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Discrimination between Arabica and Robusta Coffee Species on the Basis of Their Amino Acid Enantiomers

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This work reports the results for the composition of robusta and arabica coffee species in terms of their amino acid enantiomers in the green and roasted states. The analyses were conducted for the free amino acids, as well as for the amino acids obtained after acid hydrolysis. The amino acids were extracted/hydrolyzed and isolated by SPE on strong cation exchange columns, derivatized to their *N*-ethoxycarbonylheptafluorobutyl esters, and analyzed by gas chromatography/FID on a Chirasil L-Val column. Multivariate analyses applied to the results showed that the free amino acids can be used as a tool for discrimination between coffee species, with a special reference to L-glutamic acid, L-tryptophan, and pipecolic acid. There is also some evidence that these compounds can be used for discrimination between green coffees subjected to different postharvest processes. It is also shown that the amino acid levels observed after acid hydrolysis can be used for the same purposes, although displaying less discriminatory power.

KEYWORDS: Coffee; L- and D-amino acids; amino acid enantiomers; discriminant analysis; processing method

INTRODUCTION

Coffee is one of the most important international trade products. Among all of the known species, the most important are *Coffea arabica* L. (arabica coffee) and *C. canephora* Pierre (robusta coffee), which account, respectively, for about 75 and 24% of the total world coffee production. Besides their different chemical and organoleptic characteristics, these two species have very different commercial values, with the former attaining the highest market prices. For quality and economical reasons it is therefore very important to guarantee their authenticity (*1*).

Several studies have been conducted during the past decades in order to find chemical components that can be used as discriminators between different coffee species and as indicators of adulteration of coffee lots with beans of less valued species. More recently, interest has also been shown in applying these same principles to the discrimination of the geographical origin of coffees. It is important to note that such studies are not easy tasks because all vegetable products are very influenced by climate and local weather conditions, agronomical practices, and transport, storage, and distribution conditions, as well as initial or intermediate processing phases. For the time being the diterpene 16-*O*-methylcafestol is probably the best marker for the presence of small amounts of robusta beans in arabica coffee, either green or after roasting (*2*). The assessment of geographical origin has been based essentially on the mineral composition, trace element profile, and multivariate stable isotope analysis (*1*, *3*, *4*), but no method has yet been claimed to be capable of carrying out such an assessment with no ambiguity.

Following studies on the amino acid composition of green coffees using several methodologies, pipecolic acid and some free amino acids (e.g., β-alanine) have been proposed as a contribution for dealing with problems related to the identification of the botanical and geographical origin of coffee beans (*5*, *6*). Recent studies have also reported that the total concentration of free amino acids does not change significantly with the chemical reactions occurring during the harvest season and the postharvest processing steps, such as drying, fermentation, and storage (*7*, *8*). However, during the roasting process, the minor free amino acid fraction, usually not exceeding 0.5% dry weight, is extensively degraded, and only vestigial amounts are present in the roasted coffee and subsequent brews (*8*), a fact that is usually faced as a limitation to the utility of free amino acids as authenticity indicators. These problems are not found in relation to the protein fraction, which is known to be more stable upon roasting and represents a major coffee fraction, corresponding to ∼10% of its dry weight. However, the utilization of protein amino acids in the field of authenticity is also very limited, because they have been reported to exist in quite similar amounts in both species (*9*, *10*).

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The postharvest treatments applied to coffee beans constitute an additional important source of variation for the chemical and organoleptic coffee characteristics. Studies dealing with the identification of green coffee postharvest processing types (i.e., dry or wet processes) are scarce (*7*), and more information on this subject is therefore required. When compared, these two processing types are quite different: In the dry method, adopted mostly in Brazil and western Africa, the cherries are sun-dried with the pulp intact, for up to 4 weeks, producing the so-called "natural" coffees. In the wet processing method, the outer skin and part of the mucilage is mechanically eliminated and the remaining mucilage is hydrolyzed for 18−48 h, depending on ambient temperature and altitudes, either immersed in water (wet fermentation) or without it (dry fermentation). After washing, the beans are dried in the sun or by means of mechanical dryers. This method is usually used with arabica beans (except in Brazil), producing the "washed" or "mild" beans (*9*, *11*, *12*).

The majority of the studies on the total and free amino acid composition of coffee have focused on the L-amino acid enantiomers, but a recent report indicates that free D-amino acids are ubiquitous constituents of plants, although in low percentage values (*13*). Accordingly, the free and total D-amino acid contents in coffee have been the subject of recent investigations (*14*−*16*) that confirmed their presence in green beans and also indicated the existence of some effects of the roasting process (*16*) on the D-amino acids' profile, raising the question of the possible uses of these constituents in authenticity studies.

