# Characterization of Green and Roasted Coffees through the Chlorogenic Acid Fraction by HPLC-UV and Principal Component Analysis

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Chlorogenic acids (CGA) are a group of phenolic acid derivatives which are commonly found and differently distributed in higher plants. The CGA pattern can successfully be employed to characterize green and roasted coffees of different origins and qualities. This paper reports on the possibilities of characterizing green or roasted coffees by the CGA fraction through HPLC-UV and principal component analysis (PCA) and *comparing* the results with those from sensory evaluation. The CGA fraction was prepared by submitting the Melitta extracts of green and roasted coffees to cleanup on a $C_{18}$ cartridge, and the 40/60 methanol/water fraction was then analyzed by a RP-$C_{18}$ column at 325 nm using a methanol/citrate–hydrochloric acid mobile phase in gradient elution. Examples concern the discrimination of coffees of the same quality but from different origins or plantations, discrimination of coffee blends of different compositions, and comparison with sensory evaluation.

## INTRODUCTION

Chlorogenic acids (CGAs) are a group of compounds that derive from the esterification of one or more residues of phenolic acid (mainly caffeic, ferulic, or *p*-coumaric acid) with quinic acid and which are commonly found and differently distributed in higher plants (Figure 1).

CGAs constitute an important fraction in coffee and can successfully be used to characterize green and roasted coffees of different origins and qualities. CGAs have been used in conjunction with caffeine to characterize commercial and noncommercial species of coffee and also to guard against possible fraud (Clifford, 1986; Clifford and Jarvis, 1988; Clifford et al., 1989) and to measure the degree of roasting and its effect on coffee (Feldman et al., 1969; Trugo and Macrae, 1984a; Purdon and McCamey, 1987). Roasting processes and conditions strongly influence the qualitative and quantitative compositions of the CGA fraction. Trugo and Macrae (1984b), among others, investigated in depth the CGA fraction of Robusta and Arabica coffees before roasting and after submission to light, medium, strong, and very strong roasting processes by evaluating the variation in the percentages of seven CGAs.

Several authors have suggested a relationship between the composition of the CGA fraction and the quality of the beverage, although results have not been conclusive. Among others, Tressl (1977) reported a more direct link between CGA fraction and beverage quality, through the influence on beverage aroma of organoleptically significant CGA degradation products; Ohiokpehai et al. (1982), Clifford and Ohiokpehai (1983), and Naish et al. (1993) investigated the astrin-
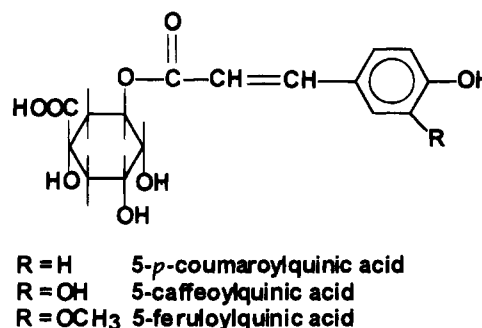


R = H          5-*p*-coumaroylquinic acid
R = OH        5-caffeoylquinic acid
R = OCH₃    5-feruloylquinic acid

**Figure 1.** General formulas of chlorogenic acids.

gency of dicaffeoylquinic acid (DCQA) in depth, and Ohiokpehai et al. (1982) reported that the caffeoylquinic acid(CQA)/DCQA molar ratio of green coffee beans may influence the beverage ultimately obtained. Moreover, Nagel et al. (1987) demonstrated that CQA is not bitter when its acid character is masked.

A previous study investigated the possibilities of using the static headspace GC (S-HSGC) pattern of roasted, ground sample or the total Mocha extract HPLC-UV pattern (alone or in combination) together with principal component analysis (PCA) to distinguish coffees of different origins or submitted to different roasting processes or in mixtures of different compositions (Bicchi et al., 1993). This paper reports on the possibilities both of discriminating green or roasted coffees by the CGA fraction, isolated after cleanup of the Melitta extract, through HPLC-UV and PCA (Wold, 1987; *Statgraphics Manual*) and of comparing the CGA fraction patterns of sets of roasted coffees with sensory evaluation. As examples, some results concerning the discrimination of coffees of the same quality but from different origins or plantations and of coffee blends of different compositions are compared with those from sensory evaluation.

