Contribution of FA Profile Obtained by High-Resolution GC/Chemometric Techniques to the Authenticity of Green and Roasted Coffee Varieties

M. Rui Alves[a], S. Casal[b], M.B.P.P. Oliveira[b,*], and M.A. Ferreira[b]

[a]Escola Superior de Tecnologia e Gestão—Instituto Politécnico de Viana do Castelo, 4900 Viana do Castelo, Portugal, and [b]Rede de Quimica e Tecnologia, Serviço, Bromatologia, Faculdade de Farmácia, Universidade do Porto Rua, Aníbal Cunha, 164, 4050-047 Porto, Portugal

ABSTRACT: Twenty-four coffee samples of different botanical and geographical origins were analyzed for their FA composition, including *trans* isomers. The analysis used high-resolution GC/FID/CP Sil 88 capillary column to separate FAME obtained by esterification with BF_3/methanol. The purpose of this work was to verify whether this parameter could be applied in the discrimination of *arabica* and *robusta* coffees, either in green or in roasted stage. Statistical approaches were applied to check the efficiencies of some univariate and multivariate procedures, and the results permitted the conclusion that the FA profile can be used as a coffee variety marker and may inform on the historical background, mainly in terms of heat-processing conditions.

Paper no. J9895 in *JAOCS 80,* 511–517 (June 2003).

KEY WORDS: Authenticity, *Coffea arabica*, *Coffea robusta*, discriminant analysis, green coffee, multivariate statistics, roast coffee, *trans* fatty acids, univariate statistics.

Of all the species of *Coffea* available, two have acquired high commercial value, namely, *C. arabica* Linn. and *C. canephora* Pierre ex Froehner var. *robusta*, and are used to prepare coffee drinks. The beans of the preferred species are easily identified by their macroscopic characteristics when in the green stage. After roasting, this distinction is still possible with whole beans, but after milling, when the anatomic characteristics are lost, identification becomes extremely difficult. Since *C. arabica* and *C. robusta* display different appeals and have different commercial values, the discrimination of these species is essential to be able to avoid adulteration and to prevent unfair commercial practices (1).

To guarantee the authenticity of coffees, several chemical and physical parameters have been tried, namely, hydroxycinnamic acid derivatives (2), unsaponifiable lipid fractions (3,4), furanic aldehydes (5), trace element profiles (6), stable isotope ratios (7), aroma profiles (8), and spectroscopic techniques (9–11).

With regard to the FA composition of the two coffee varieties, an unambiguous position among authors is not yet available, especially when roasted beans are considered (12–16).

Therefore, the approach presented in this paper is (i) a new attempt to verify the utility of FA profiles in the discrimination between *arabica* and *robusta* coffee varieties, both in the green and roasted stage, (ii) a study of the possible role played by *trans* isomers of unsaturated FA in the discrimination of the roasted coffees, and (iii) an attempt to develop a statistical model for green and roasted *arabica* and *robusta* coffee varieties for use as a starting point for the development of a database.

EXPERIMENTAL PROCEDURES

Samples. A total of 16 samples of coffee beans from *C. canephora* Pierre ex Froehner var. *robusta* and 8 samples of *C. arabica* Linn., before and after roasting, were studied. The 16 *C. robusta* samples in the green stage were identified as RG01–RG16, and the same 16 samples after roasting were identified as RR01–RR16. These samples had several geographical origins (India, Vietnam, Uganda, Amboim/Angola, Angola, Cameroon, and the Ivory Coast). Like the *C. robusta* samples, the 8 *C. arabica* samples were prepared in green and roasted stages and identified as AG01–AG08 and AR01–AR08, respectively. The countries of origin of these samples were Honduras, Brazil, Mexico, Guatemala, Colombia, and Costa Rica. A local broker and a coffee roaster importer supplied the green and roasted samples.

Samples were roasted in a local industry according to the common commercial procedure. During the initial phase of roasting, the temperature was set to 140°C and then was gradually increased to 221°C. The entire roasting procedure lasted approximately 14 min.

Sample preparation. Green and roasted beans were visually examined to confirm their variety. Following this procedure, all beans were classified as *C. arabica* or *C. robusta*. Owing to their hardness, green beans were first coarsely ground in a regular crusher. Afterward, all samples of green and roasted coffees were ground in a hammer mill to pass an 0.8-mm sieve.

Lipid extraction and quantification. Total lipids were extracted from the milled coffee seeds with petroleum ether (b.p. 40–60°C) by refluxing in a Soxhlet apparatus for a minimum of 36 h. Fat samples were recovered after solvent evaporation at low temperature (<40°C). The total fat content of samples was determined by the AOAC 920.97 method (17).