The present study was carried out to determine the composition of green and roasted coffees of arabica and robusta species in terms D- and L-amino acid enantiomers and to evaluate the opportunity of using these components in authenticity studies. The samples included in this study had different geographical origins and were known to have been subjected to different postharvest processing methods in the place of origin, and all were subjected to a standard industrial roasting procedure. The analyses were conducted for the free amino acids and also for the amino acids obtained after acid hydrolysis. This work represents a contribution to the discrimination of the most representative coffee species and provides a preliminary study for the assessment of the type of postharvest processing method applied to coffee beans (either wet or dry process), based on the D- and L-amino acids.

MATERIALS AND METHODS

Reagents. The D- and L-amino acids were all of analytical grade from Sigma (St. Louis, MO) and included alanine (Ala), glycine (Gly), valine (Val), proline (Pro), leucine (Leu), isoleucine (Ile), aspartic acid (Asp), glutamic acid (Glu), methionine (Met), phenylalanine (Phe), lysine (Lys), ornithine (Orn), tyrosine (Tyr), and tryptophan (Trp). L-Pipecolic acid (Pip), γ-amino-*n*-butyric acid (GABA), and the internal standard L-*p*-chlorophenylalanine (IS) were also from Sigma. Ethylchloroformate and pyridine were from Fluka (Neu-Ulm, Germany), and 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB), 5-sulfosalicylic acid dihydrate (SSA), and methanesulfonic acid (MSA) were from Aldrich (Steinheim, Germany). All other chemicals were of analytical grade from several other suppliers.

The strong cation exchange columns used in the cleanup procedure were Extra-Sep-SCXD, 500 mg, 3 cm^3 (Lida Manufacturing Corp., Kenosha, WI).

Coffee Samples. Coffee samples from both arabica and robusta species were studied. A local broker and industrial coffee roaster supplied all coffee samples, both green and roasted, and was able to confirm their botanical and geographical origin, as well as the general type of postharvest processing (dry/wet process). No further details were known on the samples' historical background. A standard method was used in the roasting procedure (160−220 °C, 14 min), corresponding to a dark roast, with average organic losses of ∼11% for the arabica samples and ∼13% for the robusta ones (on a dry weight basis).

The total number of samples included in the study was equal to 30 green coffee samples plus the corresponding 30 roasted beans. Samples were labeled with a first character indicating the coffee species (A = arabica, R = robusta) and a second character indicating the postharvest processing type (D = dry, W = wet). These two characters were followed by a hyphen and another character for geographical origin (using the characters italicized in the country names presented below), and finally one figure indicating the sample number within each country. The countries of origin and the numbers of samples per country, indicated in parentheses, were, for robusta species, *I*vory Coast (6), *A*ngola (5), *U*ganda (3), *C*ameroon (2), *V*ietnam (2), and In*d*ia (1); and, for arabica species, *B*razil (5), Co*l*ombia (1), Costa *R*ica (2), *G*uatemala (1), *H*onduras (1), and *M*exico (1). This sampling is representative of the coffees generally consumed in Portugal, usually for espresso blends.

Sample Preparation. *Amino Acid Extraction.* The free amino acid extraction was performed with a 2% SSA solution (1 g/50 mL) on previously defatted powdered samples with petroleum ether (*17*) and after the addition of the internal standard, L-*p*-chlorophenylalanine. For the total amino acid analysis the samples were hydrolyzed with 4 M MSA (150 mg/3 mL), under N_2, at 110 °C for 16 h on a Reacti-therm heating module (Pierce, Rockford, IL), and then diluted to 25 mL after the internal standard addition. All analyses were carried out in duplicate.

Cleanup. Coffee extracts (2 mL) were loaded into the SPE columns, after being diluted with water, to reduce the ionic strength, and the pH was adjusted to 2.2. Interferences were washed with water and the amino acids eluted with 4 M aqueous ammonia. The extracts were dried under a N_2 stream and kept below 0 °C until derivatization.

Derivatization Procedure. The dry residues were dissolved with 0.1 M HCl, transferred into silanized screw-cap vials (Supelco, Bellefonte, PA), and then derivatized using a mixture of HFB/pyridine followed by ethylchloroformate (*18*). The derivatives were extracted with chloroform and transferred to inserts adjustable to the liquid sampler vials. About 1.0 μL was injected into the gas chromatographic system.

GC Analysis. Chiral discrimination of D- and L-amino acids was achieved by gas chromatography, carried out with a Chrompack CP 9001 instrument (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector and an automatic liquid sampler (CP-9050, Chrompack). Separation was achieved on a Chirasil L-Val (25 m × 0.25 mm i.d.) fused-silica capillary column with a 0.12 μm film coating (Chrompack) with programmed temperature: increase from 80 °C (1 min hold) to 150 °C, at 5 °C/min (7 min hold), followed by an increase to 195 °C at 7 °C/min (15 min hold). The temperatures of the injector and detector were 250 and 280 °C, respectively, and splitless injection was used with a purge time delay of 0.8 min. Helium was used as the carrier gas at an initial inlet flow of 0.7 mL/min programmed to increase to 1.7 mL/min after 36 min (*18*).