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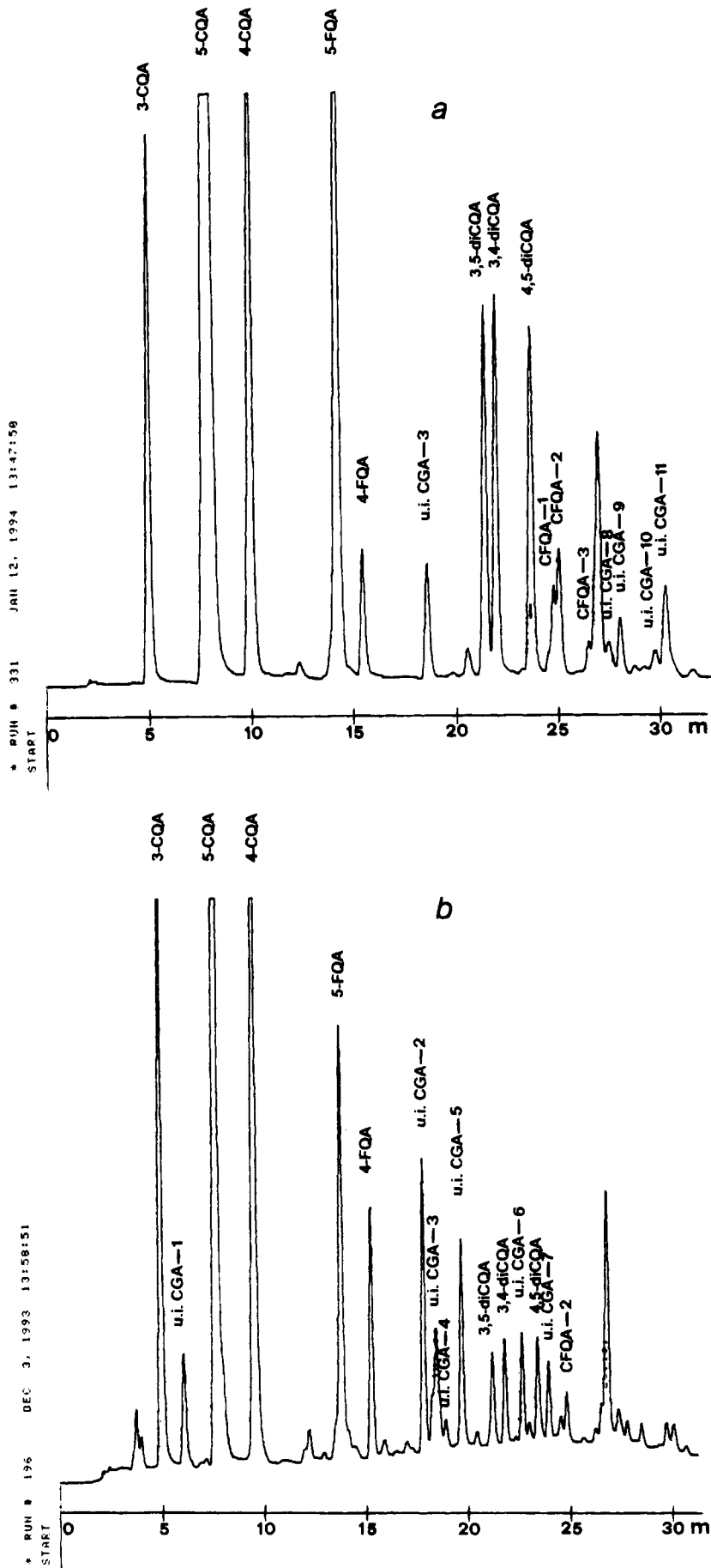† Dipartimento di Scienza e Tecnologia del Farmaco.
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**Figure 2.** (a) CGA HPLC-UV pattern of a green Robusta coffee; (b) CGA HPLC-UV pattern of the corresponding roasted coffee.

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**Table 1. CGAs Characterized and Identified in the Robusta Coffee Samples of Figure 2**

| | abbreviation | name |
|---|---|---|
| 1 | 3-CQA | 3-caffeoylquinic acid |
| 2 | ui-CGA-1 | unidentified CGA |
| 3 | 5-CQA | 5-caffeoylquinic acid |
| 4 | 4-CQA | 4-caffeoylquinic acid |
| 5 | 5-FQA | 5-feruloylquinic acid |
| 6 | 4-FQA | 4-feruloylquinic acid |
| 7 | ui-CGA-2 | unidentified CGA |
| 8 | ui-CGA-3 | unidentified CGA |
| 9 | ui-CGA-4 | unidentified CGA |
| 10 | ui-CGA-5 | unidentified CGA |
| 11 | 3,5-diCQA | 3,5-dicaffeoylquinic acid |
| 12 | 3,4-diCQA | 3,4-dicaffeoylquinic acid |
| 13 | ui-CGA-6 | unidentified CGA |
| 14 | 4,5-diCQA | 4,5-dicaffeoylquinic acid |
| 15 | ui-CGA-7 | unidentified CGA |
| 16 | CFQA-1 | caffeoylferuloylquinic acid isomer |
| 17 | CFQA-2 | caffeoylferuloylquinic acid isomer |
| 18 | CFQA-3 | caffeoylferuloylquinic acid isomer |
| 19 | ui-CGA-8 | unidentified CGA |
| 20 | ui-CGA-9 | unidentified CGA |
| 21 | ui-CGA-10 | unidentified CGA |
| 22 | ui-CGA-11 | unidentified CGA |

## EXPERIMENTAL PROCEDURES

**Coffee Samples.** Samples of green and roasted coffees of different origins, and of different blends of coffees, were supplied by Lavazza SpA, Torino, Italy. The original green coffees were stored in the areas of production. Ten samples (50 g) of each coffee variety or blend were hermetically sealed under vacuum in nonpermeable polypropylene/aluminum/polyethylene packages and stored at −20 °C after roasting until used for chemical analysis.

