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Roast effects on coffee amino acid enantiomers

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Abstract

The effects of roast on the amino acid enantiomers (D- and L-) of two green coffee samples (*arabica* and *robusta*) were determined, by gas chromatography, on a Chirasil L-Val column. The free amino acids were present in low amounts in both green samples and destroyed to a very high degree with roast. After hydrolysis, the amino acids behaved according to their thermal stabilities: some amino acids remained unchanged whilst the more thermally sensitive were slightly reduced. Generally, the mean free amino acid racemisation value was higher than that calculated after hydrolysis, and was also higher for the *robusta* sample. In both cases, an increase in the racemisation value with temperature was observed, with aspartic acid being the most sensitive amino acid. The results suggested that the *robusta* coffee matrix is more affected by roast than the *arabica*. The amount of D-amino acids ingested per coffee cup does not seem to constitute a serious food safety problem.

Keywords: Coffee; L- and D-Amino acids; Racemisation value; Roasting; Food safety

1. Introduction

The nutritional value and the possible toxic effects of D-amino acids have been raising several questions over the past decade. Although initially considered unnatural, the recent chromatographic work has proved that they are almost ubiquitous in nature. Some D-amino acids, namely aspartic acid, serine and alanine, are distributed in tissues and body fluids of vertebrates, suggesting that they may play important physiological roles.

Although some D-amino acids are detected in the human organism, their function, metabolism, nutritional importance or toxicological consequences are still unknown. The main sources of D-amino acids in humans include: (i) food and drink intake, (ii) degradation of cell walls of microorganisms in the gut or (iii) de novo synthesis (Zagon, Dehne, & Bögl, 1994). Only 10–20% of the ingested D-amino acids are excreted. The remaining 80–90% are absorbed in the intestine and metabolised into α-oxoacids by the D-amino acid oxidase, mainly present in the liver, kidney and brain (D'Aniello et al., 1993). The accumulation of D-amino acids in the organism, resulting from low D-amino acid oxidase activity or reduced degradation rate of peptides containing D-amino acids, may elicit serious damage, namely enzyme synthesis inhibition and growth suppression (D'Aniello, Di Fiore, & Fisher, 1998).

Two main pathways for D-amino acid formation in foodstuffs and beverages are known: technological enrichment during food processing (heat, pH) and microbial activity during fermentation processes (cell wall lyses, racemases) (Friedman, 1999; Zagon et al., 1994). The amino acid enantiomeric ratio is becoming increasingly popular in food industry control. The possible fields of application include the control of technological processes (fermentations and heat treatments) and detection of adulteration or microbial contamination.

Green (unroasted) coffee has no desirable taste or aroma of its own. These characteristics are developed only after the roasting procedure. During this severe heat process, the coffee beans undergo several physical and chemical modifications, especially above 180–200 °C. In quantitative terms, major chemical alterations have been described for chlorogenic acid (Trugo & Macrae, 1984), sucrose (Trugo & Macrae, 1982),

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trigonelline (Casal, Oliveira, & Ferreira, 2000a; Stennert & Maier, 1994) and amino acids (Macrae, 1987; Nehring & Maier, 1992; Roffi, Santos, Mexia, Busson, & Maigrot, 1971).

Free and total amino acid profiles have been evaluated in coffee by several authors during the past decade, mostly without enantiomer separation (Clifford, 1985; Macrae, 1987). The published results vary widely, depending on several parameters such as species/varieties, degree of maturation, storage, roasting conditions and the analytical technique used (Smith, 1987). The amino acid contents are usually expressed on a dry mass basis, without corrections for the mass loss occurring during roasting. This leads to variations more related to the amino acids thermal stabilities than to the coffee matrix itself. The real amino acid behaviour with roast is therefore less intelligible.

Under roasting conditions the amino acids partially racemise. Therefore, D-amino acids were detected in roasted coffee, either free (Brückner & Hausch, 1989; Palla, Marchelli, Dossena, & Casnati, 1989) or bound (Nehring & Maier, 1992). In particular, the D-amino acid contents after hydrolysis have been proved to be useful for the purpose of indirect organic roasting loss control, although requiring a long analysis time (Nehring & Maier, 1992).

