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Chemical composition of defective coffee beans

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Abstract

Immature-black beans (VP) and immature beans (V), known by the Brazilian coffee terminology as 'café verde-preto' and 'café verde', are defective beans and cause a reduction of the quality of the beverage. Their presence in raw coffee samples is due to a high percentage of immature fruits at harvest. In this study, some of their physico-chemical properties and chemical composition were investigated and compared with non-defective coffee beans (B). B beans were heavier and had higher humidity than VP and V. Although they did not differ with respect to pH, V beans were more acidic (titrable acidity). Sucrose was the main soluble carbohydrate in all samples and its content in B beans was higher than defective beans. Reducing sugars were found at higher concentration in V and B beans. Protein contents increased from VP to B (VP < V < B); however, there was no relationship with free amino acid contents, which were higher in V beans. Qualitative analysis showed that asparagine was the main amino acid in these beans. Denaturing electrophorectic (SDS-PAGE) profiles of proteins did not show qualitative differences among the three coffee types. The contents of 5-caffeoylquinic acid (5CQA) and soluble phenols were also higher in V beans, and their ratio increased from VP to B (VP < V < B). In this order, the reversed-phase high-performance liquid chromatography profile at 280 nm of methanolic extracts prepared similarly showed, in general, a reduction of detected peak areas. Polyphenol oxidase activity was inversely associated with 5CQA contents, V beans showing the lowest activity. Similar caffeine contents were observed in the samples. Total oil content was higher in B beans. In addition, the defective beans were individually mixed with B beans in different proportions and analysed for some constituents. The results showed that, depending on the substance analysed, its content in the B beans is significantly affected. Although the influence of each component of the raw coffee bean that determines beverage quality has never been established, our results show that the presence of defective beans can drastically change the chemical composition of the final product.

1. Introduction

In Brazil, coffee blossoms at the beginning of the rainy season (September/October) and at least three or four blossoms occur afterwards. After 8 to 12 days from the first rain, the first flowering occurs, which accounts for most of the fruits produced on the bushes (Gouveia, 1984). However, depending on the severity of the dry season, this may vary. Gouveia, 1984 followed coffee flowering for varieties of *Coffea arabica* during 3 years and observed that 47.1 to 83.1% of the flowers produced on the branches blossomed at the first flowering. Although the majority of the fruits are ripe at harvest, there will always be a significant proportion of immature and overripe fruits. Since most of the coffee in Brazil is harvested by strip-picking, all kinds of fruits will be processed together when dry processing is used.

If dry processing is adopted, beans from immature fruits will have a different aspect. In these seeds the silver skin is tightly bound to the endosperm and confers a green colour. The Brazilian terminology for them is 'café verde', or immature (quaker) bean in English. For commercial purposes they are considered defects, a term used to describe the quality factor of the amounts of defective beans or extraneous matter in a coffee sample (Clarke, 1987).

In Brazilian coffee terminology there are two types of black beans. The first are those beans that fall naturally on the ground by action of the rain or over-ripening. They can also fall during the harvest, remaining in contact with the soil and favouring microbial fermentation (Sampaio, 1967). The second are referred to as immature beans. However, these are at an earlier stage of maturation than those denominated 'café verde'

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(Sampaio, 1967). Alternatively, Clarke, 1987 indicated that black beans are mainly derived from dead beans within the cherry on the tree. These immature black beans can be differentiated from ground-fermented black beans by the shining and adherent silver skin on the bean surface. In Brazil they are referred to as 'café verde-preto' (immature-black beans).

Immature-black beans (VP) and immature beans (V) are considered serious defects because they affect the beverage quality. The first, for example, confers heavy flavour to the coffee beverage (Clarke, 1987). Garruti and Gomes (1961) showed that, contrary to mature fruits, which produced a soft beverage, seeds from immature fruits (green in colour) produced a hard beverage.

Since coffee in Brazil is manually harvested, harvesting represents the greatest cost of managing this crop. Therefore, if a coffee farmer decides to wait for the full ripening of the fruits, which is desirable for coffee quality, many labourers have to be hired at the same time, concentrating a significant fraction of the costs of the season in a single period of the year. Therefore, because of this economic problem, several coffee farmers start the harvest with a high percentage of unripe fruits on the bushes. Consequently, it is not rare to find, in the Brazilian market, samples of coffee containing even 50% of V/VP.