All of the coffee samples here considered were roasted for 6 min at 270 °C in a laboratory roasting device Probat (Emmer-ich, Germany). More details about the samples analyzed are given under Results and Discussion.

**Melitta Extract Preparation and Sample Cleanup.** Coffee beverages were prepared in a commercially available Melitta coffee pot under strictly standardized conditions. Green coffee beans were ground to pass 0.8 mm in a Petroncini conical blade grinder (Bologna, Italy). Twenty grams each of both green and roasted coffees were submitted to extraction with 200 mL of water for an extraction time of 7 min.

Sample cleanup was carried out on a $C_{18}$ Sep-Pak cartridge 51910 (Millipore Waters, Milford, MA). Six milliliters of beverage was loaded onto the cartridge, previously *conditioned* with 5 mL of MeOH and 3 mL of distilled water of HPLC grade. The cartridge was then eluted with 20 mL of MeOH/water (40/60). The eluate was diluted to 50 mL with distilled water (HPLC grade) and then directly submitted to HPLC-UV analysis. Twenty microliters of the resulting eluate from cleanup was injected.

**HPLC-UV Analysis.** The HPLC-UV analyses were carried out following the method proposed by Van der Stegen and Van Duijn (1980) and modified in the authors' laboratory. HPLC-UV was carried out on an HPLC system consisting of two Waters Associates Model 501 LC pumping units, an automated gradient controller (Waters Associates), a valve loop injector Rheodyne Model 7010 fitted with a 20 $\mu$L loop, and a UV detector Waters Associates Model Lambda-Max 481 LC spectrophotometer or a Varian Polycrom 9065 diode array detector (Varian, Walnut Creek, CA).

An ODA Hypersil (5 $\mu$m), 4.6 mm i.d. × 200 mm (Hewlett-Packard Co., Amsterdam, The Netherlands), column was used. The following elution program at room temperature (25 °C) was used: 15% methanol in a citrate−hydrochloric acid buffer solution (pH 3) to 45% methanol in 20 min of linear gradient. The composition of citrate−hydrochloric acid buffer solution at pH 3 was 8.470 g/L of citric acid, 4.523 g/L of potassium hydroxide, and 2.176 g/L of hydrochloric acid. UV detection
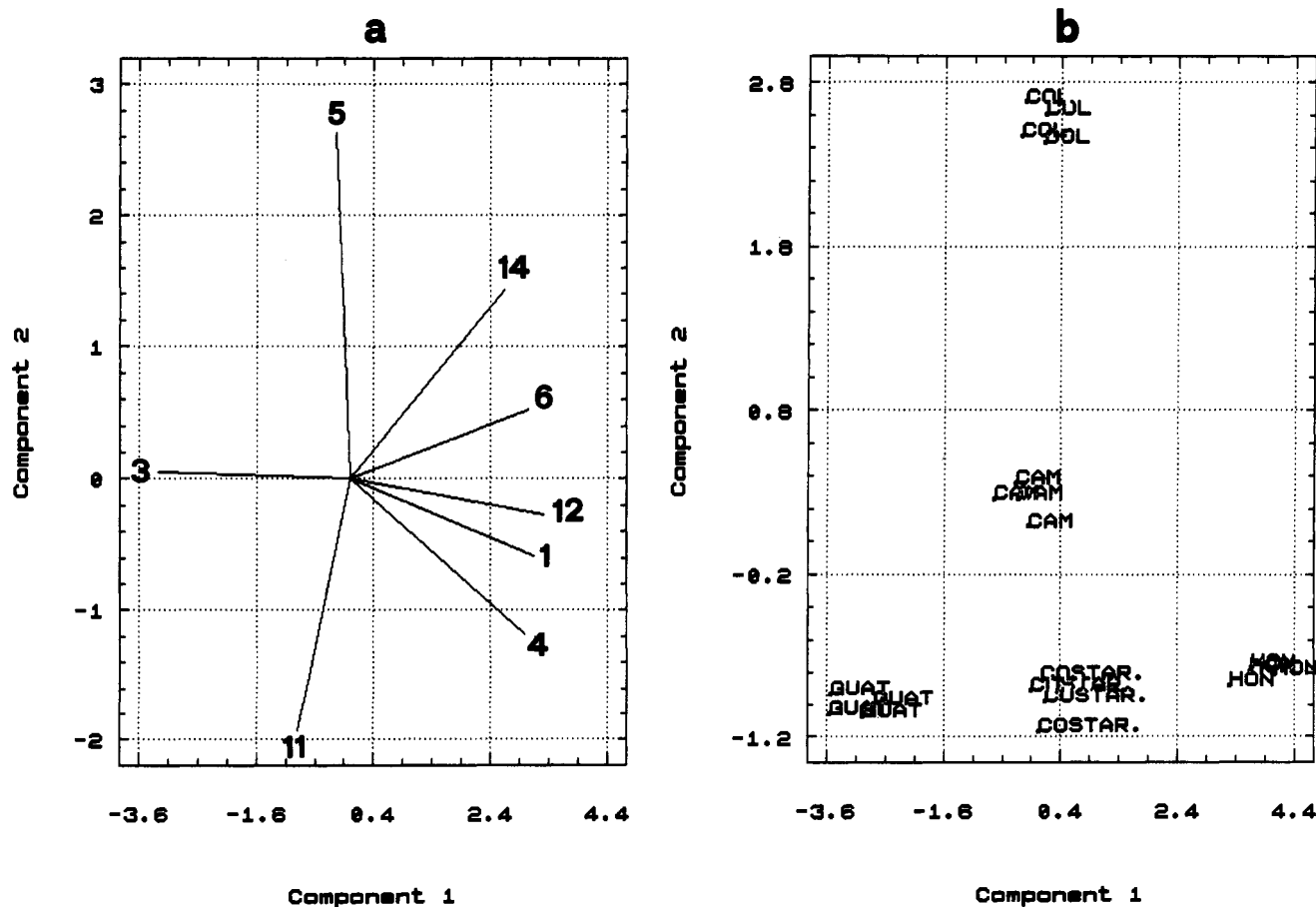