The compounds were identified by their retention times in comparison with authentic standards. Quantification was based on the internal standard method using L-*p*-chlorophenylalanine.

Statistical Data Treatment. The precision of the total procedure was evaluated by the coefficient of variation of each free amino acid calculated on the basis of five repeated analyses of the same robusta green coffee. The accuracy of the method (percent recovery) was evaluated in triplicate using the same robusta green coffee spiked with three known standard amounts.

The complete data sets with the amino acid contents were analyzed by cluster analysis (CA), discriminant analysis (DA), and canonical variate analysis (CVA). CA was carried out using Ward's method with Euclidean distances (*19*) after variable standardization to mean zero and unit variance. For DA, three groups were taken into consideration: robusta dry process, arabica wet process, and arabica dry process. DA was carried out following standard algorithms (*20*) as implemented in the Statistica for Windows package. In all DA, different "F to enter" values were tried in order to select the minimum number of amino acids necessary for total discrimination between groups, that is, with no misclassifications of sample units. The reduced data values (with only the amino acids selected by DA) were further analyzed by CVA, which was carried out as implemented in the same statistical software.

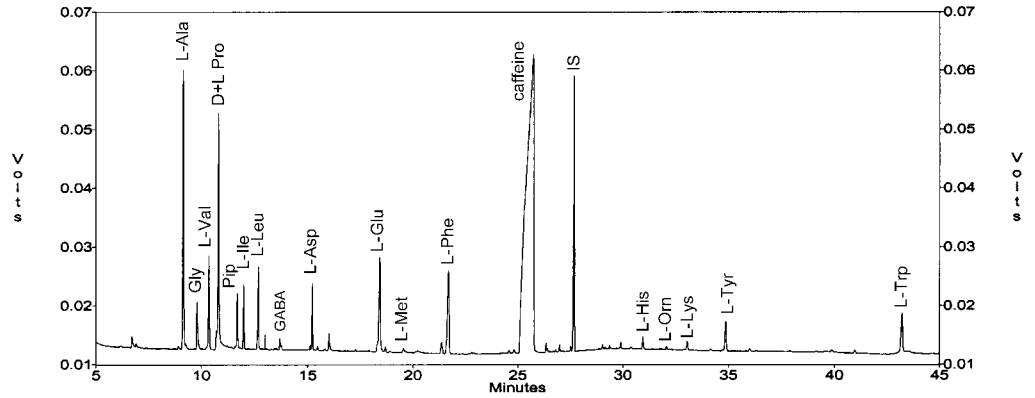

Figure 1. Free amino acids from an arabica green coffee from Brazil.

Table 1. Reproducibility of the Amino Acid Quantification Method

amino acid	precision CV% ($n = 5$)	recovery mean[a] ($n = 3 \times 3$)	quantification limit (mg/kg)
Ala	0.7	98.9 (1.4)	0.4
Gly	1.1	97.9 (1.3)	0.4
Val	0.4	98.1 (0.6)	0.3
Pro	0.6	101.9 (1.7)	0.3
Pip	0.2	93.4 (1.4)	0.4
Ile	0.2	97.3 (1.6)	0.3
Leu	0.4	97.0 (1.2)	0.3
GABA	0.9	96.8 (1.1)	7.9
Asp	1.7	99.0 (1.6)	2.1
Glu	2.7	102.6 (3.1)	3.1
Met	0.7	98.7 (1.0)	0.8
Phe	0.4	97.4 (1.3)	0.3
His	0.3	94.6 (1.4)	2.3
Orn	0.2	91.6 (1.7)	12.0
Lys	0.8	90.5 (1.7)	1.3
Tyr	0.4	99.6 (1.0)	0.4
Trp	0.6	96.4 (0.7)	0.8

[a] Standard deviation is shown in parentheses.

RESULTS AND DISCUSSION

Reproducibility of the Method. The method's precision gave results inferior to 3% for all of the compounds analyzed, as described in **Table 1**. Overall recoveries were >95% except for ornithine, lysine, and pipecolic acid, with recoveries of 91.6, 90.5, and 93.4%, respectively. These low recoveries are probably due to the very low amounts of these compounds present in the samples, which are close to the quantification limit of the technique used. Summary results are displayed in **Table 1**, where amino acids are listed following their chromatographic order of appearance.

Results for Free Amino Acids. A typical chromatogram of free amino acids obtained with an arabica green sample from Brazil (AD-B5) is shown in **Figure 1**. Whereas the green coffee samples presented values clearly falling within the method's detection range (see below), the roasted coffee samples showed only trace amounts of free amino acids, as expected (*10*), and their analysis was not performed.