Gialluly (1959) defined coffee quality as the overall characteristics of intrinsic components of the bean, which determines the degree of acceptability of the product within a given scale of comparison established by the consumer market. In this respect, flavour and taste are caused by a complex mixture of compounds, several formed after roasting. However, the influence of each component of the raw coffee bean determining the beverage quality has never been established and only a few studies have been carried out.

Ohiokpehai, Brumen, and Clifford (1987), Clifford and Kazi (1987) and Clifford, Kazi, and Crawford (1987) demonstrated a relationship between chemical composition of immature cherries and chlorogenic acid, suggesting that they might affect coffee beverage, conferring astringency. Ohiokpehai et al. (1982) showed that addition of dicaffeoylquinic acids conferred a disagreeable flavour to coffee beverage which disappeared on subsequent addition of monocaffeoylquinic acid. Menezes (1994) showed that the inclusion of beans from partially ripe fruits negatively affects coffee beverage flavour due to their lower ratios of monocaffeoylquinic acid to dicaffeoylquinic acids. Chagas (1994) observed that good beverage was associated with coffee beans with high concentrations of reducing and non-reducing sugars.

The aim of this work was to obtain more information on the chemical composition of VP and V defective beans. Their contents of several compounds and enzyme activities were compared with those of normal non-defective (B) beans. The beverages of these samples were not assessed since their low quality is well known.

2. Materials and methods

2.1. Coffee samples

Raw coffee beans were obtained from Luma Comercial de Café e Cereais Ltda, Arapongas, Paraná State, Brazil. The sample used here came from a farm near Arapongas where the harvest was carried out when 60–70% of the fruits were at the berry stage. The fruits were dry-processed and the seeds were subjected to selection in an electronic sorter. V and VP were manually selected from the beans rejected by the sorting machine. The samples were powdered to a meal before analysis. For some analyses, different proportions of VP and V were mixed with B (60% VP/V + 40% B; 20% VP/V + 80% B; 5% VP/V + 95% B).

2.2. Analytical methods

The humidity of the beans was determined after 48 h at 105°C. The pH and titratable acidity of the coffee samples were obtained according to Angelucci, Arima, Mantovani, and Figueiredo (1982). Ground coffee (2.25 g) was mixed with 50 ml of hot (80°C) water, cooled to room temperature, and the pH determined. For the determination of acidity 10 g of ground coffee was mixed with 75 ml of 80% ethanol and maintained under gentle agitation for 16 h. 25 ml of this extract was diluted to 100 ml with distilled water and the titratable acidity determined with 0.1 N NaOH, using phenolphthalein as pH indicator.

Caffeine, 5-caffeoylquinic acid (5CQA), phenols, reducing sugars and sucrose were determined in 80% methanolic extracts. Extraction (100 mg per 5 ml 80% methanol) was carried in a boiling water-bath for 2 h with occasional agitation. After cooling at room temperature, the extracts were diluted with distilled water to 10% of methanol. Caffeine and 5CQA concentrations were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) (Mazzafera, 1997) and soluble phenols (Swain & Hillis, 1959), reducing sugars (Nelson, 1944) and sucrose (van Handel, 1968) were determined colorimetricaly. Pure standards of caffeine and 5CQA were used in the HPLC analysis for the determination of the contents in the seeds. Phenol (phenic acid), sucrose and glucose were used, respectively, as standards for colorimetric determinations of soluble phenols, sucrose and reducing sugars. Using the original methanolic extracts, qualitative analysis of soluble sugars was carried out by descending paper chromatography on Whatman 1MM, using n-butanol:pyridine:water (100:30:30, v/v/v) as solvent. The

spots were visualised by dipping the chromatograms in diphenylamine/aniline/phosphoric acid reagent and heating at 105°C (Chaplin, 1986). The original extracts were also diluted to 4% methanol and phenols analysed by RP-HPLC using UV detection at 280 nm (Banwart, Porter, Granato, & Hassett, 1985).

Amino acids (50 mg/3 ml) were extracted with methanol:chloroform:water (12:5:3, v/v/v) (Bielesk & Turner, 1966). After one week at 4°C, chloroform and distilled water were added to the extracts and the upper phase formed after 24 h at 4°C was recovered, diluted with distilled water and freeze-dried. Amino acids were dissolved in distilled water, filtered through 0.22 μm filters and stored at −20°C until analysis. Amino acids were determined quantitatively by colorimetry (Cocking & Yemm, 1954) and qualitatively analysed with fluorimetric detection of the *o*-phthaldialdehyde derivatives in HPLC (Jarret, Coosky, Ellis, & Anderson, 1986).