Methylation. Fat samples were hydrolyzed with a boiling methanolic potassium hydroxide solution (11 g L^{-1}). The FA

*To whom correspondence should be addressed. E-mail: beatoliv@ff.up.pt

formed were converted to methyl esters by esterification with BF_3/MeOH while heating was maintained. The methyl esters were then extracted with *n*-heptane (18).

Chromatography. The FA composition and *trans* isomers of unsaturated FA were analyzed by high-resolution GC (HRGC)/FID in a Chrompack CP-9001 gas chromatograph equipped with a split-splitless injector and a 50 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack, Middelburg, The Netherlands). Helium was used as carrier gas at an inlet pressure of 14 kPa. The temperatures of the detector, injector, and oven were 250, 230, and 185°C, respectively. The split ratio was 1:50, and the injected volume was 0.8 μL.

Standards. FAME (>99%) used for identification were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Standards included the following FAME: dodecanoate (12:0); tetradecanoate (14:0); pentadecanoate (15:0); hexadecanoate (16:0); 9-palmitoelaidate (16:1*t*); *cis*-9-hexadecenoate (16:1*c*); heptadecanoate (17:0); octadecanoate (18:0); *trans*-9-octadecenoate, elaidate (18:1*t*); *cis*-9-octadecenoate, oleate (18:1*c*); *trans*-9,*trans*-12-octadecadienoate (18:2*tt*); *cis* 9,*trans*-12-octadecadienoate (18:2*ct*); *trans*-9,*cis*-12-octadecadienoate (18:2*tc*); *cis*-9,*cis*-12-octadecadienoate (18:2*cc*); eicosanoate (20:0); 9,12,15-octadecatrienoate (18:3); 11-eicosenoate (20:1); heneicosanoate (21:0); docosanoate (22:0); 13-docosenoate (22:1); and tetracosanoate (24:0).

Statistics. Box and whisker plots were developed based on minimum and maximum values and first, second, and third quartiles. Student's *t*-tests for independent and dependent samples were performed according to conventional techiques (19). Tests for independent samples were used to compare *arabica* with *robusta* coffee varieties, and tests for dependent samples were used to compare differences in FA levels before and after roasting. Sign tests were performed according to the methods of Conover (20) by considering the percentage differences between green and roasted samples: For each coffee sample and for each FA, a plus sign is attributed if there is an increase after processing in FA percentage and a minus sign if there is a decrease. The number of plus signs is compared to the appropriate tables to check for significant increases or decreases in FA percentage. A cluster analysis (CA) was applied with Euclidean distances and Ward's clustering method. Canonical variates analysis (CVA) and discriminant analysis (DA) were carried out to search for the FA most useful for discrimination among the groups "green *arabica*," "roasted *arabica*," "green *robusta,*" and "roasted *robusta*." Discrimination functions were developed for each of these four groups until no misallocations were observed. All these multivariate analyses were performed in the standard way (21), as implemented in the Statistica for Windows (Statsoft, Tulsa, OK) software package.

RESULTS AND DISCUSSION

The total fat content of coffee beans varied according to variety and treatment. The mean fat contents (and minimum and maximum values), expressed as weight of fat in grams per 100 g of freshly ground coffee, for green *arabica*, roasted *arabica*, green *robusta,* and roasted *robusta* were 8.7 (6.5–11.7), 14.1 (8.8–16.3), 4.0 (2.7–6.3), and 8.1 (6.1–11.6) g/100 g, respectively.

The FA percentages are presented in Figure 1A for green (left in each column) and roasted (right in each column) *arabica* samples and in Figure 1B for green and roasted *robusta* samples. These results are based on nonparametric statistics (minimum and maximum values and quartiles) displayed as box and whisker plots. This type of representation enables the observation of the actual dispersion in data values and is therefore more useful than the usual way of summarizing results in the form of mean values affected by confidence intervals based on SD, i.e., intervals of the form mean ± *ts* or mean ± *ts*/√*n*, *t* being the appropriate value of the *t*-Student distribution, and *s* referring to the sample SD. To build these figures, values from each FA were previously standardized, so that all boxes would come in reasonable sizes, be well adjusted in the graphs, and in such a way that both figures could be superimposed to compare results for the two coffee varieties, both as green and roasted samples. It is important to that owing to the standardization applied, the standardized percentage values are positive if the original values are higher than the mean FA value and are negative if the original values are lower than the mean. To relate the standardized values to the observed percentage values, the minimum and maximum values obtained for each FA for each of the groups considered are shown on the bottom and the top of the corresponding box plot, respectively.

