQA; peak 5 = 5-CQA; peak 6 = caffeic acid; peak 7 = unknown; peak 8 = 5-CoQA; peak 9 = unknown; peak 10 = 5-FQA; peaks 11 and 12 = unknowns; peak 13 = 3,4-diCQA; peak 14 = unknown; peak 15 = 3,5-diCQA; peak 16 = unknown; peak 17 = 4,5-diCQA; peak 18 = unknown; peak 19 is probably caffeoyltryptophan; peak 20 is probably 4F5CQA; peak 21 = unknown; peak 22 is probably 4C5FQA; peak 23 = unknown.

The content of the second group of chlorogenic-acid like components increased progressively. From correspondence with Professor Kido of Wakayama Medical College, Japan (see also Iwahashi *et al.*, 1985; Morashita *et al.*, 1986, 1987), it is suggested that three members of the second group are 4C5FQA, 4F5CQA and caffeoyltryptophan. The other members of this group had relative retentions of 1·98, 2·32 and 2·63 (IC 503) and 1·96, 2·20 and 2·40 (IC 182). The most immature liberica indenie sample appeared to contain traces of the six compounds observed in IC 182 but, in contrast to the robusta, these disappeared as the liberica matured.

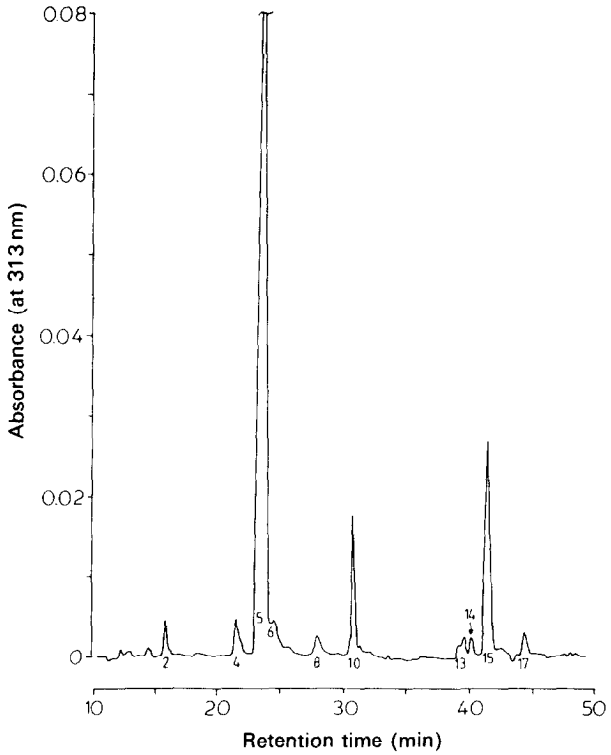

Fig. 4. Chromatogram of a 70% methanol extract of the most immature sample of Arabica maragogipe. Peak assignments as in Fig. 3.

The composition of the beans obtained from abscissed maragogipe fruit did not differ significantly from that of mature beans, and accordingly these data have not been presented. The least mature arabica maragogipe was characterised (see Fig. 4) by very small 3-CQA, 4-CQA, 3,4-diCQA and 4,5-diCQA contents. As the seed developed the contents of these CQA and diCQA isomers increased progressively to levels that are typical of commercial arabicas. Since the biosynthetic origin of these particular

isomers remains unknown, there has been speculation that they might be artifacts. If that is the case, it is difficult to explain why these isomers are virtually absent from the most immature sample. However, such changes in the relative proportions of isomers within the CQA and diCQA subgroups were not seen during the maturation of either robusta or the liberica. Previous data on the chlorogenic acids content of immature coffee beans are sparse and detailed comparisons are not possible, because different methods of extraction (Ohiokpehai *et al.*, 1982) or analysis (El Hamidi & Wanner, 1964) were used.

Caffeine and trigonelline behave similarly to the diCQA and FQA subgroups and the contents either change very little with seed development, or increase linearly and account for some 1% to $2\frac{1}{2}$% and 0·2% to 1%, respectively, of total dry matter production. Previous data for the accumulation of caffeine have been related to the whole fruit rather than the seed (Raju *et al.*, 1981), or have been expressed on a fresh weight basis (Suzuki & Waller, 1985), and detailed comparisons with the present data are not possible.

There is a general belief that when immature beans are used in commercial roasts, there is a reduction in beverage quality, which presumably is due to the different physical and/or chemical characteristics of such beans. Regrettably, lack of material prevented test-roasting of the immature beans and sensory evaluation of the beverage, and thus the critical evaluation of this belief. It is, however, interesting to note that for a given severity of roasting, beverage CGA content is a function of green bean CGA content (see Clifford, 1985*b*, p. 189–91), and that the sensory character of the beverage may be influenced adversely by low CQA–diCQA molar ratios (Clifford & Ohiokpehai, 1983). The large increases in total CQA content relative to total diCQA content over the last five weeks (35% to 40% in the indenie and IC 182) are probably sufficient to modify the beverage CQA–diCQA molar ratio and thus possibly to have an effect upon beverage acceptability. This possible relationship merits further investigation.

In contrast the scant change in the contents of caffeine and trigonelline over this same period, and their minor contribution to beverage bitterness (see Clifford, 1985*a*, p. 353) make it almost certain that changes in the contents of these alkaloids are not directly responsible for any change in beverage acceptability associated with bean maturity.

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