**Figure 3.** (a) Distribution of the loadings of washed Arabica samples from five different geographical areas (Colombia, Cameroon, Honduras, Guatemala, and Costa Rica) corresponding to the CGA detected by HPLC-UV. (b) Scatterplot of principal components of the same samples. PC1 = 63.24%, PC2 = 24.25%.
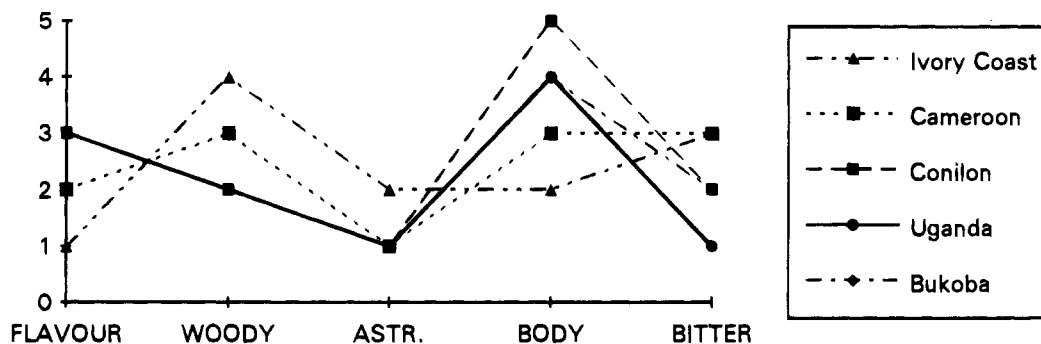
**Figure 4.** Panel's description of the Robusta coffee samples under analysis (astr., astringency): 1 = low; 2 = medium low; 3 = medium; 4 = medium high; 5 = high.
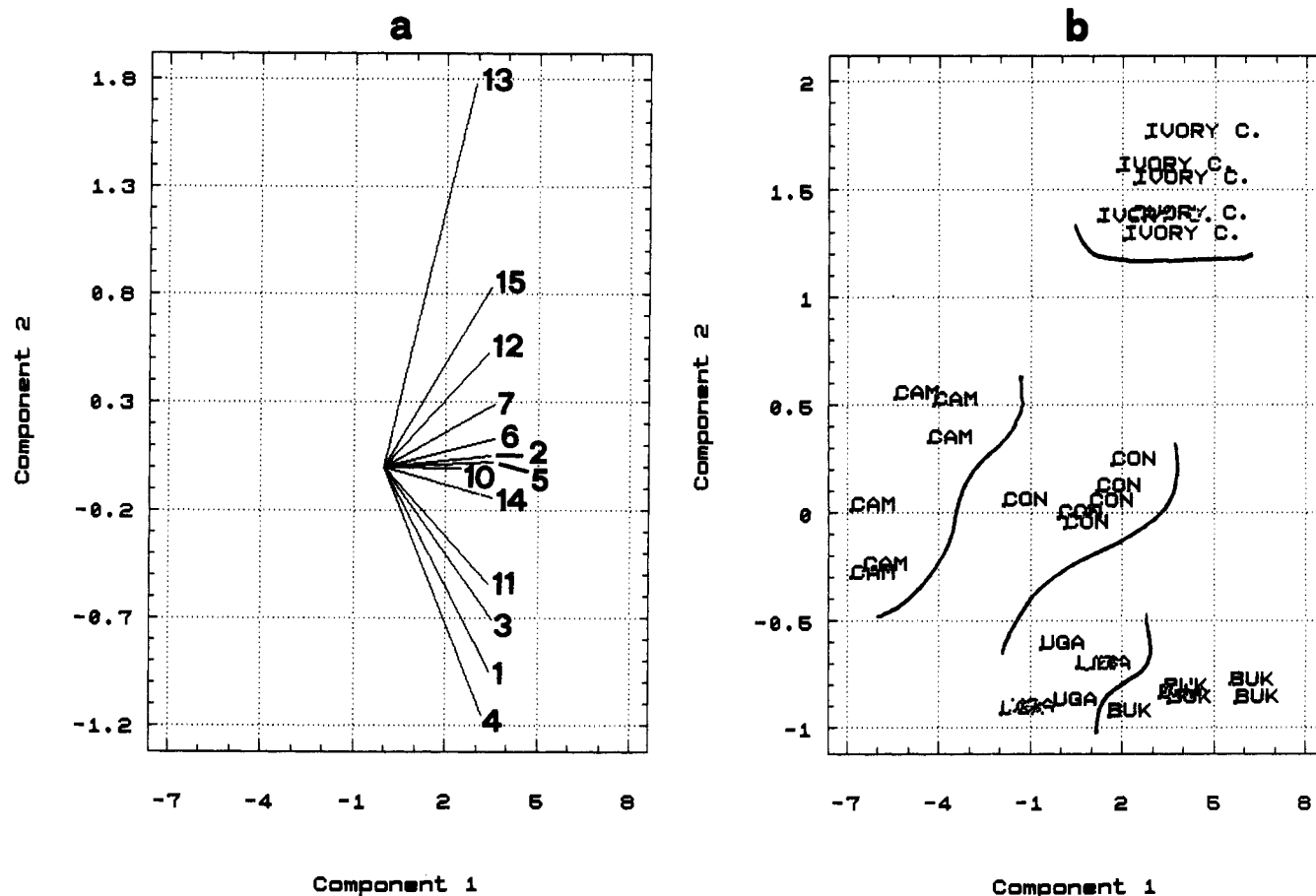


