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Distinction between Arabica and Robusta Coffee Beans by Hydroxycinnamic Acid Derivatives, Especially by *p*-Coumaroyltryptophan

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Eleven chlorogenic acids, caffeoyltryptophan (CaT), and *p*-coumaroyltryptophan (CoT) in coffee beans (*Coffea canephora* var. *robusta* (Robusta) and *C. arabica* (Arabica)) and instant coffees on the market were analyzed by HPLC. Robusta beans contained more caffeoylferuloylquinic acids and CaT than Arabica beans. CoT was detected only in Robusta beans. The more intensely the coffee beans were roasted, the more all the chlorogenic acids decreased. CoT decreased the most slowly among phenols during roasting. Only instant coffees made of Robusta beans contained CoT. CoT becomes an indicator of Robusta beans.

Keywords: coffee, *Coffea*, polyphenol, phenol, chlorogenic acid, roasting

Coffee beans are the seeds of an evergreen shrub belonging to the family Rubiaceae and the genus *Coffea*. Two species are important commercially, *Coffea arabica* (Arabica) and *Coffea canephora* var. *robusta* (Robusta). However, there is no clear chemical distinction method between the two, although Arabica beans contain more trigonelline, lipids and polysaccharides than Robusta beans and Robusta beans contain more caffeine and total chlorogenic acids than Arabica beans (Smith, 1985).

Here we observed differences in phenols between Arabica and Robusta beans. Coffee beans contain many kinds of phenols such as hydroxycinnamic acid derivatives or chlorogenic acids (Fig. 1; Clifford, 1985). Chlorogenic acids are a family of mono- and di-acyl quinic acids. The common acylating residues are caffeic acid, ferulic acid, and *p*-coumaric acid. The main compound among chlorogenic acids is 5-chlorogenic acid, a so-called chlorogenic acid (5-*O*-caffeoylquinic acid, 5-CQA; Clifford & Wright, 1976). These compounds are considered to be precursors of the brown pigments of coffee (Nakabayashi & Mano, 1975; Nakabayashi & Watanabe, 1975) and are astringent (Naish *et al.*, 1993). In addition to chlorogenic acids such as caffeoylquinic acids (CQAs), dicaffeoylquinic acids (di-CQAs; Clifford, 1986) and caffeoylferuloylquinic acids (CFQAs; Iwashita, *et al.*, 1985; Clifford *et al.*, 1989a), the existence of hydroxycinnamoyl amino acid derivatives (Fig. 2) such as caffeoyltryptophan (CaT; Morishita *et al.*, 1987) and caffeoyltyrosine (Clifford *et al.*, 1989b) in Robusta green coffee beans was reported. Recently, we also found a new hydroxycinnamoyl amino acid derivative, *p*-coumaroyl-(L)-tryptophan (CoT), in a Robusta coffee bean (Murata *et al.*, 1995) during studies on metal-chelating compounds from roasted coffee beans (Asakura *et al.*, 1990; Nakamura-Takada *et al.*, 1994; Sekiguchi *et al.*, 1994) and on the change in phenols due to roasting.

The purpose of this study is to examine the difference in hydroxycinnamic acids, especially in CoT, between Arabica and Robusta green or roasted beans, because there is no report on the difference in CoT content among coffee beans. Here we describe that CoT was detected only in Robusta beans and decreased the most slowly among the phenols in coffee beans on roasting. All the phenols except CoT were detected in both Arabica and Robusta green coffee beans, although Robusta beans contained several tens of greater CFQAs and CaT contents than Arabica beans. CoT was detected only in instant coffees made of Robusta beans.

Materials and Methods

Samples Green beans of six kinds of *Coffea canephora* var. *robusta* (four from Indonesia, Thailand, Vietnam) and eight kinds of *C. arabica* (Jamaica, Brazil, Indonesia, Guatemala, Cuba, Columbia, Ethiopia, and Honduras) were provided by Nestle Japan Ltd. (Tokyo), Nittoh Coffee Ltd. (Tokyo), and UCC Uejima Coffee Ltd. (Tokyo). Six brands of instant coffees were purchased on the local market, Tokyo, in 1995. Two brands among them contained *C. robusta* according to their labels.