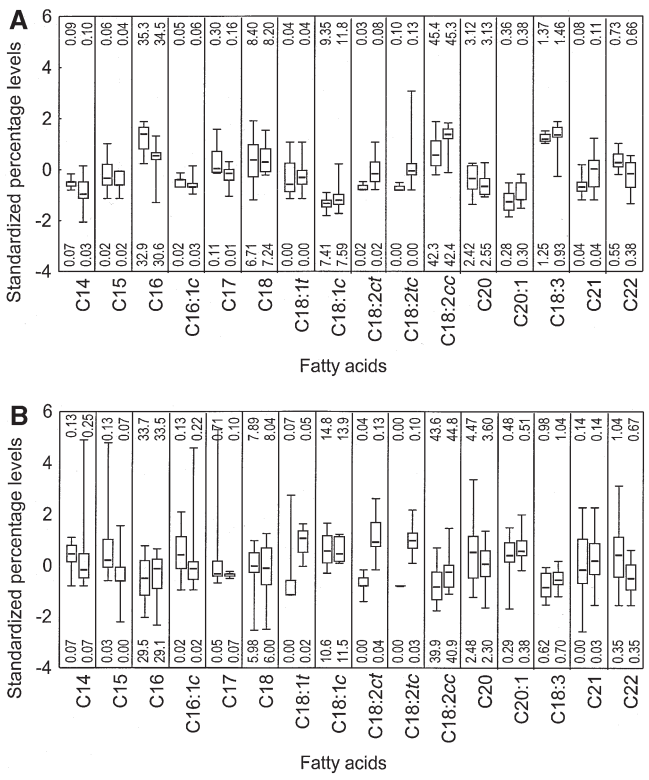

FIG. 1. Univariate nonparametric analysis of FA profiles (A) in green (left) and roasted (right) *arabica* coffee samples, and (B) in green (left) and roasted (right) *robusta* coffee samples.

From inspection of Figure 1A and 1B, the following features are evident: (i) The dispersion in the results is, in general, very high and tends to be higher for the *robusta* than for the *arabica* coffees; (ii) for many FA, the two whiskers of very different sizes show a great asymmetry in the observed results, which indicates high heterogeneity within each group; (iii) *robusta* coffees show higher levels of $C_{18:1c}$ and $C_{20:1}$, and lower levels of $C_{18:2cc}$ and $C_{18:3}$ than *arabica* coffees; (iv) higher levels of *trans* isomers ($C_{18:1t}$, $C_{18:2ct}$, and $C_{18:2tc}$) in roasted coffees when compared with the corresponding green coffees are also generally seen in these figures. These distinct features refer to situations where there is almost no overlapping of boxes and whiskers.

By themselves, these figures, are not sufficient to determine the differences in FA levels between the groups under consideration, unless these are very evident (no overlapping of boxes and whiskers), as pointed out in the preceding paragraphs. Student's *t*-tests for independent samples (Table 1) were carried out to determine all the FA responsible for significant differences between coffee varieties (green or roasted *arabica* and *robusta*) and *t*-tests for dependent samples (Table 2) to check for differences caused by the roasting process within the *arabica* and the *robusta* samples.

From analysis of these tables, the following conclusions can be drawn: (i) Comparison of differences between *robusta* and *arabica* varieties, irrespective of treatment (Table 1), shows that the former have consistently ($P < 0.05$) higher levels of C_{14}, $C_{18:1c}$, and $C_{20:1}$ and lower levels of C_{16}, $C_{18:2cc}$, and $C_{18:3}$ than the latter. (ii) Table 1 also shows that green *robusta* samples have higher levels of $C_{16:1c}$ (a feature not observed after roasting) and lower levels of $C_{18:1t}$, which seems to increase during the same process. $C_{18:2ct}$ also seems to increase more in the *robusta* than in the *arabica* samples during roasting. $C_{18:2tc}$ is higher in green *arabica,* but the reverse was observed for roasted samples. (iii) In Table 2, which reports tests carried out on the differences observed before and after the roasting process, it can be seen that more FA are affected by roasting in the *robusta* samples than in the *arabica* ones, but both samples show increases in $C_{18:2ct}$ and $C_{18:2tc}$ during roasting as a common feature. (iv) Also, in *robusta* samples, $C_{18:1t}$, $C_{18:2cc}$, and $C_{18:3}$ are higher and C_{15} and C_{20} are lower in the roasted than in the green stage. (v) For the *arabica* samples, C_{17} is reduced and C_{21} is increased in the roasted stage. This apparent C_{21} increase possibly may be due to changes in the levels of $C_{18:3}$ isomers, which may overlap in the chromatograms. It was not possible to examine this situation because adequate standards and a mass spectrometric detector were unavailable. Therefore, some caution is necessary regarding this observation.