Total proteins were extracted by grinding each sample in a mortar with 0.1 N NaOH (100 mg/2×5 ml). The concentrations were determined using a ready-to-use reagent from Bio-Rad (Bradford, 1976). Bovine serum albumin was used as standard.

For the electrophoretic analysis, 2 g of each sample were defatted with hexane in a Soxhlet apparatus and the proteins were extracted from 100 mg in mortar with 5 ml of cold (4°C) 0.1 M Na-borate buffer, pH 8.0, containing 50 mM diethyldithiocarbamic acid, 50 mM ethylenediaminetetraacetic acid, 0.3 M NaCl and 2% ascorbic acid. Polyvinylpolypyrrolidone (100 mg) was also added to the extraction slurry. The extracts were centrifuged at 4°C and the protein concentration was determined in the recovered supernatants with the Bio-Rad reagent. The protein profiles of these extracts were obtained by discontinuous SDS-PAGE using 17% polyacrylamide in the main gel (Laemmeli, 1970). The proteins were stained with Coomassie Blue R250.

Total lipid content was determined using a Soxhlet apparatus with hexane (Folstar, 1985).

Polyphenol oxidase (PPO) activity was determined by extracting 500 mg of ground coffee with 15 ml of cold (4°C) 100 mM Na-phosphate buffer, pH 7.0. Polyvinylpolypyrrolidone (50 mg) was included in the extraction slurry. The extracts were centrifuged at 27 200 *g* at 4°C and the supernatants passed through Sephadex G25 PD-10 mini-columns (Pharmacia). Proteins were eluted with 10 mM Na-phosphate buffer, pH 7.0, and their concentrations determined with the Bio-Rad reagent. The reaction mixture contained 100 μg of protein, substrate (65 mM pyrogallol or 5 mM 5CQA) and the final volume (3 ml) was adjusted with 10 mM Na-phosphate buffer, pH 7.0. The reaction mixture was incubated for 30 min (pyrogallol) or 20 min (5CQA) at 37°C in the dark and the absorbance read at 420 nm. As blanks, protein was omitted from the assays. The activity was expressed as absorbance units mg^{-1} $protein^{-1}$ min^{-1}.

3. Results and discussion

The coffee sample used in this study was obtained by a dry processing method, and therefore, the characteristics are expected to represent the green coffee commonly found in the Brazilian coffee market.

Data on humidity and weight of the seeds (Table 1) show that, besides their effect on the quality of the beverage, the presence of VP and V also leads to a loss in weight. Although significant differences were not observed for pH, the lowest value observed with V beans was associated with the highest acidity. High titratable acidity was also found with VP beans. Data in the literature show that low coffee quality is not associated with pH but with high acidity. High acidity has been attributed to fruit fermentation during the dry process (Carvalho, Chalfoun, & Chagas, 1989; Chagas, 1994; Leite, 1991; Miya, Garruti, Angelucci, Figueiredo, & Shirose, 1973). Miya et al. (1973) have shown that the black bean, resulting from fermentation (Sampaio, 1967), was the main cause of the increase of acidity in coffee samples obtained from several Brazilian localities. Since VP defective beans represent coffee seeds collected in an inappropriate developmental stage of the fruits and, therefore, do not result from fermentation or deterioration due to bad storage conditions, it is not possible to compare our data with those of previous studies.

Fig. 1(A)–(C) shows that an increase of sugar content is associated with the developmental stage. VP beans seeds showed the lowest content of reducing sugars and sucrose and mixed with B seeds they caused a marked decrease of these compounds. With regard to V seeds, this is also true for sucrose but not for reducing sugars. It is noteworthy that the ratio between sucrose and reducing sugars increased from around 4 in the VP to almost 16 in the B beans. This might indicate that, during seed filling, most of the carbohydrate metabolism is directed to accumulation of storage polysaccharides in the coffee seed, such as glucomannans (Wolfrom, Plunbelt, & Laver, 1960). The higher humidity found in the B seeds (Table 1) might also be related to the higher sugar content.