The compositions of robusta and arabica green coffee samples, expressed in terms of their mean values and standard deviations over all samples of the same species, are presented in **Table 2**. Broadly speaking, the mean free amino acid compositions are very similar in both species, with L-Ala, Pro, L-Asp, L-Glu, L-Phe, and L-Trp as the most abundant amino acids. With the exception of L-Glu, which is higher in the arabica samples, robusta coffees presented higher contents of all amino acids. The mean concentrations of total free amino acids are not significantly different in both species: 3.65 ± 0.65 g/kg of dry weight (dw) for robusta green samples ($n = 19$) and 3.83 ± 0.48 g/kg of dw for arabica ($n = 11$). In general, these results are in agreement with the values presented in the literature (*8*, *10*, *21*, *22*), although most authors have reported slightly higher values for both arabica and robusta beans. The inability of this derivatization method to determine some amino acids, such as arginine, serine, and threonine, might contribute to these differences. Nevertheless, this method was chosen because the derivatization is performed at ambient temperature, avoiding racemization during the usual heating steps (*18*).

On the basis of the discussion presented in the Introduction about the consequences of the two most common types of postharvest processes on coffee composition, one would expect to find higher free amino acid levels in the wet-processed samples due to the naturally present coffee enzymes and the action of microorganisms. Nevertheless, a statistical difference in the sum of the individual free amino acid levels between the dry-processed arabicas (4.11 ± 0.36 g/kg of dw) and the wet-processed arabicas (3.51 ± 0.40 g/kg of dw) was not found in this work.

D-Amino acids were present in the green coffee samples in very low amounts (with values of <2 mg/kg of dw) except for Ala, Asp, Glu, Phe, and Lys that were slightly higher but <30 mg/kg. These values are in accordance with the 0.2−8% relative ratio to the L-amino acid as reported by Brükner (*13*) for plants in general. The total D-amino acid content was higher ($p > 0.05$) in the green robusta samples (46.6 ± 5.0 mg/kg of dw) than in the green arabica samples (30.7 ± 3.9 mg/kg of dw). One would also expect to find higher D-amino acid contents in the wet-processed samples, particularly D-Ala, D-Asp, and D-glu, naturally constituents of microbial cell walls (*14*, *23*). Nevertheless, the wet-processed arabicas presented a lower D-amino acid content (24.4 ± 4.0 mg/kg of dw) than the dry-processed ones (36.0 ± 7.8 mg/kg), in accordance with the sum of the individual amino acids already discussed.

An explanation for these results might be the fact that during the long drying stage of the dry process some fermentation is inevitable (*12*). Furthermore, it is important to take into account that an additional washing step in the wet process is performed after fermentation, to remove the remaining mucilage, and any free D- or L-amino acids can be washed out at this stage. In accordance with this is the fact that the wet coffee samples are characterized by a smaller amount of total soluble solids (*9*).

Cluster analysis was carried out in order to search for natural groupings based on the D- and L-amino acid compositions. Because the variables in the initial data set were standardized to mean zero and unit variance, all amino acids, even those existing in very low amounts, have an equal opportunity to influence the results. Several linkage methods were tried, as is

Table 2. Mean Free Amino Acid Profiles of Robusta and Arabica Green Coffee Samples[a]

amino acid	robusta ($n = 19$)			arabica ($n = 11$)		
	L (mg/kg of dw)	D (mg/kg of dw)	D (%)	L (mg/kg of dw)	D (mg/kg of dw)	D (%)
Ala	363.5 (56.8)	5.5 (4.6)	1.5	343.9 (74.2)	3.2 (1.2)	0.9
Gly[b]	69.5 (15.2)			53.7 (11.4)		
Val	120.6 (21.7)	0.6 (0.3)	0.5	100.1 (16.3)	0.6 (0.3)	0.6
Pro[c]	255.5 (71.0)			271.2 (60.1)		
Ile	71.7 (16.0)	1.1 (0.7)	1.6	65.6 (10.3)	0.6 (0.4)	0.9
Leu	98.7 (22.7)	0.8 (0.8)	0.8	80.0 (15.2)	0.4 (0.2)	0.5
Asp	539.0 (47.4)	14.0 (7.0)	3.1	515.3 (56.4)	11.0 (4.9)	2.1
Glu	924.2 (268.0)	12.7 (4.8)	1.3	1314.7 (118.2)	16.0 (6.2)	1.2
Met	15.7 (8.3)	nd		22.9 (7.5)	nd	
Phe	260.5 (75.2)	4.9 (3.8)	1.8	165.4 (32.0)	3.8 (3.1)	2.2
His	70.6 (20.0)	nd		64.7 (17.2)	nd	
Orn	34.4 (15.2)	nd		26.7 (9.8)	nd	
Lys	100.6 (43.0)	2.6 (1.6)	2.5	76.3 (14.6)	1.6 (0.8)	2.1
Tyr	124.4 (24.6)	1.9 (1.4)	1.5	69.2 (21.5)	1.5 (0.9)	2.1
Trp	337.7 (113.9)	nd		178.8 (61.0)	nd	
(Pip)	17.3 (9.8)			79.5 (17.5)		
(GABA)	844.9 (387.0)			620.9 (309.3)		
Σ	3288.7 (234.8)	49.7 (5.0)	1.4	3348.5 (331.4)	39.9 (3.9)	1.0