TABLE 1
Significant Differences in FA Levels Between *arabica* and *robusta* Coffee Samples[a]

	Green samples			Roasted samples		
	Mean values			Mean values		
FA	*robusta*	*arabica*	*P*	*robusta*	*arabica*	*P*
C_{14}	.108	.079	.000	.105	.068	.030
C_{16}	31.8	34.3	.000	32.05	33.1	.047
$C_{16:1c}$	.073	.038	.009	.056	.034	.203
$C_{18:1t}$	.010	.015	.505	.036	.016	.001
$C_{18:1c}$	12.6	8.41	.000	12.6	8.98	.000
$C_{18:2ct}$	.021	.023	.781	.082	.044	.001
$C_{18:2tc}$	.000	.003	.039	.062	.036	.040
$C_{18:2cc}$	41.4	43.6	.000	42.2	44.5	.000
$C_{20:1}$	.415	.316	.000	.433	.335	.000
$C_{18:3}$	.809	1.30	.000	.876	1.31	.000

[a]In either the green or roasted stage, as revealed by *t*-tests for independent samples.

TABLE 2
Significant Differences in FA Levels Between *arabica* and *robusta* Coffee Samples, Before and After the Roasting Treatment[a]

Variety	FA	Stage	Mean	SD	*P*
robusta, $n = 16$	C_{15}	Green	.053	.023	.040
		Roasted	.036	.015	
	$C_{18:1t}$	Green	.010	.018	.000
		Roasted	.036	.0109	
	$C_{18:2ct}$	Green	.021	.012	.000
		Roasted	.082	.023	
	$C_{18:2tc}$	Green	.000	.000	.000
		Roasted	.062	.019	
	$C_{18:2cc}$	Green	41.4	1.11	.008
		Roasted	42.2	1.04	
	C_{20}	Green	3.23	.538	.027
		Roasted	3.01	.384	
	$C_{18:3}$	Green	.809	.118	.005
		Roasted	.876	.090	
	C_{22}	Green	.646	.206	.002
		Roasted	.506	.089	
arabica, $n = 8$	C_{17}	Green	.164	.074	.024
		Roasted	.100	.045	
	$C_{18:2ct}$	Green	.023	.005	.031
		Roasted	.044	.020	
	$C_{18:2tc}$	Green	.003	.005	.048
		Roasted	.036	.040	
	C_{21}	Green	.056	.012	.029
		Roasted	.073	.023	

[a]*t*-tests for dependent samples.

A general conclusion from these analyses is that FA of *robusta* beans seem to be more affected by the roasting process than those of *arabica*. An explanation for the fact that some FA are affected by the roasting treatment in one coffee variety and not in the other, as suggested by the statistical tests, is not yet fully established. A possible explanation is that *robusta* beans, having smaller dimensions, offer less resistance to heat transfer, and consequently changes in FA occur at a higher rate for this variety than for the larger *arabica* beans.

It can be reasoned, however, that the statistical analyses, which are based on ratios of differences in group mean values against differences within groups, may not be able to put forward some smaller differences. In other words, if the dispersion within groups is very high, small differences in group means may not be detected by the analyses used. As pointed out in the discussion of Figures 1A and 1B, the dispersion in the results is very high, and consequently, this high dispersion may be hiding small changes that occur mainly in FA that exist at low levels.

TABLE 3
Results of Sign Tests Checking for Significant Changes in the Roasting Process[a]

FA	C_{14}	C_{15}	C_{16}	$C_{16:1c}$	C_{17}	C_{18}	$C_{18:1t}$	$C_{18:1c}$
robusta	=	=	=	−	=	=	+	=
arabica	=	=	−	=	−	=	=	=

FA	$C_{18:2ct}$	$C_{18:2tc}$	$C_{18:2cc}$	C_{20}	$C_{20:1c}$	$C_{18:3}$	C_{21}	C_{22}
robusta	+	+	=	=	+	+	=	−
arabica	+	+	=	=	+	=	+	=

[a]+, increase with roasting; –, decrease with roasting; =, no significant difference upon roasting. Results for *robusta* are significant at $\alpha = 0.0106$ and for *arabica* at $\alpha = 0.0352$.