Analysis of hydroxycinnamic acid derivatives in green coffee beans and instant coffee Hydroxycinnamic acid derivatives in coffee beans and instant coffees were analyzed according to the previous paper (Murata *et al.*, 1995). Phenols were extracted by boiling ground coffee beans in 70% methanol and were analyzed by 3D-HPLC; column YMC-pak R-ODS-10 (Yamamura, Kyoto); eluent, a mixture of acetonitrile and 5% acetic acid (2 : 92→30 : 40). Each phenol was determined from the peak area at 320 nm as 5-CQA. The mean value of triplicate determinations was expressed as dry weight. Each variation was less than 10%. Statistical analyses (Mann-Whitney *U* test) of the contents of phenols between Robusta and Arabica beans were performed using StatViev

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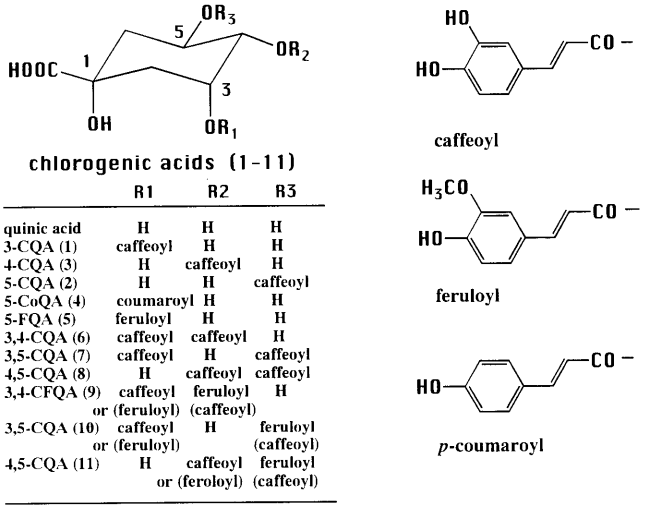
4.0 (Abacus Concepts, Inc., Berkley, CA).

Roasting of green coffee beans Cinnamon, medium, and city roast beans prepared from the same green beans (*C. canephora* var. *robusta* Indonesia or *C. arabica* Colombia) in the industry were used. The densities of the green beans, and cinnamon, medium and city roast beans were 1.33, 0.70, 0.68, and 0.63 g/cm^3 for Robusta beans and 1.16, 0.71, 0.60, and 0.58 g/cm^3 for Arabica beans, respectively. In addition to these beans, 50 g of green coffee beans (*C. canephora* var. *robusta* Indonesia) was roasted in a Muffle furnace (FP31, Yamato Kagaku, Tokyo) in our laboratory at 200 or 220°C for 30 min. The densities of the roasted beans after 5, 10, 15, 20, and 30 min at 200°C were 1.09, 0.94, 0.91, 0.84, and 0.79 g/cm^3, respectively, and those after 5, 10, 15, 20, and 30 min at 220°C were 0.98, 0.92, 0.80, 0.67, and 0.61 g/cm^3, respectively.

Identification of hydroxycinnamic acids Thirteen peaks detected on this HPLC at 320 nm were identified as described in the previous paper (Murata *et al.*, 1995). Peaks **1**-**13** on HPLC (Fig. 3) were identified as 3-CQA (**1**), 5-CQA (**2**), 4-CQA (**3**), *p*-coumaroyl-quinic acid (**4**), 5-feruloylquinic acid (5-FQA, **5**), 3,4-diCQA (**6**), 3,5-diCQA (**7**), 4,5-diCQA (**8**), 3,4-CFQA (**9**), 3,5-CFQA (**10**), 4,5-CFQA (**11**), CaT (**12**), and CoT (**13**).

Results and Discussion

Differences between Arabica and Robusta green coffee beans in hydroxycinnamic acids and CoT Thirteen peaks were detected in Robusta beans and eight or nine peaks in Arabica beans on the same scale. Figure 3 shows typical chromatograms of Robusta and Arabica beans. Each peak was identified according to the previous paper (Murata *et al.*, 1995). The contents of total hydroxycinnamic acid derivatives calculated as the sum of each phenol were about 8-9 g/100 g (%) in six Robusta and 5-6% in eight Arabica beans (Table



	R1	R2	R3
quinic acid	H	H	H
3-CQA (1)	caffeoyl	H	H
4-CQA (3)	H	caffeoyl	H
5-CQA (2)	H	H	caffeoyl
5-CoQA (4)	coumaroyl	H	H
5-FQA (5)	feruloyl	H	H
3,4-CQA (6)	caffeoyl	caffeoyl	H
3,5-CQA (7)	caffeoyl	H	caffeoyl
4,5-CQA (8)	H	caffeoyl	caffeoyl
3,4-CFQA (9)	caffeoyl or (feruloyl)	feruloyl (caffeoyl)	H
3,5-CQA (10)	caffeoyl or (feruloyl)	H	feruloyl (caffeoyl)
4,5-CQA (11)	H	caffeoyl or (feroloyl)	feruloyl (caffeoyl)

Fig. 1. Structures of chlorogenic acids.