[a] Standard deviation is shown in parentheses. %D = D/(D + L) × 100. nd = not detected; Σ = sum without Pip and GABA. [b] Nonchiral amino acid. [c] Proline enantiomers not separable; arginine, threonine, serine, and cysteine not determinable.

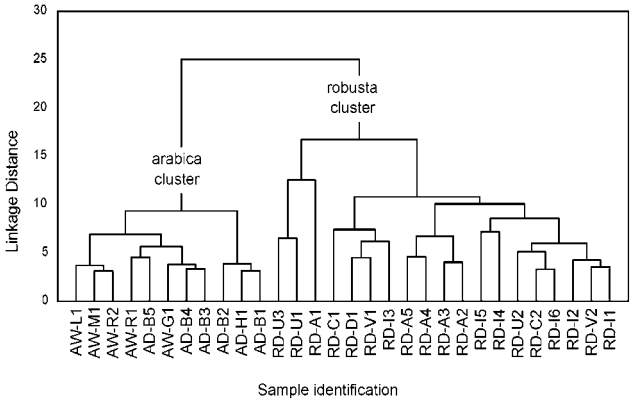

Figure 2. Dendogram expressing the result of cluster analysis on the free amino acid content of green coffee samples.

usual with this type of analysis, and it was observed that Ward's linkage method provided results consistent with the experimental design. A dendrogram expressing the results of this cluster analysis is shown in **Figure 2**.

It is immediately obvious that there is a sharp distinction between robusta and arabica coffees, forming two distinct clusters, which means that the amino acids can provide a distinction between these two coffee species. In general terms, the distance linkages between members of each cluster (expressed as the length of the vertical lines joining isolated or clustered samples) fall within the same range, indicating an apparently similar dispersion for both clusters. However, because the robusta cluster has almost double the number of samples in comparison to the arabica cluster, it must be concluded that that there is some evidence that dispersions may be higher for the arabica than for the robusta cluster, indicating higher homogeneity for the latter species in the amino acid composition.

On the basis of the same cluster analysis, a subdivision of the arabica cluster in a "wet process" cluster and a "dry process" cluster is not evident, although there is a clear distance between some of these samples, as is the case of samples AD-B1, AD-B2, and AD-H1, on the one hand, and AW-L1, AW-M1, and AW-R2, on the other. Further studies with an increased number of samples and with samples from the same origin and processed by different methods are necessary on this subject.

Cluster analysis shows how different samples or clusters of samples are from each other but does not inform on the reasons underlying such differences. To determine the most important free amino acids for discrimination between the three groups considered, a DA was carried out, using the forward method. Following this analysis, Pip, L-Ala, L-Asp, L-Trp, L-Glu, D-Glu, Pro, and L-His were selected as the most discriminatory compounds (listed by decreasing discriminatory power) among the three groups These eight amino acids were then used for a canonical variate analysis, which enables a further study of amino acid discriminatory power, as well as an observation of the main structures in the data values. As there are three groups defined, CVA defines only two canonical variates, with a relative importance given by the corresponding eigen value. **Figure 3** shows the plot of the two canonical variates. It is seen that the first variate, with an eigenvalue equal to 31.02, represents the main structure (97% of the total variation in the data values) and explains the separation between robusta and arabica species. This separation is a consequence of the higher levels of L-Trp in robusta species and the higher levels of pipecolic acid and L-Glu in the arabica samples. Therefore, these three compounds can be used for species discrimination. The presence of some particular free amino acids has been proposed as a parameter for coffee type's differentiation (*10*). In fact, pipecolic acid was initially found only in arabica coffee (*6*), but as already reported by Arnold (*21*), it was also found in low amounts in robusta samples. On the basis of the definition rule imposed in this work (of zero misclassifications), pipecolic acid cannot be used alone for species discrimination.

The second variate, with an eigenvalue of 1.48 (roughly 3% of the total variation), shows that the second data structure is the discrimination between samples of the arabica species subjected to different postharvest treatments. Dry-processed arabicas are distinguished by higher levels of L-Ala, Pro, and L-His and lower levels of L-Tyr and D-Glu, emphasizing that these observations must be viewed with caution due to the reduced number of samples used, as already discussed.