To try to highlight these possible changes, sign tests were carried out for each FA, considering only differences between treatments (green/roasted) for *arabica* and *robusta* varieties separately. The sign tests only indicate whether there is an increase or decrease between the green samples and their roasted counterparts and are independent of the magnitude of the differences. Therefore, if a change in percentage level of any FA consistently exists in the samples analyzed, though small, it will be detected. Table 3 shows the results and summarizes the conclusions, which can be formulated as follows: (i) During the roasting of *robusta* coffees, the levels of $C_{16:1c}$ and C_{22} decrease and the levels of $C_{18:1t}$, $C_{18:2ct}$, $C_{18:2tc}$, $C_{20:1}$, and $C_{18:3}$ increase; and (ii) during the roasting of *arabica* coffees, the levels of C_{16} and C_{17} decrease and the levels of $C_{18:2ct}$, $C_{18:2tc}$, $C_{20:1c}$, and C_{21} increase.

These results show that both tests, parametric and nonparametric, used to analyze the effect of the roasting process on the FA composition (Tables 2 and 3) lead to the same conclusions when only the main FA are considered. However, with respect to FA present in smaller amounts, the tests diverge. In our opinion, the results obtained by the use of the sign test are better since they take into consideration only the increase or decrease of a given FA, and not the amount of the difference. For example, owing to this effect, the sign test indicates that the effect of the roasting process on the FA composition of *arabica* coffees may be more extensive than what could be believed if only the results of the Student's *t*-tests were considered.

In this respect, a word of caution is important regarding $C_{18:3}$. This FA is known to isomerize at a high rate; therefore, levels of $C_{18:3}$ *trans* isomers could be important, and theoretically the best indicators of heat treatments or processing history. However, since the levels of this FA are already very low in the green samples (around 1% of the total FA), the levels of the *trans* isomers would exist at levels of the order of 0.001%, which are impossible to detect by the techniques used in this work. For this reason, increases or decreases of $C_{18:3}$ reported in this work must be faced as changes on the total $C_{18:3}$ content, all isomers included.

Thus, it can be concluded that isomerization of unsaturated FA occurs during the roasting process for both *robusta* and *arabica* varieties, although the rate at which it occurs is higher for coffees of the *robusta* variety. This phenomenon was observed under very mild roasting conditions (roasting during 14 min with temperature ranging from 140 to 210°C). A greater level of isomerization should be expected under "dark" industrial roasting conditions.

The univariate aspects discussed so far are not useful for classification purposes, since the percentage level of any FA, by itself, is not enough for the classification of a given coffee sample. For any means of classification, a multivariate approach is therefore necessary. Since the initial data set was very large, consisting of the percentage composition in terms of 16 FA (or isomers) in a total of 48 coffee samples (24 samples each in the green and in the roasted stage), a first search for any patterns was done *via* CA, using several clustering methods and different distance measures. The best clustering method, in terms of results that provide the best approach to the experimental design in four different groups, was found to be Ward's method with Euclidean distances (with linkage distances measured as the overall difference in FA percentage) and with data previously standardized to mean zero and unit variance. Results are shown in Figure 2. This standardization

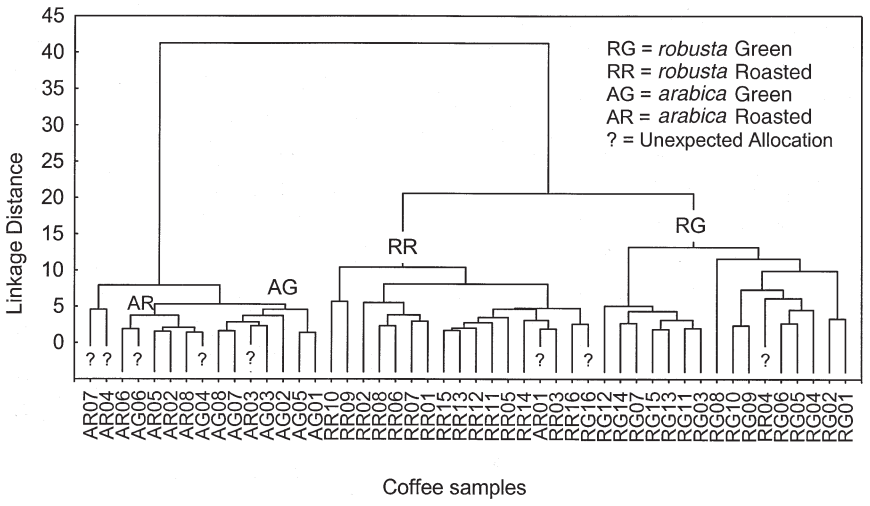

FIG. 2. Cluster analysis of *arabica* and *robusta* coffee samples. Linkage distance is measured as the overall difference in FA percentage.

can be viewed as an attempt to give FA (or their isomers) that exist in small percentages an equal chance to influence results.