**Figure 5.** (a) Distribution of the loadings of five Robusta coffee samples of different origin (Ivory Coast, Cameroon, Conilon, Uganda, and Bukoba cultivations) corresponding to the CGA detected by HPLC-UV. (b) Scatterplot of principal components of the same samples. PC1 = 88.53%, PC2 = 5.77%

was carried out at a wavelength of 325 nm corresponding to the CGA absorption maximum.

**Data Elaboration.** The peaks in the HPLC-UV pattern to be submitted to PCA were selected on the basis of CGA UV spectra obtained by diode array detection. Twenty-two components were confirmed as belonging to the CGA class and 11 identified.

The peak areas from HPLC-UV were processed on HP 3396A computing integrators and then transferred on-line to an HP Vectra 386SX personal computer (Hewlett-Packard, Grenoble, France), where they were elaborated through a PCA program (Statgraphics, Statistical Graphics Corp., Rockville, MD). A homemade program was used to make the file format from a computing integrator compatible with the statistic program.

Routine statistical analyses were carried out on the CGA peaks with detectable and reproducibly measurable areas in all of the samples under investigation. PCA was applied to the mean areas (calculated over six HPLC-UV analyses) of

each peak considered from each sample, so as to obtain a clearer scatterplot.

**Sensory Evaluation.** The beverages prepared with each sample of each group of coffees were evaluated under the same conditions by a panel of 15 tasters trained in the sensory evaluation of coffee (Gillette, 1990). The terms used to evaluate each group of coffees were selected on the basis of the coffees under investigation. The reference value for each of the parameters was 3 (i.e. the medium value). The beverages for sensory evaluation were prepared according to the Melitta method under the strictly standardized conditions described and tested at a temperature of 55 °C.

