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The relationship between the state of maturity of raw coffee beans and the isomers of caffeoylquinic acid

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A high level of statistical significance was found between the ratio % monocaffeoylquinic acid:% dicaffeoylquinic acid and the level of maturity in samples of *Coffea arabica* cv Catuai vermelho prepared by both the dry and wet processing methods. Results indicated that the inclusion of partially green berries could negatively affect beverage flavour due to their lower ratios. It was also shown that processing methods significantly affected the ratio, the wet method always presenting higher values than the dry method.

INTRODUCTION

It is well known that coffee beans from the species *Coffea arabica* vary in quality according to the area of the world in which they are produced. This difference in quality is attributed mainly to the climatic conditions prevailing in the production areas (Northmore, 1965; Amorim *et al.*, 1967; Amorim, 1970; Amorim *et al.*, 1973) resulting in differences in the methods of harvesting and processing.

In Brazil the harvesting season is considered to be clearly defined, occurring in the autumn, which is relatively dry, and thus strip-picking followed by dry processing is generally employed. However, in reality the harvesting season is not as clearly defined as is usually imagined and at any one time cherries at all stages of maturity can be found on most bushes. Thus, when harvested by strip-picking, although the majority of the cherries may be ripe or almost ripe, there is always a measurable proportion of underripe and overripe cherries present, which are usually all processed together. It is supposed that this is one of the reasons for the lower quality of Brazilian arabica coffee.

Previous studies (Ohiokpehai *et al.*, 1982) have indicated that the molar proportions of caffeoylquinic acid (CQA) and dicaffeoylquinic acid (diCQA) vary during maturation, with an initial decrease followed by a significant increase at the end of the maturation process. Using the triangular test, these same studies showed that the addition of diCQA conferred a disagreeable flavour to the coffee beverage and that the subsequent addition of CQA could mask the disagreeable flavour. Thus it appears that the quantitative relation between CQA and diCQA could well be one of the factors determining the quality of coffee beans.

The object of this study was to determine variations in the levels of the different isomers of caffeoylquinic acid in Brazilian-grown commercial green coffees during maturation. The cherries were strip-picked, separated into seven different maturation groups on the basis of colour and processed by both the dry and wet methods.

MATERIALS AND METHODS

Materials

The raw coffee used in this study was *Coffea arabica* cv Catuai vermelho supplied by the Instituto Brasileiro de Cafe, Brazil. All bushes were cultivated under the same conditions of soil, fertilizer, sunlight and irrigation. Bushes were strip-picked when the majority of the cherries were fully ripe and immediately classified into seven groups on the basis of colour (Table 1). Beans were harvested on six separate occasions during two consecutive harvests.

After classification, half of each sample was processed by the wet method and half by the dry method. Depulping was effected by hand and drying by the sun, to approximately 11% moisture.

5-Caffeoylquinic acid was obtained from Sigma Chemical Company Ltd, Poole, UK.

Methods

Extraction

Samples were ground to pass a 0.7 mm mesh and approximately 1.00 g samples were extracted by refluxing

Table 1. Cherry classification by colour

Classification	Colour
Unripe	Green/hard
Semi-ripe	Half green/half pink
Almost ripe	Light red
Ripe	Cherry red
Very ripe	Dark red
Slightly overripe	Almost black
Overripe	Black/wrinkled

in a Soxhlet apparatus for 4 h with boiling 70% isopropanol.After cooling, the extracts were made up to 200 ml with 70% isopropanol and filtered.

Determination of total chlorogenic acid

Extracts were analysed for total chlorogenic acid by the method of Clifford and Wight (1976), using the sodium metaperiodate reagent.

Chromatography

High performance chromatography was carried out using a Waters Associates (Milford, Massachusetts, USA) liquid chromatograph consisting of a model 440 absorbance detector operating at 313 nm and Data Module, using a 30 cm × 3.9 mm stainless-steel column containing Bondapak C_{18} (10 Qmm pore size) (Waters). A two-step isocratic system was used with an initial solvent of 27% methanol in 0.1 M potassium dihydrogen phosphate up to the completion of the elution of the monoisomers, followed by 30% methanol in 0.1 M potassium dihydrogen phosphate up to the elution of 3,5-diCQA. Solvents were degassed under vacuum and the flow rate was 1.5 ml/min. Peak area was determined using the Data module. An external standard of 5 CQA (Sigma Chemical Co. Ltd) was used for quantification, the remaining isomers being quantified by comparison of their peak areas with that of 5-CQA and the use of already calculated correction factors (Clifford *et al.*, 1985).