From inspection of Figure 2 one can conclude that, in general, samples cluster according to variety and stage, forming four clusters: green *arabica*, roasted *arabica*, green *robusta,* and roasted *robusta*. However, some exceptions were found and identified with the symbol "?" in Figure 2. The following speculations can be advanced: (i) With respect to *robusta* clusters, sample RR04 clustered with RG04 and other green *robusta* coffees, suggesting that this coffee sample was not greatly affected by the roasting treatment, whereas RG16 clustered with RR16 and other roasted *robusta* samples, suggesting that this sample in the green stage was already altered. All other samples are clustered in the correct clusters, and green and roasted clusters are distinct. (ii) The *arabica* green and roasted clusters are not clearly separated, as happened with the *robusta* clusters, which reflects previous observations that *arabica* beans seem to be less affected by the roasting process. (iii) Sample AR03 clustered with AG03 and other green *arabica* samples, probably because it was not greatly affected by the roasting treatment. AG04 clustered with roasted *arabica,* and AR04, the roasted counterpart, was at a greater distance from other *arabica* samples, which means that initial quality was altered and for this reason the sample was more affected during roasting. (iv) AG07 was similar to other green *arabica* samples, whereas AR07 was very different from the other *arabica*, meaning that this sample was very prone to the deleterious effects of roasting. The same type of observation applies to AR01, which clustered with roasted *robusta*, whereas AG01 was clustered in the correct position.

CA, which considers similarities/differences between samples in the overall FA content, supports previous observations but does not provide an answer to the question of which FA are responsible for the clustering observed. As discussed above, the results from CA indicate that a general model can be defined, but some difficulties are to be expected in defining classification functions for the four coffee groups considered, mainly with respect to the *arabica* varieties.

A CVA was carried out with all 16 FA and with coffee samples assigned to the four groups (green *robusta*, roasted *robusta*, green *arabica*, and roasted *arabica*). Of the three canonical dimensions derived by the analysis, only the first two were significant and are shown in Figure 3. Displayed in this figure along the axes are the FA for which the correlations with the canonical axes (or dimensions) were at least superior to 0.25 in absolute value ($|r| \geq 0.25$, $P = 0.05$). These FA were used to give meaning to the canonical dimensions, as is usually done. The eigenvalues, indicated in Figure 2 as percentages of the total variance, were used to observe the relative importance of each dimension. It became obvious that 98% of the total information was concentrated in the two canonical dimensions. The first dimension represents the differences between *robusta* (on the right half) and *arabica* coffee varieties (on the left half of the figure), which are explained by differences in the levels of $C_{18:1c}$ and $C_{20:1}$ (higher in *robusta*) and $C_{18:3}$ and $C_{18:2cc}$ (higher in *arabica*). The second dimension, which reflects 27% of the total variation, represents the effects of roasting, with green samples in the top half and roasted samples in the bottom half of the figure. This dimension shows that the roasting process causes increases in the levels of $C_{18:1t}$, $C_{18:2ct}$, and $C_{18:2tc}$, i.e., in general terms, roasted coffees show a higher isomerization of unsaturated FA than their green counterparts.

Interpretation of Figure 3 leads to the following remarks: (i) There is a slightly higher heterogeneity in roasted than in green samples; (ii) all *robusta* coffees are affected by roasting, which sharply increases the amount of *trans* isomers, whereas separation of green and roasted *arabica* coffees is less apparent when compared to the *robusta* varieties; (iii) two *arabica* samples (Costa Rica and Brazil) are strongly affected by the roasting process, i.e., the former is not well defined in this 2-D