Paper chromatography of methanolic extracts of the seeds showed the massive predominance of sucrose. Very faint bands corresponding to glucose could be

Table 1
Physico-chemical properties, caffeine and polyphenol oxidase activity of the VP, V and B coffee beans[a]

Coffee	Humidity (%)	Weight 100 seeds (g)	pH	Titratable acidity (ml NaOH g^{-1})
VP	8.8c	9.20c	5.98a	2.67b
V	9.4b	9.56b	5.85a	3.52a
B	11.4a	10.74a	5.91a	2.28b

[a] Means of three replicates. Different letters indicate significant differences at Tukey 5%.

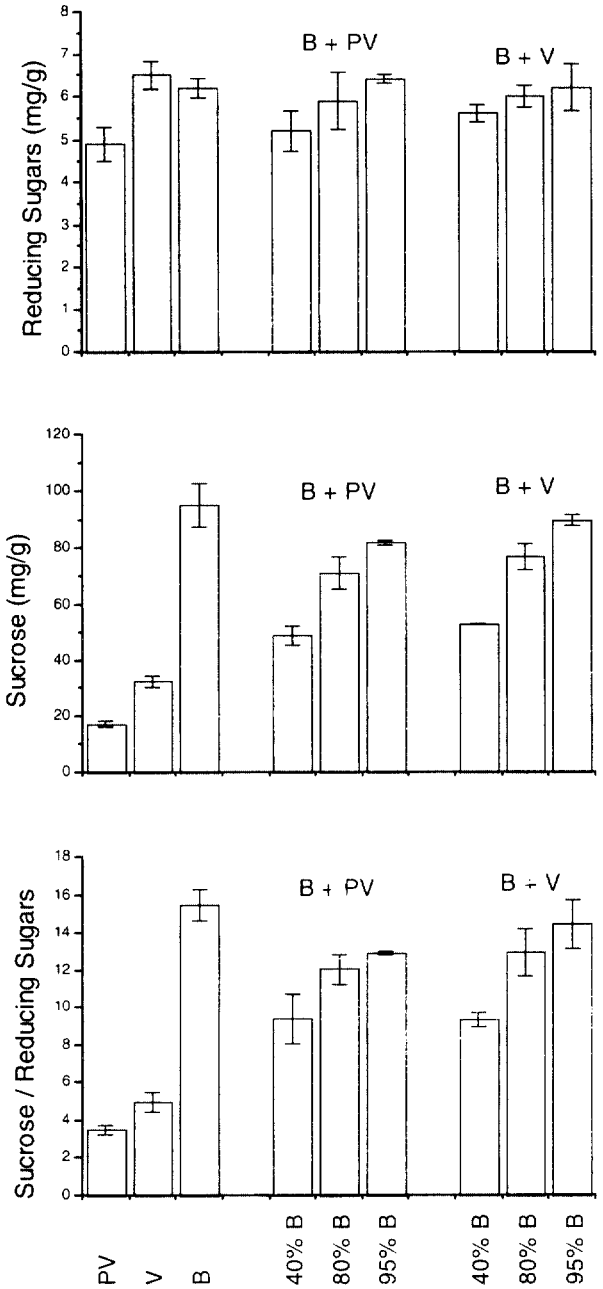


Fig. 1. (A) Reducing sugars and (B) sucrose contents, and (C) their ratio in VP, V and B beans.