Moisture content

Samples were dried to constant weight at 105°C.

Statistical analysis

The data were analysed using a block model for the analysis of variance and two classification criteria. For each factor considered the average values for the ratio % CQA:% diCQA were compared using Duncan's test.

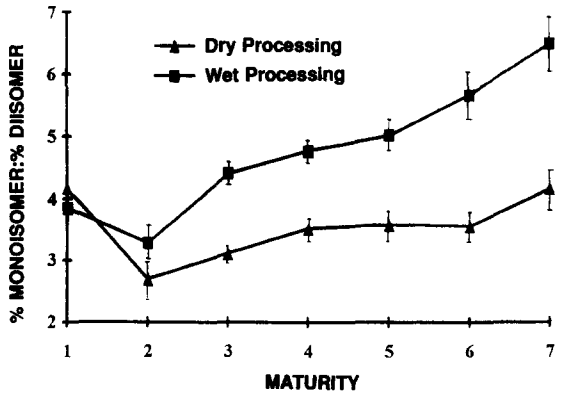

Fig. 1. Variation in the ratio % monoisomer:% diisomer with maturity.

RESULTS AND DISCUSSION

According to the results of the periodate test (data not shown)for total chlorogenic acid, little change in average values occurred during maturation (as judged by fruit colour) in beans processed by either the dry (up to 18% change)and wet (up to 22% change) methods There was, however, considerable variation between the data obtained for beans from cherries of a given maturity/colour but harvested on different days. It was thus concluded that no clear relation could be drawn between the state of maturity and the % total chlorogenic acid as measured by the periodate test.

With respect to the monoisomers of caffeoylquinic acid, the distribution between the three monoisomers was found to be essentially constant in all samples, being approximately 75% 5-CQA, 16% 4-CQA and 9% 3-CQA, similar to values reported previously (Clifford, 1985). However, no defined sequence could be determined either for the range in content of individual monoisomers with maturity or for the sum of the three monoisomers with maturity, and once again there was considerable variation in the values for individual days.

Only two of the three diisomers of caffeoylquinic acid were quantified by HPLC (3,5-diCQA and 3,4-diCQA), since 4,5-diCQA did not elute in the 2-step isocratic system used. Although 3,5-diCQA was nearly always dominant, there were considerable differences between the proportions of each in the different samples, and no correlation was discernible between this variation and maturity or method of procesing. Similarly, changes in the sum of the two diisomers could not be clearly related to maturity, although there was a general tendency to decrease in value up to maturity and then increase. Once again, considerable differences were encountered between different harvesting dates.

Table 2. Statistical analysis of the data for the ratio % monoisomer: % diisomer

Source of variation	Degree of freedom	Sum of squares	Mean square	*F* value	Probability >*F*
Maturity	6	78·994 6	13·165 77	105·72	0·000 1
Procesing method	1	54·438 2	54·438 17	437·15	0·000 1
Harvesting day	5	0·1241 3	0·024 826	0·200 0	0·962 3
Maturity × processing method	6	21·334 3	3·555 716	28·550	0·000 1

Table 3. Duncan's test comparing average values for % monoisomer: % diisomer with the level of maturation

Level of maturation	Average values monoisomer: diisomer	Decision ($p \leq 0.05$)
7 Overripe	5·550	A
6 Slightly overripe	4·540	B
5 Very ripe	4·180	C
4 Ripe	4·050	D
1 Unripe	4·012	D
3 Almost ripe	3·756	E
2 Semi-ripe	2·978	F

Table 4. Duncan's test comparing average values for % monoisomer: % diisomer with the processing method

Processing method ($p \leq 0.05$)	Average monoisomer: diisomer	Decision
2 Wet	4·726	A
1 Dry	3·539	B

Despite the lack of correlation between the contents of mono- and diisomers of caffeoylquinic acid and maturity, a positive relation was found between the ratio % monoisomer:% diisomer and maturity, % monoisomer being the sum of 3-CQA+4-CQA+5-CQA and % diisomer the sum of 3,4-diCQA+3,5-diCQA. Figure 1 shows the change in this ratio with maturity for both methods of processing. Little variation in this ratio was encountered between beans from cherries of a given maturity/colour but harvested on different days (average variation 23%).

Table 2 shows the statistical analysis of these data (ANOVA). From an analysis of these data it can be affirmed that there was a highly significant relationship between the ratio % monoisomer:% diisomer and the levels of maturity, that the processing method had a significant influence on the ratio % monoisomer:% diisomer and that the interaction between maturity level and processing method was highly significant.