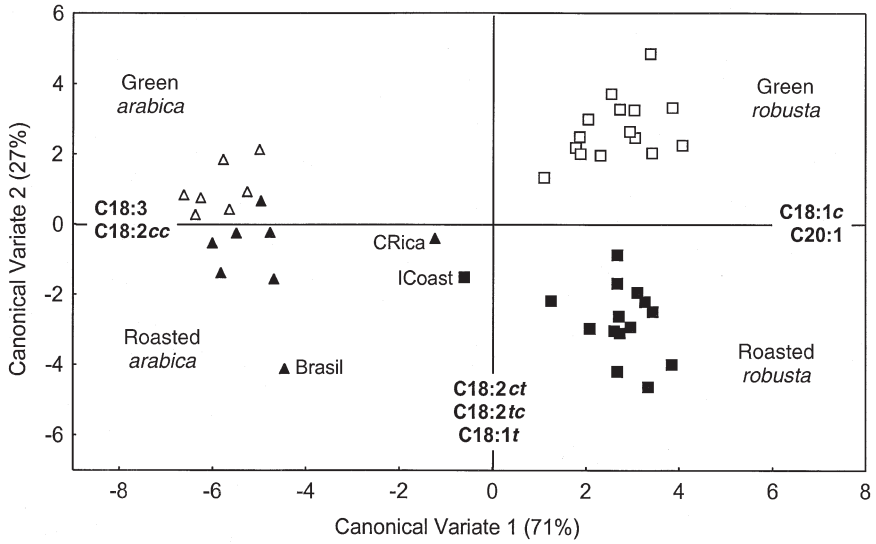

FIG. 3. Canonical variates analysis of coffee samples based on 16 FA.

representation, and the latter shows very high levels of *trans* isomers; (iv) one *robusta* sample (Ivory Coast) displays a profile similar to that of the *arabica* from Costa Rica.

Although the origin of samples was known in the beginning of the work, no geographical relationships could be found that enabled any discrimination of coffee samples by origin (geographical proximity) based on FA profiles. Therefore, in Figure 3, three samples are referred to by country of origin for ease of discussion only, and the other samples are not identified in order to keep the figure as clear as possible with only pertinent information.

This model, derived by a CVA, must be seen as a general model, mainly because it takes into consideration the contribution of all FA for the separation of the groups under study. A more specific study can be done by a forward stepwise DA. DA, in comparison to CVA, will search for the FA with the highest discriminant power, then for a second one, given that a FA is already chosen, then for a third FA, given that there are already two FA in the model, and so on. At each stage of the analysis, the FA introduced in the model is the one showing the highest power to maximize the differences between groups. DA was carried out with several different '*F* to enter' values, in order to check the minimum number of FA necessary to produce a classification of all individual samples in the correct groups (according to variety and treatment).

With an '*F* to enter' value of 1.28, a model with 11 FA can be developed, and a set of four classification functions can be determined (one for each of the coffee groups considered) that guarantee a correct classification of all individual samples in the group to which they belong. Normally, such a high number of variables would not be necessary, but owing to the problems noticed in the group of roasted *arabica* coffees, all these FA were necessary to allocate one sample from Honduras and another from Brazil correctly. The classification functions are shown in Table 4, with FA displayed in decreasing order of discriminating power.

It should be remembered that these functions are used in the following way: Given the results obtained for any new coffee sample, the percentage levels of the 11 FA are multiplied by the corresponding coefficients presented in the first column, and the sum is calculated, adding the constant. The procedure is repeated for the remaining columns, obtaining at the end for values, one for each group. Finally, allocation of any new coffee is done for the group for which the sum is smaller.

TABLE 4
Classification Functions Derived by Discriminant Analysis

	Green *robusta*	Roasted *robusta*	Green *arabica*	Roasted *arabica*
Constant	−1790.75	−1841.61	−2101.32	−2007.72
C_{14}	−671.87	−607.39	−778.34	−740.43
C_{15}	1190.70	994.01	1221.57	1154.45
C_{16}	77.26	77.38	83.92	81.39
C_{18}	78.42	79.43	84.13	82.75
$C_{18:1t}$	0.85	215.10	140.52	139.71
$C_{18:2tc}$	−1279.64	−1155.98	−1471.44	−1329.11
$C_{18:2ct}$	2071.60	2222.42	2369.27	2311.00
$C_{20:1}$	262.39	300.46	171.99	203.34
$C_{18:3}$	217.27	242.40	279.87	283.34
C_{21}	842.60	800.15	946.07	929.16
C_{22}	264.89	249.83	277.98	256.03

ACKNOWLEDGMENTS

The authors wish to thank Fundação para a Ciência e a Tecnologia (PBIC/C/TPR/2565/95 and BD/9580/96) for the financial support for this work.