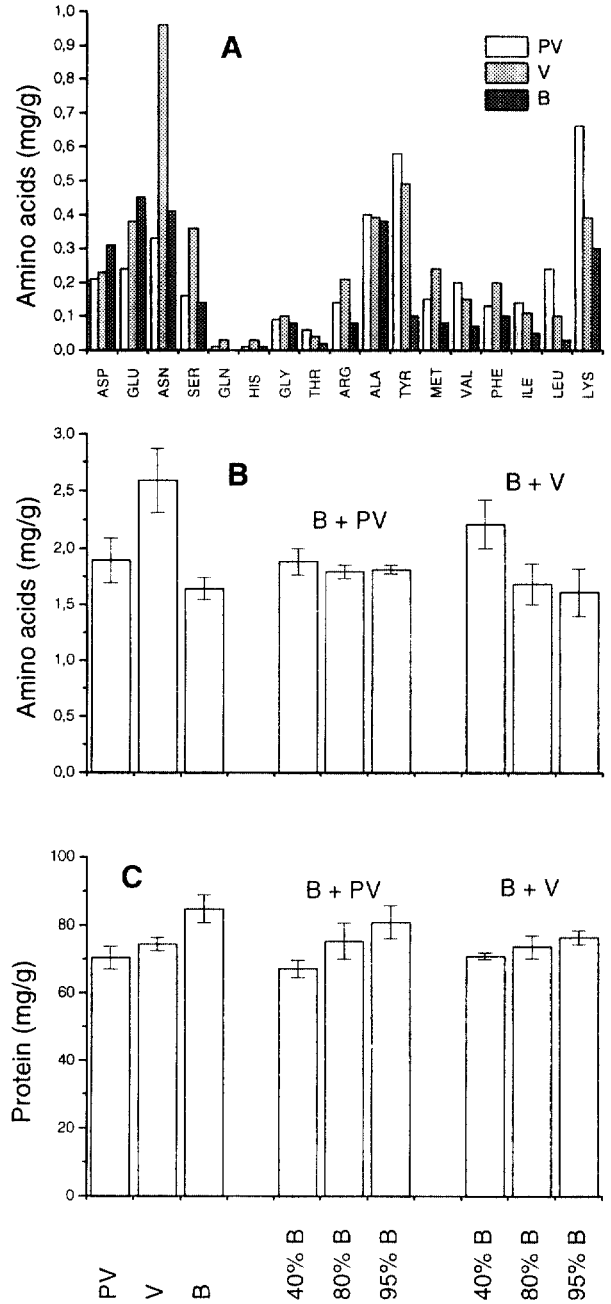


Fig. 2. (A) Amino acid composition, (B) soluble amino acids and (C) protein contents of VP, V and B beans.

observed only after overloading samples (data not shown).

Chagas (1994) found a positive association between coffee quality and content of reducing and non-reducing sugars in coffee beans from several Brazilian coffee regions. The highest values were found in coffee from the Triângulo Mineiro/Alto Paranaíba region, where the climatic conditions lead to a more gradual and uniform maturation. Coffees from the Zona da Mata region had the lowest scores for beverage quality and also the lowest values for reducing and non-reducing sugars.

Protein extracted with 0.1 N NaOH increased from VP to B (Fig. 2(C)). The low content in VP beans resulted in measurable changes when mixed with B. However, the protein content could not be correlated with amino acid content (Fig. 2(B)). V beans showed a higher amino acid content than the other two samples, which could be explained largely by the variation observed for asparagine (Fig. 2(A)). Serine, arginine, methionine and phenylalanine may also have contributed to this higher content. Although VP and B presented similar contents of total amino acids, qualitative changes were observed. Tyrosine and lysine were

outstanding in VP, while aspartic and glutamic acid, asparagine were the main amino acids found in the B seeds.

Only quantitative differences were observed in the protein SDS-PAGE profile of the three coffee samples (data not shown). Two main bands, as observed by Luthe (1992), represented the main proteins in the coffee seeds. It is noteworthy that the protein extracts obtained for SDS-PAGE had 22.5, 32.3 and 48.6 mg g^{-1} of proteins for VP, V and B, respectively. These data are lower than those for NaOH extraction but followed the same tendency.

The content of soluble phenols and 5CQA was higher in V seeds, although their proportions did not change significantly among the coffee samples (Fig. 3(A)–(C)). This higher content altered the contents of the mixed samples inversely. B + VP showed an increase of soluble phenols and 5CQA, corresponding to an increase of B in the mixture. On the other hand, the opposite occurred with V + B mixtures. However, the ratio between 5CQA and soluble phenols always increased with addition of B in the mixtures.

Clifford et al. (1987) and Clifford and Kazi (1987) followed the content of total caffeoylquinic acids during fruit development in different coffee species and found that, for arabica coffee, the weight of fruits was still increasing when the highest values of these compounds was reached. This occurred between 25 and 28 weeks after flowering. After this period, there was a decrease in total caffeoylquinic acids. They also observed a marked increase in the ratio between mono and di-isomers of caffeoylquinic acids, suggesting that this might be sufficient to influence the beverage ratio and, therefore, organoleptic properties. This was later confirmed by Menezes (1994) who determined the ratio between mono and di-caffeoylquinic acids in arabica coffee fruits collected at seven different stages of maturity. She showed that this increase was more pronounced in wet processed coffees. At the ripe stage (cherry red fruits), this ratio was about 3.5 in dry-processed coffee beans and near 5 in wet-processed beans. Since we did not determine the contents of the different chlorogenic acid isomers, it is not possible to compare our data with those of Clifford et al. (1987), Clifford and Kazi (1987) and Menezes (1994). However, V and B showed higher contents of 5CQA, and the ratio between this monoisomer and soluble phenols increased from VP to B seeds.