## RESULTS AND DISCUSSION

Figure 2a reports the CGA HPLC-UV pattern of a green Robusta coffee, while Figure 2b reports that of the corresponding roasted coffee. Table 1 lists the CGAs identified in the coffee sample of Figure 2.
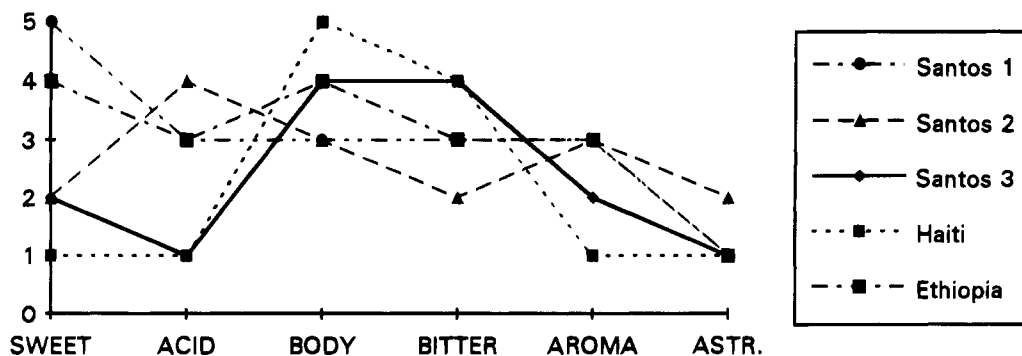
**Figure 6.** Panel's description of the Arabica coffee under analysis (astr., astringency): 1 = low; 2 = medium low; 3 = medium; 4 = medium high; 5 = high.
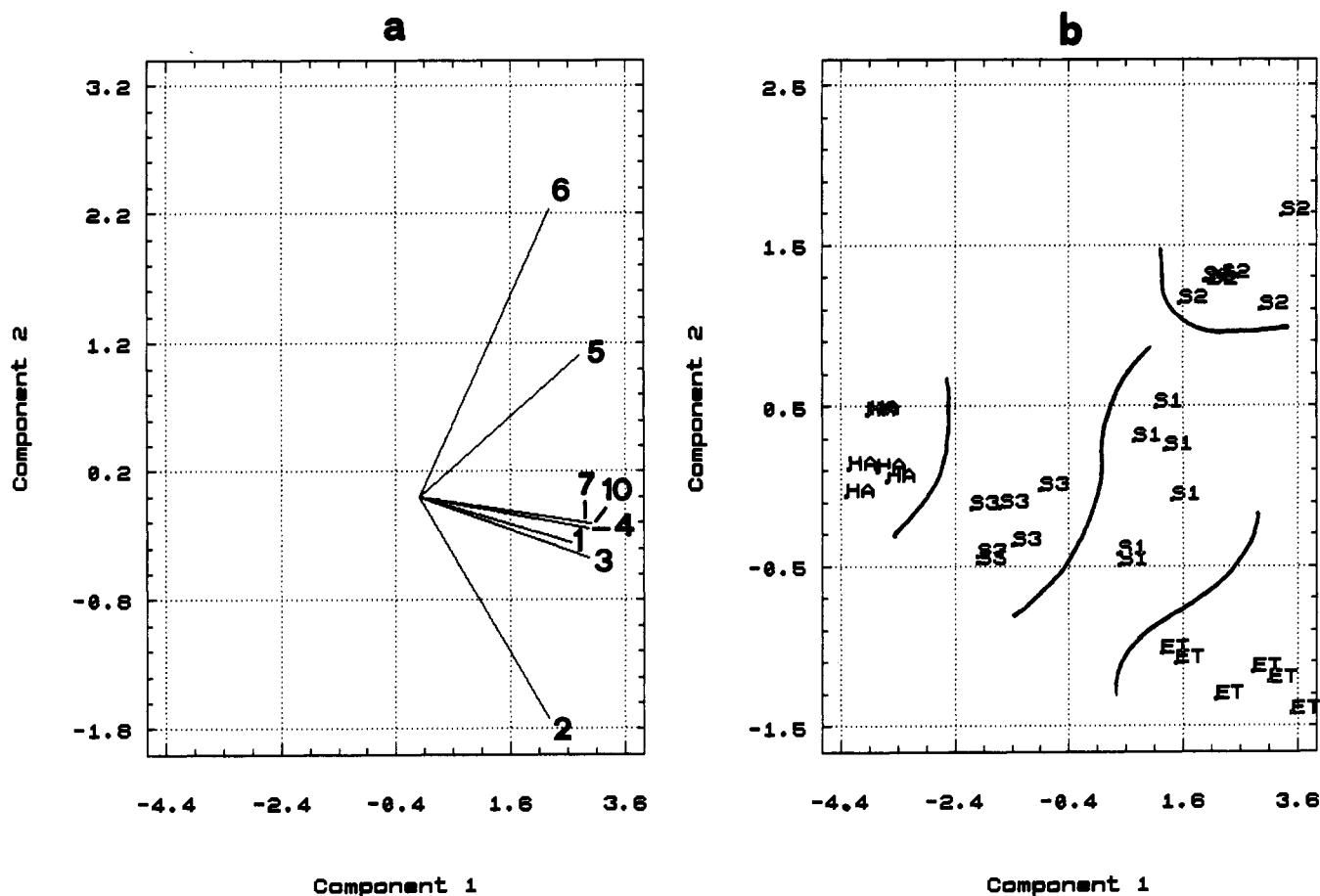


**Figure 7.** (a) Distribution of the loadings of five Arabica coffee samples [one from Haiti, one from Ethiopia, and three Santos samples from different plantations in the same region (Soul de Minas, Brazil)] (for details see text) corresponding to the CGA detected by HPLC-UV. (b) Scatterplot of principal components of the same samples. PC1 = 79.54%, PC2 = 9.68%

To make the results as far as possible comparable to those of sensory evaluation, water was chosen for CGA extraction, although methanol/water or 2-propanol extracts are more exhaustive and avoid isomerization of 5-CQA to 3-CQA and 4-CQA (Trugo and Macrae, 1984a; Clifford et al., 1985).

In the examples reported below, all peaks of the HPLC-UV pattern of the CGA fraction with detectable and reproducibly measurable areas in all of the samples under investigation were considered for the routine statistical analyses (PCA).

The possibility of distinguishing different green coffees through the CGA fraction has already been reported by several authors (Clifford, 1986; Clifford and Jarvis, 1988; Clifford et al., 1989; Feldman et al., 1969; Purdon and McCamey, 1987; Trugo and Macrae, 1984a,b). The CGA fraction is often qualitatively and/or quantitatively

richer in Robusta than in Arabica coffee samples (Clifford, 1985).