REFERENCES

1. Prodolliet, J., Authenticity of coffee, in *Food Authentication*, edited by P.R. Ashurst and M.J. Dennis, Blackie Academic & Professional, London, 1996, pp. 304–338.
2. Andrade, P.B., R. Leitão, R.M. Seabra, M.B.P.P. Oliveira, and M.A. Ferreira, 3,4-Dimethoxycinnamic Acid Levels as a Tool for Differentiation of *Coffea canephora* var. *robusta* and *Coffea arabica*, *Food Chem. 61*:511–514 (1998).
3. Trouche, M.-D., M. Derbesy, and J. Estienne, Contribution à l'identification de melanges d'espéces Arabica et Robusta par étude du 16-*O*-méthylcafestol, *Ann. Falsif. Exp. Chim. Toxicol. 90*:121–132 (1997).
4. Frega, N., F. Bocci, and G. Lercker, High Resolution Gas Chromatographic Method for Determination of Robusta Coffee in Commercial Blends, *J. High Resolut. Chromatogr. 17*:303–307 (1994)
5. Chambel, P., M.B.P.P. Oliveira, P.B. Andrade, R.M. Seabra, and M.A. Ferreira, Development of an HPLC/Diode-Array Detector Method for Simultaneous Determination of 5-HMF, Furfural, 5-*o*-Caffeoylquinic Acid and Caffeine in Coffee, *J. Liq. Chromatogr. Rel. Technol. 20*:2949–2957 (1997).
6. Krivan, V., P. Barth, and A. Feria Morales, Multielement Analysis of Green Coffee and Its Possible Use for the Determination of Origin, *Mikrochimica Acta 110*:217–236 (1993).
7. Danho, D., N. Naulet, and G.J. Martin, Deuterium, Carbon, and Nitrogen Isotopic Analysis of Natural and Synthetic Caffeines. Authentication of Coffees and Coffee Extracts, *Analusis 20*:179–184 (1992).
8. Bicchi, C.P., A.E. Binello, M.M. Legovich, G.M. Pellegrino, and A.C Vanni, Characterization of Roasted Coffee by S-HSGC and HPLC-UV and Principal Component Analysis, *J. Agric. Food Chem. 41*:2324–2328 (1993).
9. Downey, G., R. Briandet, R.H. Wilson, and E.K. Kemsley, Near- and Mid-infrared Spectroscopies in Food Authentication: Coffee Varietal Identification, *Ibid. 45*: 4357–4361 (1997).
10. Downey, G., J. Boussion, and D. Beauchêne, Authentication of Whole and Ground Coffee Beans by Near Infrared Reflectance Spectroscopy, *J. Near Infrared Spectroscopy 2*:85–92 (1994).
11. Kemsley, E.K., S. Ruault, and R.H. Wilson, Discrimination Between *Coffea arabica* and *Coffea canephora* Variant *Robusta* Using Infrared Spectroscopy, *Food Chem. 54*:321–326 (1995).
12. Clarke R.J., and R. Macrae, *Coffee*, Elsevier Applied Science, New York, 1985, Vol. 1.
13. Ratnayake, W.M.N., R. Hollywood, E.O. Grady, and B. Stavric, Lipid Content and Composition of Coffee Brews Prepared by Different Methods, *Food Chem. Toxicol. 31*:263–269 (1993).
14. Muratore, G., M.C.C. Lupo, F. Fiorenza, and C.N. Asmundo, La frazione lipidica del caffè in relazione al processo di tostatura, *Ind. Aliment. 37*:161–164 (1998).
15. Casal, S., M.B. Oliveira, and M.A. Ferreira, Discrimination of *Coffea arabica* and *Coffea canephora* var. *robusta* Beans by Their Fatty Acid Composition, in *Proceedings of Euro Food*

Chem IX, Interlaken, Switzerland, edited by R. Amadò and R. Battaglia, Swiss Society of Food and Environmental Chemistry, Wädenswil, 1997, Vol. 3, p. 685.
16. Oliveira, M.B., S. Casal, P.C. Gomes, and M.A. Ferreira, Efectos de la tostacion sobre el contenido de grasa y acidos grasos en café, *Inf. Tecnol. 9*:135–138 (1998).
17. AOAC, *Official Methods of Analysis of AOAC International,* 16th edn., Vol. 2, AOAC International, Gaithersburg, MD, 1996, 920.97.
18. Oliveira, M.B.P.P., and M.A. Ferreira, Capillary Gas Chromatographic Evaluation of *trans*-Fatty Acid Content of Food Produced Under the Traditional Conditions of Semi-industrial Frying, *J. High Resolut. Chromatogr. 19*:180–182 (1996).
19. Dagnelie, P., *Théorie et Méthodes Statistiques,* Vol. 2 (translated to Portuguese by St. Aubyn), Publicações Europa-América, Mem Martins, 1973, pp. 367–374.
20. Conover, W.J., *Practical Nonparametric Statistics*, John Wiley & Sons, New York, 1980.
21. Mardia, K.V., J.V. Kent, and J.M. Bibby, *Multivariate Analysis*, Academic Press, London, 1979, pp. 213–254, 281–299.

[Received February 14, 2001; accepted February 28, 2003]