The same extracts of VP, V and B used for the determination of soluble phenols were analysed by HPLC using a reversed-phase system and UV detection at 280 nm. A representative chromatogram is shown in Fig. 4. Since the extraction was identical for the different coffee types, the relative peak areas obtained are comparable. Peak 3 co-chromatographed with caffeine. Except for peak 2, all others showed a reduction in area. These data suggest that the high content of soluble phenols in

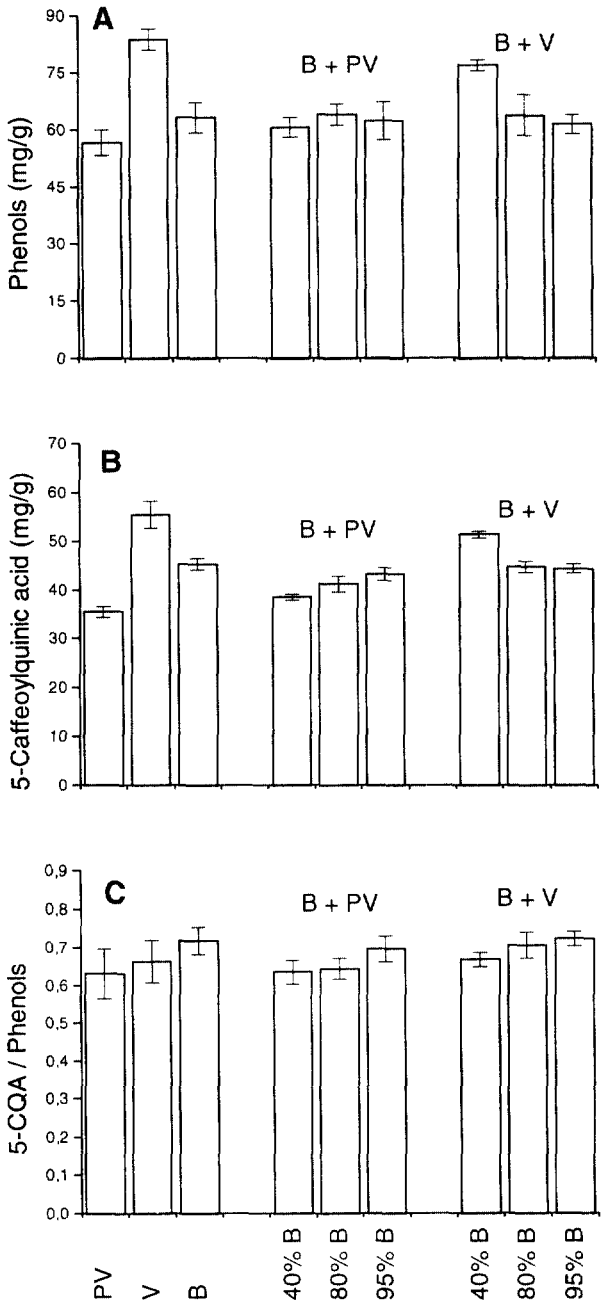
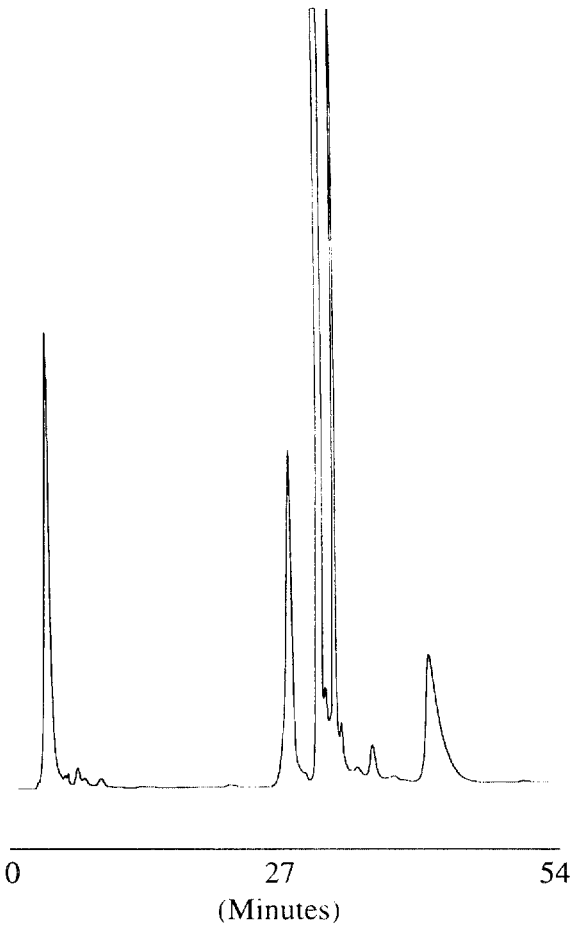