For green coffees, the elaboration of the HPLC-UV profiles by PCA further increases the characterizing ability of the CGA fraction, as is shown by the next example, which concerns the discrimination of five washed Arabica samples from different geographical areas (Colombia, Cameroon, Honduras, Guatemala, and Costa Rica). Four different samples from each of these geographical areas were investigated. From the HPLC-UV pattern of the CGA fractions, eight CGA peaks were considered for the routine statistical analyses. The CGAs selected were 3-CQA, 5-CQA, 4-CQA, 5-FQA, 4-FQA, 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA. Figure 3a reports the distribution of the loadings corresponding to the CGA detected by HPLC-UV. Figure 3b reports
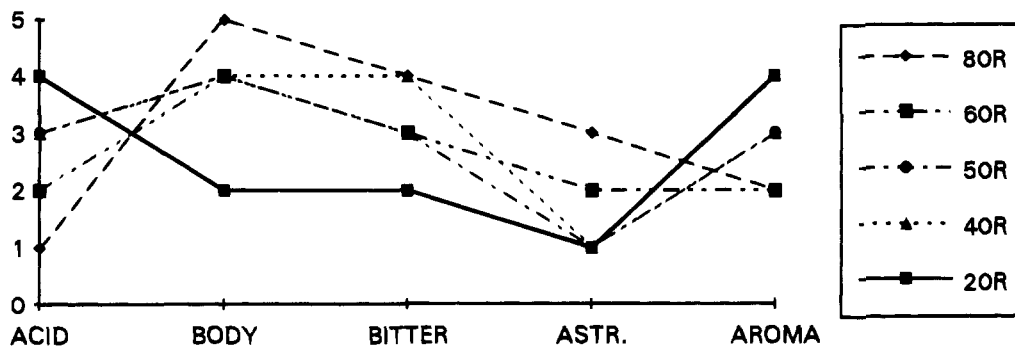
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**Figure 8.** Panel's description of the coffee blends under analysis (astr., astringency): 1 = low; 2 = medium low; 3 = medium; 4 = medium high; 5 = high.
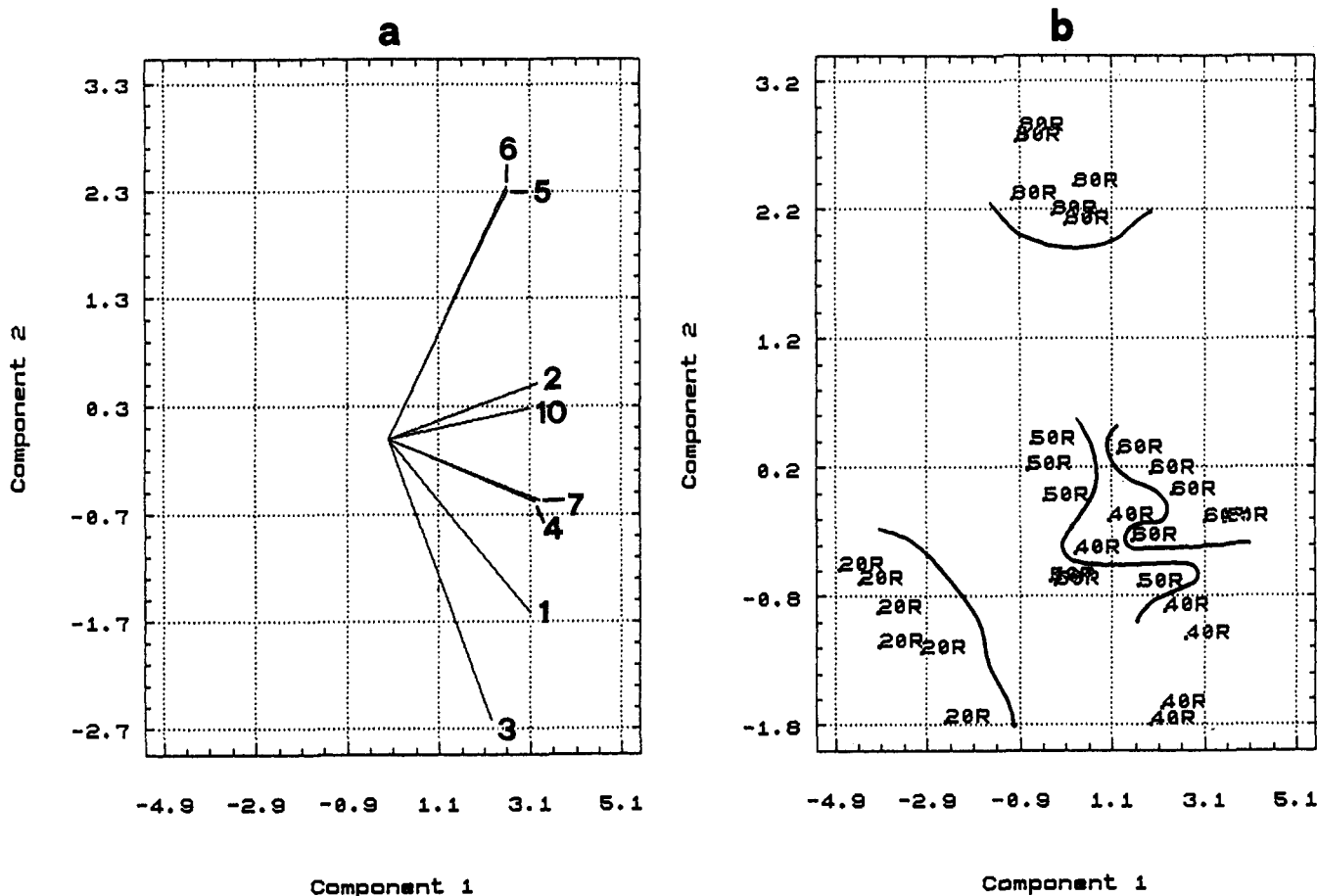


**Figure 9.** (a) Distribution of the loadings of five different blends obtained from pure washed Arabica and pure Robusta coffees (for composition see text) corresponding to the CGA detected by HPLC-UV. (b) Scatterplot of principal components of the same samples. PC1 = 64.03%, PC2 = 23.92%.

the scatterplot of principal components of the five washed Arabica samples from different geographical areas.