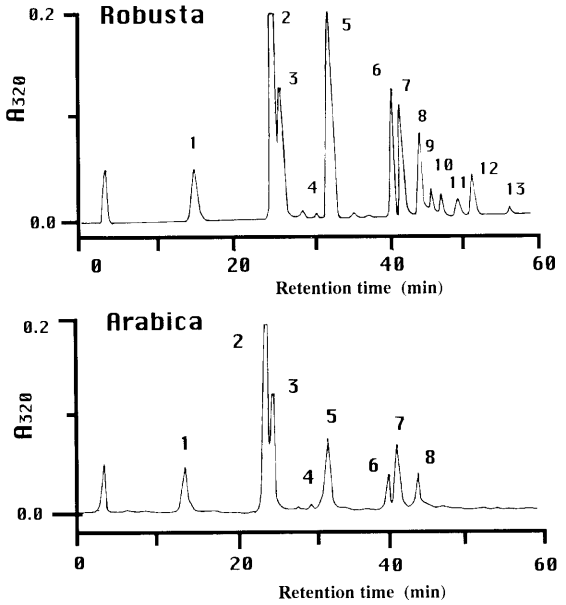


Fig. 3. HPLC of hydroxycinnamic acid derivatives in green coffee beans (*Coffea canephora* var. *robusta* and *C. arabica*).

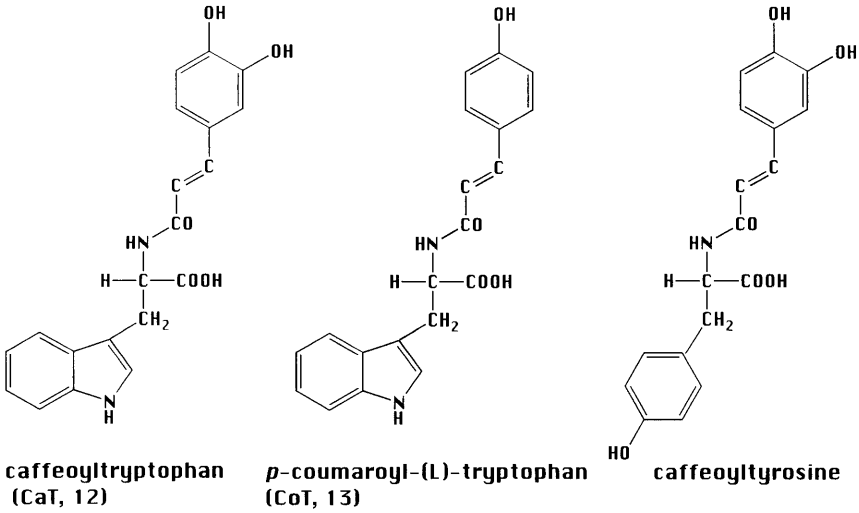


Fig. 2. Structures of hydroxycinnamoylamino acids.

Table 1. Content of hydroxycinnamic acid derivatives in green coffee beans (*Coffea canephora* var. *robusta* (Robusta) and *C. arabica* (Arabica)).

	CQAs*	5-CoQA	5-FQA*	diCQAs*	CFQAs*	CaT*	CoT*	Total phenols*
Robusta (n=6)	5.64±1.23	0.023±0.015	1.14±0.30	1.60±0.19	0.36±0.14	0.22±0.06	0.033±0.010	8.78±1.78
Arabica (n=8)	4.28±0.40	0.025±0.005	0.34±0.05	0.66±0.05	trace	trace	n.d.	5.30±0.45

The values show means±standard variations (g/100 g dry weight).
* indicates that Robusta beans contain it significantly more than Arabica beans ($p<0.01$).
trace, 2-6 mg%; n.d. not detected.

Table 2. Content of CQAs and diCQAs in green coffee beans (*Coffea canephora* var. *robusta* (Robusta) and *C. arabica* (Arabica)).