Fig. 3. (A) Soluble phenols and (B) 5CQA contents, and (C) their ratio in VP, V and B beans.

V seeds (Fig. 3(A)) may be directly correlated with the increase of 5CQA (Fig. 3(B)). However, a detailed analysis of chlorogenic acid isomers is needed to confirm this hypothesis.

Caffeine content decreased very little from VP to B, and the differences were not statistically different (Table 2). Higher caffeine contents in the endosperm of immature fruits (Mazzafera, Crozier, & Magalhães, 1991) or in the whole immature fruit (Suzuki & Waller, 1984) have been reported. Clifford et al. (1987) and Clifford and Kazi (1987) reported very small changes in caffeine during the development of the coffee fruit. In



Peak areas					
1	2	3	4	5	6
VP - 9,873	VP - 8,186	VP - 52,995	VP - 16,593	VP - 669	VP - 6,972
V - 7,525	V - 7,369	V - 53,138	V - 19.962	V - 526	V - 4,428
B - 5,846	B - 8,224	B - 49,914	B - 15,326	B - 475	B - 3,870

Fig. 4. Typical HPLC profile at 280 nm of a methanolic extract together with peak areas of the main peaks. Peak 3 co-chromatographed with caffeine.

Table 2
Total oil content, caffeine content and polyphenol oxidase activity of the VP, V and B coffee beans[a]

Coffee	Caffeine (mg g^{-1})	Polyphenol oxidase (Uabs mg $prot^{-1}$ min^{-1})		Oil content (%)
		pyrogallol	5CQA	
VP	13.6a	29.2a	109.8a	11.04b
V	13.5a	26.9b	65.2c	11.71b
B	12.6a	29.0a	80.4b	12.98a

[a] Means of three replicates. Different letters indicate significant differences at Tukey 5%.

addition to this, the minor contribution of caffeine to beverage bitterness led these authors to conclude that this alkaloid is not responsible for any change in beverage quality associated with fruit maturity.

Pyrogallol and 5CQA were used as substrates to determine PPO activity in the coffee samples (Table 2). Even at lower concentrations in the reaction mixture, activities of PPO were higher with 5CQA (5 mM) than pyrogallol (65 mM). However, in both cases V beans showed the lowest activity. It is noteworthy that PPO activities and 5CQA contents (Fig. 3(C)) are inversely related. This suggests that PPO might control the

content of this compound in the coffee seeds during their development.

Several reports in the literature showed a relationship between chlorogenic acid content and PPO activity (reviewed in Amorim & Amorim, 1977; Amorim & Melo, 1989). Good quality was associated with high activities. However, a study more closely related to ours was carried out by Arcila-Pulgarin and Valência-Aristizabal (1975). They collected fruits at different stages of maturation (from green to cherry) and observed that there was a reduction of PPO activity with maturation.

With respect to the oil content, B beans presented the highest value (Table 2). Although not a significant difference, V beans had a higher oil content than VP beans.

VP and V beans are the only defects directly related with development of coffee fruits. It is generally accepted that the presence of immature beans in commercial roasts causes a reduction of the quality of the beverage. Indeed, this was demonstrated by Garruti and Gomes (1961) many years ago. However, to our knowledge, investigations on the chemical composition of immature beans are not available in the literature. Although nowadays such defective beans can be eliminated by electronic sorting machines, mainly in those markets where higher quality is required, they are not eliminated in commercial roast in some countries. Therefore, our data clearly show that defective beans cause marked changes in the chemical composition of the green coffee and probably change the acceptability of the roast product.

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