Our work was intended to study the effect of roast on the free and bound amino acids, both D- and L-enantiomers, to quantify them and to compare *arabica* and *robusta* coffee susceptibilities regarding amino acid racemisation.

2. Materials and methods

2.1. Chemicals

The D- and L-amino acid standards 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), tryptophan (Trp) and hydroxyproline (OH-Pro). L-pipecolic acid (Pip), γ-amino-*n*-butyric acid (GABA), β-alanine (β-Ala) and the internal standard L-*p*-chlorophenylalanine (IS) were also from Sigma. Ethylchloroformate (ECF) and pyridine (Py) were from Fluka (Neu-Ulm, Germany), 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB), 5-sulphosalicylic acid dihydrate (SSA) and methylsulphonic acid (MSA) were from Aldrich (Steinheim, Germany). All other chemicals were of analytical grade from several suppliers. The strong cation exchange columns used in the clean-up procedure were Extra-Sep-SCXD, 500 mg, 3 cm^3 (Lida Manufacturing Corp., Kenosha,WI).

2.2. Coffee samples

Two green (unroasted) coffee samples were provided by a local industrial coffee roaster – one from Brazil (*Coffea arabica* L., var. *typica*, dry-processed) and another from the Ivory Coast (*Coffea canephora* Pierre, var. *robusta*, dry-processed). The samples were roasted in our laboratory, in a WTC blinder stove (Tuttlinger, Germany) for 15 min at different temperatures (140, 160, 180, 200, 220 and 240 °C).

2.3. Sample characterisation

In order to fully characterise the samples under study, several physical and chemical parameters were evaluated. Water content was determined by oven-drying the fresh ground roasted coffee at 102 ± 2 °C to constant weight; fat content was determined by Soxhlet extraction with petroleum ether according to the AOAC method 920.97 (2000); crude protein was determined according to the Kjeldahl method, using the conversion factor 6.25; caffeine and trigonelline were determined by HPLC (Casal, Oliveira, & Ferreira, 1998). All the chemical parameters were corrected for the roast weight loss.

2.4. Amino acids 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. Extraction was achieved by magnetic stirring at room temperature, for 2×15 min, after the addition of the internal standard, L-*p*-chlorophenylalanine (Casal, Oliveira, Alves, & Ferreira, 2001).

In order to determine the total amino acid profile, fresh ground coffee samples (150 mg) were hydrolysed with 4 M MSA (3 ml) in pyrolised vials (490 °C, 4 h). Oxygen was previously removed by bubbling N_2 for about 15 min. The vials were closed and heated in a "Reacti-therm" heating block (Pierce, USA) at 110 °C for 16 h. After achieving room temperature, the internal standard was added and the volume adjusted to 25.0 ml.

2.5. Solid phase extraction

After dilution with water, in order to reduce the ionic strength, and adjustment to pH 2.2, coffee extracts, prepared for both free and total amino acid analysis, were loaded into the strong cation exchange SPE columns. Interferences were eliminated with water and the amino acids were collected with 4 M aqueous ammonia. The extracts were dried at 40 °C, under a N_2 stream, and kept below 0 °C until derivatisation.

2.6. Derivatisation procedure

In order to determine the amino acid enantiomers by gas chromatography, *N*-ethoxycarbonylheptafluorobutyl ester derivatives were prepared (Casal et al., 2000b). Briefly, the dry amino acid residues were dissolved in 0.1 M HCl, transferred into silanised screw-cap vial (Supelco, Bellefonte, PA) and then derivatised using a mixture of HFB-Py (2:1 v/v), followed by ECF. The vial was tightly capped and vortexed. Subsequently, chloroform and sodium chloride were added and the vial shaken for extraction of the derivatives into the organic layer. About 1.0 μl of the organic phase was injected into the gas chromatographic system.

2.7. 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 ionisation 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: 80 °C (1 min hold), 5 °C/min to 150 °C (7 min hold), 7 °C/min to 195 °C (15 min hold). Helium was used as carrier gas at an initial inlet flow of 0.7 ml/min and increase to 1.7 ml/min after 36 min. Splitless injection was used with a purge time delay of 0.8 min. The temperatures of the injector and detector were 250 and 280 °C, respectively.