	3-CQA*	4-CQA**	5-CQA*	CQAs**	3,4-diCQA**	4,5-diCQA**	3,5-CQA**	diCQAs**
Robusta	0.52±0.20	0.69±0.27	4.44±0.90	5.64±1.23	0.51±0.08	0.54±0.11	0.55±0.10	1.60±0.19
(n=6)	(9.32)	(12.41)	(78.26)	(100)	(32.26)	(33.64)	(34.10)	(100)
Arabica	0.36±0.06	0.29±0.05	3.63±0.34	4.28±0.40	0.16±0.02	0.22±0.04	0.28±0.04	0.66±0.05
(n=8)	(8.31)	(6.83)	(84.85)	(100)	(24.39)	(32.69)	(42.92)	(100)

The values show means±standard variations (g/100 g dry weight).
n and values in parentheses show sample numbers and % against total CQAs or total diCQAs, respectively.
* and ** indicate that Robusta beans contain these compounds significantly more than Arabica beans ($p<0.05$ and $p<0.01$), respectively.

1). Robusta and Arabica beans contained 5.6±1.2% and 4.3±0.4% of CQAs, respectively. Robusta beans contained slightly more CQAs than did Arabica beans. CQAs occupied about 60-80% of the total phenols. In CQAs, 5-CQA was major, occupying about 80% of the total CQAs (Table 2). The ratio of 4-CQA in CQAs was larger in Robusta beans (12.4±4.2% of total CQAs) than in Arabica beans (6.83±0.68%). On the other hand, Robusta beans contained 2-3 times as much diCQAs as did Arabica beans (Table 1). Robusta and Arabica beans contained 1.60±0.19% and 0.66±0.05% of diCQAs, respectively. This result corresponded with that of Clifford *et al.* (1989c), showing that there was no difference in total chlorogenic acids between Arabica and Robusta beans but that Robusta beans contained 2-3 times as much diCQAs as did Arabica beans. In Robusta beans, each diCQA occupied about 33% of the total diCQAs, while it seemed that 3,5-diCQA was the highest among the diCQAs (42.92±5.66% of total diCQAs) in Arabica beans and 3,4-diCQA was the least (24.39±1.56%).

Although Robusta beans contained about 0.36% of CFQAs and 0.22% of CaT, Arabica beans contained little (Table 1). Trace amounts (2-6 mg%) of them were detected in Arabica beans. Caffeoyltyrosine (Clifford *et al.*, 1989b) was not detected in this study. CoT, found recently in a Robusta bean by us (Murata *et al.*, 1995), was detected in all the Robusta beans (0.033±0.010%) but not in any Arabica beans examined here. These results suggest that CoT can be used as an indicator for Robusta beans.

Decrease in hydroxycinnamic acids by roasting Chlorogenic acids are known to decrease on roasting, and part of them is polymerized to form brown pigments. Here the change in each hydroxycinnamic acid in Robusta and Arabica green coffee beans was examined. Figure 4 shows the content of hydroxycinnamic acid derivatives in a Robusta green coffee bean and three kinds of roasted ones (cinnamon, medium, and city roast, which were prepared in a factory). City roast was roasted most intensely and cinnamon roast was done the least. City roast contained the lowest amounts of all the hydroxycinnamic acid derivatives. The degree of decrease

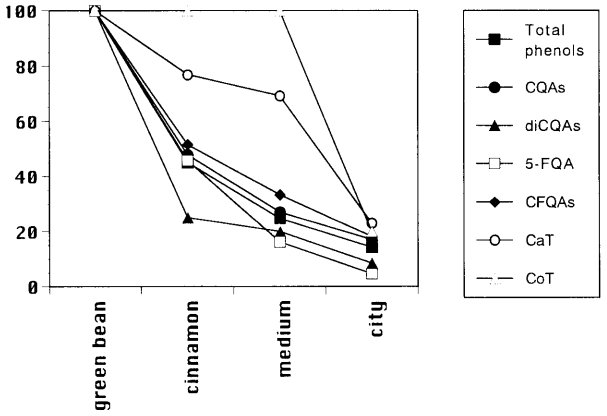


Fig. 4. Contents of hydroxycinnamic acid derivatives in Robusta (*Coffea canephora* var. *robusta*) green and cinnamon, medium, and city roast beans.

in each hydroxycinnamic acid derivative was variable. About 80% of the phenols were lost in city roast beans. CoT decreased the most slowly among all the phenols in coffee beans during roasting. There was no definite difference in the pattern of decreasing phenols between Robusta and Arabica beans (data are not shown).