Results of the Amino Acids after Hydrolysis. The mean amounts for each amino acid analyzed after hydrolysis of the green and roasted samples are presented in **Table 3**. A typical

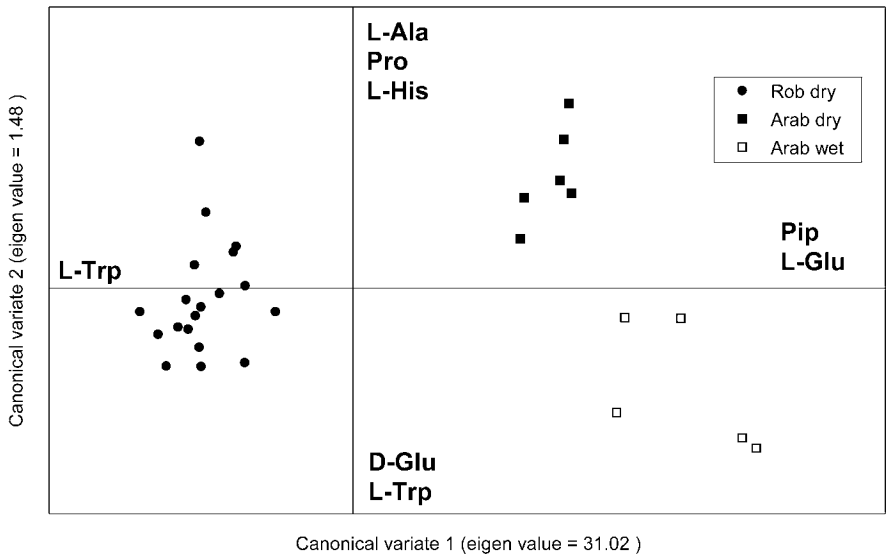

Figure 3. Canonical variate 1 versus 2 of free amino acid data.

Table 3. Mean Amino Acid Profiles of Robusta and Arabica Samples Obtained after Protein Hydrolysis[a]

	robusta						arabica					
	green ($n = 19$)			roasted ($n = 19$)			green ($n = 11$)			roasted ($n = 11$)		
amino acid	L (g/100 g of dw)	D (g/100 g of dw)	D (%)	L (g/100 g of dw)	D (g/100 g of dw)	D (%)	L (g/100 g of dw)	D (g/100 g of dw)	D (%)	L (g/100 g of dw)	D (g/100 g of dw)	D (%)
Ala	0.425 (0.094)	0.007 (0.003)	1.7	0.313 (0.057)	0.040 (0.009)	11.3	0.325 (0.102)	0.004 (0.002)	1.2	0.317 (0.075)	0.029 (0.011)	8.3
Gly[a]	0.553 (0.179)			0.355 (0.056)			0.459 (0.198)			0.364 (0.066)		
Val	0.358 (0.110)	0.002 (0.002)	0.6	0.348 (0.050)	0.020 (0.005)	5.4	0.276 (0.047)	0.002 (0.001)	0.8	0.365 (0.094)	0.014 (0.007)	3.6
Pro[b]	0.709 (0.089)			0.682 (0.101)			0.650 (0.136)			0.708 (0.080)		
Ile	0.392 (0.089)	0.004 (0.005)	1.0	0.418 (0.061)	0.005 (0.002)	1.1	0.331 (0.045)	0.005 (0.005)	1.4	0.435 (0.078)	0.006 (0.004)	1.3
Leu	0.908 (0.121)	0.007 (0.003)	0.8	0.720 (0.089)	0.036 (0.007)	4.8	0.680 (0.121)	0.009 (0.004)	1.3	0.788 (0.118)	0.028 (0.010)	3.5
Asp	0.717 (0.223)	0.013 (0.011)	1.7	0.513 (0.100)	0.263 (0.098)	33.9	0.665 (0.179)	0.014 (0.009)	2.0	0.595 (0.202)	0.296 (0.098)	33.3
Glu	1.392 (0.293)	0.040 (0.029)	1.8	0.748 (0.126)	0.160 (0.023)	17.6	1.450 (0.215)	0.038 (0.008)	2.8	1.078 (0.250)	0.159 (0.078)	12.8
Met	0.038 (0.017)	0.002 (0.002)	4.9	0.048 (0.013)	0.007 (0.003)	12.3	0.037 (0.018)	0.002 (0.002)	5.7	0.053 (0.018)	0.06 (0.004)	10.4
Phe	0.480 (0.080)	0.004 (0.002)	0.8	0.315 (0.053)	0.022 (0.004)	6.4	0.416 (0.086)	0.004 (0.003)	0.9	0.335 (0.081)	0.015 (0.008)	4.4
His	0.074 (0.023)	nd		0.037 (0.013)	nd		0.052 (0.045)	nd		0.034 (0.021)	nd	
Orn	nd	nd		0.013 (0.012)	nd		nd	nd		0.011 (0.013)	nd	
Lys	0.605 (0.150)	0.008 (0.005)	1.3	0.081 (0.016)	0.010 (0.018)	11.3	0.550 (0.097)	0.006 (0.004)	1.0	0.111 (0.091)	0.004 (0.004)	3.2
Tyr	0.374 (0.170)	0.006 (0.006)	1.5	0.270 (0.094)	0.008 (0.006)	2.9	0.269 (0.055)	0.006 (0.007)	2.3	0.223 (0.062)	0.005 (0.005)	2.3
Σ	7.025 (0.632)	0.093 (0.030)	1.3	4.862 (0.414)	0.571 (0.108)	10.5	6.163 (0.486)	0.114 (0.040)	1.8	5.411 (0.617)	0.567 (0.143)	9.5