Due to the high level of significance, the average values of the ratio % monoisomer:% diisomer were compared with each factor considered using Duncan's test. Table 3 shows the results of this test for the maturation level and Table 4 for the processing method.

The statistical data show that the overripe beans presented the highest value for the ratio % monoisomer:% diisomer followed by, in decreasing order, slightly overripe and very ripe, then ripe and unripe with no significant difference between them and finally almost ripe and semi-ripe. The two extremes, overripe (highest) and semi-ripe (lowest) gave values well separated from the adjacent stage of maturity on the scale (slightly overripe and almost ripe respectively).

Duncan's test also showed a significant difference between the two processing methods, wet processing always producing a higher ratio than dry processing.

The data show that the highest ratios were reached in the completely overripe and completely green beans, beans which would obviously be removed anyway due to both their chemical composition and physical nature, which impede processing. Of those beans usually present in strip-picked samples (semi-ripe to slightly overripe) it can clearly be seen that it is the semi-ripe beans that affect the quality the most negatively, it being assumed that the lower the ratio the worse the flavour. Thus it can be concluded that, independent of the processing method, the quality of the coffee can be improved by removal of all beans with any percentage of green colour, a process which is relatively easy both manually and electronically. The fact that wet processing gave consistently higher ratios implies that it would be advantageous to wet-process strip-picked cherries after removal of the partially green ones.

It is interesting to note the tremendous variation in individual values (both for total CQA and individual isomers) on different harvesting days and especially between different years, and it can only be assumed that this was caused by variations in the climatic conditions. The ratio between mono- and diisomers was the only item studied that gave a significant relationship with maturity, independent of harvesting day.

It is not possible to compare previous data published on the relationship between maturity and chlorogenic acid (Ohiokpehai *et al.*, 1982; Clifford *et al.*, 1987) with the present data since, quite apart from varietal differences, the cherries used in the previous studies had been cultivated in greenhouses and frozen in liquid nitrogen immediately after harvesting. In addition, maturation was measured in terms of weeks after flowering. In this study, the objective was to study beans as they are available on the market, and thus samples were dried and processed according to standard commercial practices, thus preventing a comparison of the data obtained with those from specially treated beans.

REFERENCES

Amorim, H. V. (1970). Nutritional status of the coffee plant and beverage quality. *Indian coffee*, **34**(12), 331–5.

Amorim, H. V., Scoton, L. C., Castilho, A., Pimentel-Gomes, F. & Malavolta, G. (1967). Estudo sobre a alimentaçao mineral do cafeeiro XXI. Efeito de adubaçao N,P,K e organica na composiçao mineral do grao e na qualidade da bebida (2a nota). *Anais da E.S.A., 'Luiz de Queiroz' USP*, **24**, 215–28.

Amorim, H. V., Teixeira, A. A., Morais, R. S., Reis, A. J., Pimentel-Gomes, F. & Malavolta, E. (1973). Estudos sobre a alimentacao mineral do cafeeiro, XXVII. Efeito da adubaçao N,P,K no teor de macro e micronutrients do fruto e na qualidade da bebida. *Anais da E.S.A. 'Luiz de Queiroz' USP*, **30**, 323–33.

Clifford, M. N. (1985). Chemical and physical aspects of green coffee and coffee products. In *Coffee, Botany, Biochemistry and Production of Beans and Beverage*, ed. M. N. Clifford & K. C. Willson. Croom Helm, London, pp.331.

Clifford, M. N. & Wight, 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**, 73–84.

Clifford, M. N., Ohiokpehai, O. & Menezes, H. C. (1985).

The influence of extraction method on the chlorogenic acid content of green coffee beans. In *11th Int. Scientific Colloquium on Coffee*, Lome. ASIC, Paris, pp.252–62.

Clifford, M. N., Kazi, T. & Crawford, S. (1987). The content and washout kinetics of chlorogenic acid in normal and abnormal green coffee beans. In *12th Int. Scientific Colloquium on Coffee*, Montreux. ASIC, Paris, pp.221–8.

Northmore, J. M. (1965). Some factors affecting the quality of Kenya coffee. *Turrialba*, **15**(3), 184–93.

Ohiokpehai, O., Brumen, G. & Clifford, M. N. (1982). The chlorogenic acids content of some peculiar green coffee beans and the implications for coffee beverage quality. In *10th Int. Scientific Colloquium on Coffee*, Salvador. ASIC, Paris, pp.177–86.