Next, the effects of roasting temperature and time on the decrease in phenols were examined on a laboratory scale. Figure 5 shows that all of the hydroxycinnamic acid derivatives decreased more rapidly as the roasting temperature became higher and that they decreased more as the roasting time became longer. Trugo and Macrae (1984a) followed the degradation of seven chlorogenic acids such as CQAs, diCQAs and 5-FQA and showed that all chlorogenic acids decreased during roasting, although some differences in degradation rates of the isomers were observed. Our results closely corresponded to theirs. Here we point out that CoT decreased the most slowly among all the phenols in coffee beans during roasting.

Distribution of hydroxycinnamic acid derivatives in instant coffee Because CoT seemed to become an indicator

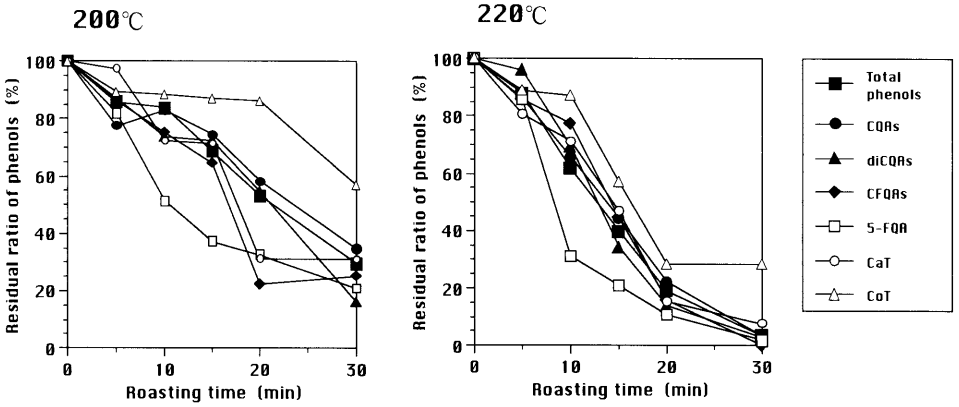


Fig. 5. Decrease in hydroxycinnamic acids in green coffee beans (*Coffea canephora* var. *robusta*) on roasting at 200 and 220°C.

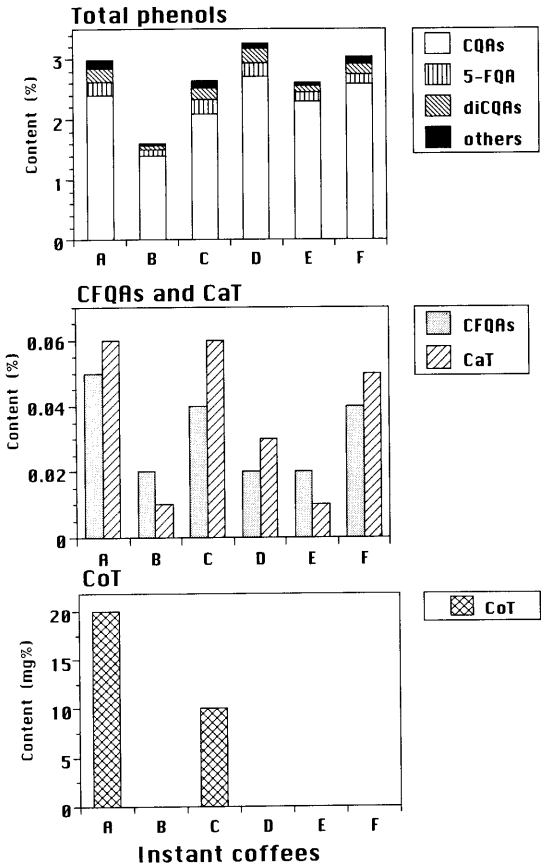


Fig. 6. Contents of hydroxycinnamic acid derivatives in instant coffees.

of coffee made of Robusta beans, CoT and other hydroxycinnamic acid derivatives in several instant (soluble) coffees on the market were examined. There was no definite difference in the total hydroxycinnamic acids (2.69±0.58%). Trugo and Macrae (1984b) reported that the total amount of chlorogenic acids was 3.6–10.7% and that 5-CQA accounted for about 30% of the total chlorogenic acids using 13 commercial instant coffees. The ratio of 5-CQA (32.3% of total phenols) was similar to their results, although our total was less than theirs. Only two brands of instant coffee were found to contain CoT (Fig. 6), which were considered to be partly made of Robusta beans according to their labels, while diCQAs, CFQAs, and CaT were detected in all the instant coffees examined. These results suggest that CoT becomes an indicator of Robusta beans in instant coffees.