[a] Standard deviation is shown in parentheses. %D = D/(D + L) × 100. nd = not detected. [b] Nonchiral amino acid. [c] Proline enantiomers not separable; arginine, threonine, serine, and cysteine not determinable.

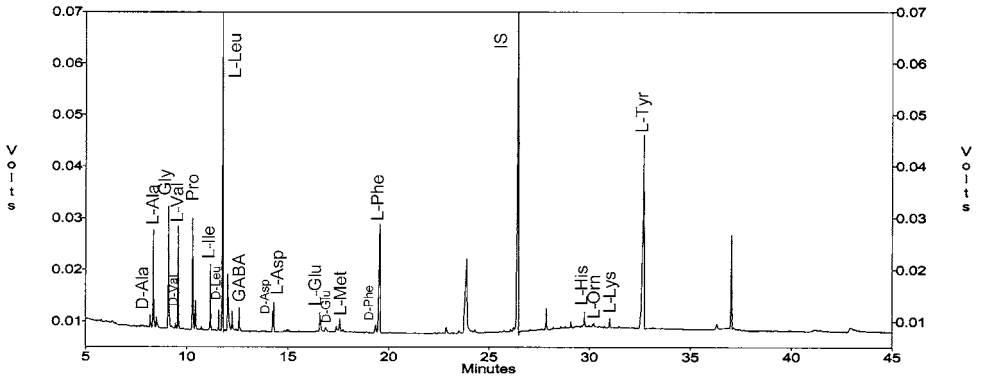

Figure 4. Amino acids from a roasted robusta coffee from Angola (RD-A1), obtained after protein hydrolysis.

chromatogram from a roasted coffee sample is presented in **Figure 4**.

As with the free amino acids, the robusta green coffees, in comparison with the arabica ones, presented higher levels of all individual amino acids. Comparison of the sum of the individual amino acids showed significant differences ($p < 0.05$) between the two species: robusta samples showed a higher mean content (7.17 ± 0.65 g/100 g of dw) than arabica samples (5.91

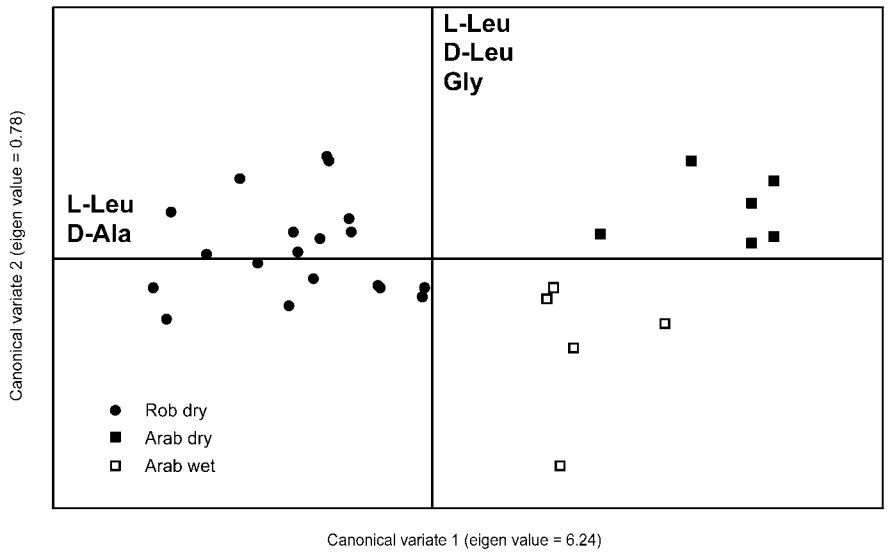

Figure 5. Canonical variate 1 versus 2, depicting the main relationships of amino acid data obtained after hydrolysis of green coffee.