The characterization of roasted coffees through the composition of the CGA fraction is less immediate, since the roasting process produces a qualitative–quantitative variation of CGA (Clifford, 1985). The next examples aim to show the agreement between the evaluation of roasted coffees by the panel and the discrimination obtained by PCA elaboration of the HPLC-UV pattern of the CGA fraction.

The first example concerns the discriminations between five Robusta coffee samples coming from plantations in the Ivory Coast, Cameroon, Conilon, Uganda, and Bukoba. The resulting beverages were evaluated both by the panel and by HPLC-UV/PCA analysis of the CGA fraction. The five samples were defined by the test

panel as reported in Figure 4. The panel described the samples from Cameroon and Conilon and from Uganda and Bukoba as being very similar to each other, while the Ivory Coast coffee was described as different from the previous two groups.

From the HPLC-UV pattern of CGA fractions, 13 CGA peaks were considered for the routine statistical analyses (PCA). The selected CGAs were 3-CQA, ui-CGA-1, 5-CQA, 4-CQA, 5-FQA, 4-FQA, uiCGA-2, uiCGA-5, 3,5-diCQA, 3,4-diCQA, uiCGA-6, 4,5-diCQA, and ui-CGA-7. Figure 5a reports the distribution of the loadings corresponding to the CGA detected by HPLC-UV. Figure 5b reports the scatterplot of the principal components of the five coffees under investigation. For clarity, the different areas of origin have been outlined by hand. The PCA scatterplot clearly shows both the discrimination between the different origins of the Robusta samples

and a data distribution in agreement with the sensory evaluation.

The second example concerns the discrimination between five Arabica samples, i.e. one from Haiti, one from Ethiopia, and three Santos samples cultivated in the same region (Soul de Minas, Brazil) under the same conditions of soil and climate but in three different plantations and classified as being of the same quality. Here again the resulting beverages were evaluated, both by the same panel and by the HPLC-UV/PCA analysis of the CGA fraction. The panel's description of the Arabica samples is reported in Figure 6. The panel classified the Haiti sample as similar to Santos 3, the Ethiopia sample similar to Santos 1, Santos 1 and 2 more similar to each other than to Santos 3, and Santos 1 better than Santos 2 or 3. From the HPLC-UV pattern of CGA fractions, eight CGA peaks were considered for the routine statistical analyses (PCA). The CGAs selected were 3-CQA, ui-CGA-1, 5-CQA, 4-CQA, 5-FQA, 4-FQA, ui-CGA-2, and ui-CGA-5. Figure 7a reports the distribution of the loadings corresponding to the CGA detected by HPLC-UV. Figure 7b reports the scatterplot of the principal components of the five coffees under investigation, which clearly distinguishes the samples in question. For clarity, the different samples have been outlined by hand. The scatterplot clearly shows how PCA discriminates between the different Arabica samples and how the data distribution was in agreement with the sensory evaluation.

The third example concerns the distinction of five different coffee blends obtained from pure washed Arabica and pure Robusta in the following ratios: 80% Robusta/20% Arabica (80R), 60% Robusta/40% Arabica (60R), 50% Arabica/50% Robusta (50R), 40% Robusta/ 60% Arabica (40R), and 20% Robusta/80% Arabica (20R).

The sensory discrimination between 80R and 20R was clear, while that of the blends with intermediate composition was difficult because of their close properties (Figure 8).

From the HPLC-UV pattern of the CGA fraction, eight CGA peaks were considered for the routine statistical analyses (PCA). The CGAs selected were 3-CQA, ui-CGA-1, 5-CQA, 4-CQA, 5-FQA, 4-FQA, ui-CGA-2, and ui-CGA-5. Figure 9a reports the distribution of the loadings corresponding to the CGA detected by HPLC-UV. Figure 9b reports the scatterplot of the principal components of the five coffees under investigation, which clearly distinguishes the 80R blend from the 20R blend and from those of intermediate composition and separates 60R clearly enough from 50R and 40R. For clarity, the different blends have been outlined by hand. In this example too, the distribution of the data of the different coffee blends by HPLC-UV/PCA is not only in agreement with the sensory evaluation but also provides a better discrimination of the coffee blends with intermediate composition.

Although a direct relationship between the CGA fraction composition and the beverage quality has not yet been fully demonstrated, our results indicate that it is possible to compare the classification of a set of coffee samples based on the evaluation of a panel with the discrimination obtained by applying PCA to the HPLC-UV patterns of their CGA fractions. Unfortunately, we are still unable to correlate with certainty the beverage quality, or a specific sensory attribute such as astringency, with the presence of specific chlorogenic acid(s).

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