In conclusion, CoT was detected only in Robusta green and roasted coffee beans and in instant coffees made of Robusta beans.

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References

Asakura, T., Nakamura, Y., Inoue, N., Murata, M. and Homma, S. (1990). Characterization of zinc chelating compounds in instant coffee. *Agric. Biol. Chem.*, **54**, 855–862.

Clifford, M.N. (1985). Chlorogenic acids. In "Coffee, Vol. 1: Chemistry," ed. by R. J. Clarke & R. Macrae. Elsevier Applied Science Publishers, London and New York, pp. 153–202.

Clifford, M.N. (1986). Coffee bean dicaffeoylquinic acids. *Phytochemistry*, **25**, 1767–1769.

Clifford, M.N. and Wright, J. (1976). The measurement of feruloylquinic acids and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans. *J. Sci. Food Agric.*, **27**, 37–84.

Clifford, M.N., Kellard, B. and Birch, G.G. (1989a). Characterization of chlorogenic acids by simultaneous isomerization and transesterification with tetramethylammonium hydroxide. *Food Chem.*, **34**, 81–88.

Clifford, M.N., Kellard, B. and Ah-Sing, E. (1989b). Caffeoyltyrosine from green robusta coffee beans. *Phytochemistry*, **28**, 1989–1990.

Clifford, M.N., Williams, T. and Bridson, D. (1989c). Chlorogenic acids and caffeine as possible taxonomic criteria in *Coffea* and *Psilanthus*. *Phytochemistry*, **28**, 829–838.

Iwashita, H., Morishita, H., Osawa, N. and Kido, R. (1985). 3-O-Feruloyl-4-O-caffeoylquinic acid from coffee beans. *Phytochemistry*, **24**, 630–632.

Morishita, H., Takai, Y., Yamada, H., Fukuda, F., Sawada, M., Iwashita, H. and Kido, R. (1987). Caffeoyltryptophan from green robusta coffee beans. *Phytochemistry*, **26**, 1195–1196.

Murata, M., Okada, H. and Homma, S. (1995). Hydroxycinnamic acid derivatives and *p*-coumaroyl-(L)-tryptophan, a novel hydroxycinnamic acid derivative, from coffee beans. *Biosci. Biotech. Biochem.*, **59**, 1887–1890.

Naish, M., Clifford, M.N. and Birch, G.G. (1993). Sensory astringency of 5-O-caffeoylquinic acid, tannic acid and grape-seed tannin by a time-intensity procedure. *J. Sci. Food Agric.*, **61**, 57–64.

Nakabayashi, T. and Mano, S. (1975). Chemical studies on the quality

of coffee. III. Qualitative and quantitative changes of chlorogenic acids in the course of roast. *Shokuhin Kogyo Gakkaishi*, **22**, 549–553 (in Japanese).

Nakabayashi, T. and Watanabe, C. (1975). Chemical studies on the quality of coffee. IV. Formation of brown pigments from chlorogenic acid by roast. *Shokuhin Kogyo Gakkaishi*, **24**, 124–129 (in Japanese).

Nakamura-Takada, Y., Shata, H., Minao, M., Ogawa, H., Sekiguchi, N., Murata, M. and Homma, S. (1994). Isolation of a zinc-chelating compound from instant coffee by the tetramethyl murexide method. *Food Sci. Technol.*, **27**, 115–118.

Sekiguchi, N., Yata, M., Murata, M. and Homma, S. (1994). Identification of iron-binding compound in instant coffee. *Nippon Nôgeikagaku Kaishi*, **68**, 821–827 (in Japanese).

Smith, A.W. (1985). Composition. In "Coffee, Vol. 1: Chemistry," ed. by R.J. Clarke & R. Macrae. Elsevier Applied Science Publishers, London and New York, pp. 32–34.

Trugo, L.C. and Macrae, R. (1984a). A study of the effect of roasting on the chlorogenic acid composition of coffee using HPLC. *Food Chem.*, **15**, 219–227.

Trugo, L.C. and Macrae, R. (1984b). Chlorogenic acid composition of instant coffees. *Analyst*, **109**, 263–266.