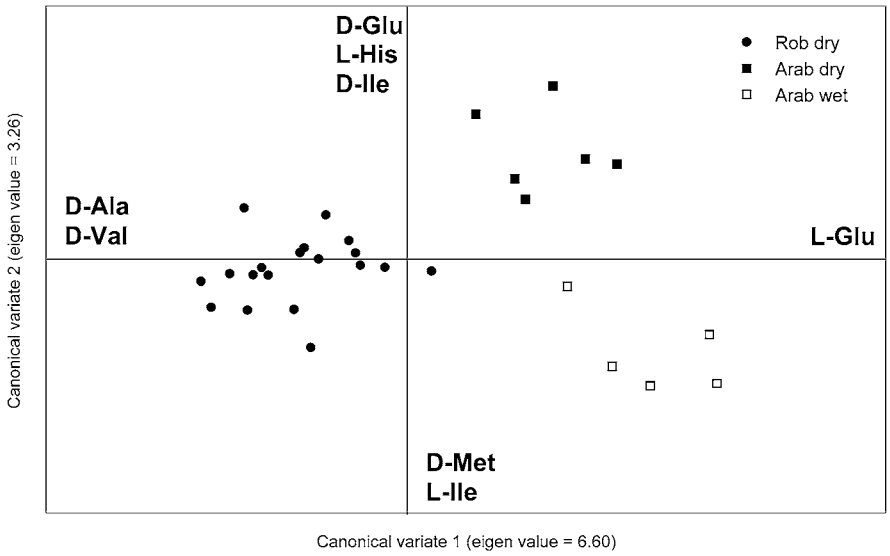

Figure 6. Canonical variate 1 versus 2, depicting the main relationships of amino acid data obtained after hydrolysis of roasted coffee.

± 0.68 g/100 g of dw), with no statistical differences between the dry-processed arabicas (6.03 ± 0.80 g/100 g of dw) and the wet (5.76 ± 0.56 g/100 g of dw).

The high temperatures achieved during the roasting process are expected to have a dramatic effect on the protein present in coffees (*10*), with reported protein losses in the range of 20−40% or more (*9*). In the robusta roasted coffee samples analyzed, the mean level of total amino acids was lower by ∼20% when compared with the green samples, whereas in the arabica samples this effect could not be observed. Generally speaking, the levels of all individual amino acids decreased during the roasting process, with the exceptions of valine, proline, and isoleucine that remained constant or increased slightly due to the mass loss naturally occurring during roasting. Lysine, a particularly reactive amino acid, presented the highest degradation during roasting, with ∼80% reduction in both coffee species. The D-amino acid content in roasted samples increased significantly ($p < 0.01$). The higher increase was observed for D-Asp, achieving a 50% D/L ratio after roast, followed by D-Glu, with 15%. All other D-forms presented ratios inferior to 10%.

As previously done for free amino acids, DA was first used for the selection of a reduced number of amino acids by their discriminatory power, followed by a CVA. For green samples, the selected amino acids listed by decreasing order of discriminatory power were L-Leu, D-Leu, D-Ala, D-Asp, D-Ile, D-Lys, Gly, L-Val, L-Ile, and D-Met. The results for the CVA are shown in **Figure 5**. Again, two main structures are visible. The first canonical variate shows that high levels of L-Leu and D-Ala characterize robusta green samples, a fact that represents 89% of the total variation in the results. The second canonical variate displays the remaining 11% of the information, with dry-processed arabica samples exhibiting higher levels of L-Leu and D-Leu, followed by Gly and D-Met. This last structure is less significant as shown by an eigenvalue of <1, which reflects a lower discrimination between groups in comparison to the dispersion within both arabica groups. As a consequence, one must conclude that the total amino acids are still useful for discrimination between species in the green state, but the discrimination between wet and dry green arabica samples is doubtful.

For roasted coffee samples the amino acids selected by DA, by decreasing discriminatory power, were L-Glu, D-Ala, L-His,

D-Ile, D-Glu, L-Ile, L-Asp, D-Val, D-Met, and Gly. **Figure 6** presents results from the CVA carried out with these amino acids. The first canonical variate, representing ~67% of the total information, is characterized by an opposition between D-Ala and D-Val (higher in robusta coffees) and L-Glu (higher in arabica coffees). The second canonical variate, resuming 33% of the total variation in the data values, can be explained by the existence of higher levels of D-Glu, L-His, and D-Ile in dry arabica samples, as opposed to the wet arabica samples, which show higher contents in D-Met and L-Ile. In this case, robusta and arabica coffee species are again well discriminated, whereas the distinction between dry- and wet-processed arabica samples is much more pronounced than with the green samples.

As a general conclusion one can say that D- and L-amino acids show high potential to be used as coffee species' discriminators. This includes discrimination with free and total amino acids, although the former seem to be more promising. Regarding the discrimination between coffees subjected to the two different postharvest treatments assayed, again the free amino acids proved to be superior.

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