2.8. Amino acids quantification

Response factors relating to the internal standard were determined for all the amino acids, after subjecting the standard solutions to the SPE extraction. Calibration curves were also determined. The relative amount of the D-enantiomer, or racemisation value, was determined according to the following equation: $\%D = A_D/(A_D + A_L) \times 100$, where A_D and A_L are the peak areas of the D- and L-enantiomers, respectively.

3. Results and discussion

3.1. General

Complete isolation of free amino acids from the green coffee beans is difficult, particularly if quantitative data are required (Macrae, 1987). Based on literature reports, several extracting agents were tested (ethanol, methanol/picric acid, methanol/water and SSA), with or without preliminary lipids removal. The best results, corresponding to higher chromatographic peak areas and cleaner chromatograms, were achieved with SSA after previous fat extraction (data not shown), as already reported by Arnold, Ludwig, Kühn, and Möschwitzer (1994).

Protein hydrolysis is required in order to determine the total individual amino acids. Conventional acidic hydrolysis uses 6 M HCl for 20–24 h at 110 °C (Weiss, Manneberg, Juranville, Lahm, & Foutoulakis, 1998). These conditions inevitably result in partial racemisation of the amino acids, proportional to the hydrolytic conditions applied (Fountoulakis & Lahm, 1998; Weiss et al., 1998). When the naturally occurring enantiomers are the main subject, the hydrolysis must be performed with caution: less drastic hydrolysis conditions than the classical ones should be used, without compromising the protein hydrolysis (Stenberg, Marko-Varga, & Öste, 2001).

In order to investigate the degree of racemisation introduced by the hydrolysis on coffee, a green coffee sample was hydrolysed at 110 °C, from 12 to 24 h, with both 6 M HCl and 4 M MSA, the latter being described as inducing less isomerisation (Fountoulakis & Lahm, 1998). The results showed that the individual amino acid chromatographic areas increased from 12 to 16 h, remaining almost constant or slightly reduced until the 24th h tested (data not shown). The relative amount of the D-enantiomer also increased with the hydrolysis time, achieving higher intensities after 18 h. Some amino acids, namely Asp, Glu, Lys and Ala, presented higher racemisation. After 16 h, Asp racemisation, using MSA, produced lower amounts of the D-form (2.4%) than using the HCl hydrolysis (5.7%). Phenol or tryptamine addition did not influence the racemate formation (Fountoulakis & Lahm, 1998).

The SPE performance was also adjusted for the extracts under analysis. In order to find the maximum loading capacity, increasing volumes of acidic coffee solution were loaded on to the SCX columns and the amino acid peak areas compared. A linear increase was observed until the equivalent of 45 mg green coffee was loaded. Additionally, and due to the high ionic strength of the acidic coffee extracts, several dilutions were performed in order to find the optimal value. The highest recoveries were achieved with 0.005 M extracts (data not shown).

The samples where fully characterised, both physically and chemically, using several standard determinations. These results are shown in Table 1. As discussed in Section 1, and in order to understand what really happens within the coffee beans, the results where all normalised for the mass weight loss.

The protein, calculated directly from total Kjeldhal nitrogen, is of limited value, as there are many other nitrogen components in coffee (Macrae, 1987). Therefore, the protein content reported in Table 1 was calculated as 6.25 × (Kjeldhal N − caffeine N − trigonelline N).

Table 1
Characterisation of the coffee samples (%)

Roasting temperature (°C)	Moisture content		Weight loss (dw)		Fat (dw[a])		Caffeine (dw[a])		Trigonelline (dw[a])		Protein (dw[a,b])	
	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
Green	8.5	8.3	–	–	13.8	10.0	1.24	2.08	0.89	0.63	12.8	13.6
140	6.8	6.6	5.0	7.7	10.9	7.3	1.29	2.08	0.82	0.60	11.5	12.3
160	5.1	5.3	7.8	15.8	11.2	7.1	1.26	1.89	0.79	0.51	10.7	11.4
180	4.0	4.4	10.1	18.0	10.9	7.6	1.19	1.70	0.78	0.50	11.0	10.0
200	2.7	2.4	15.5	22.7	10.2	6.6	1.16	1.66	0.70	0.45	9.7	10.8
220	1.6	1.4	17.7	23.9	10.6	7.4	1.07	1.65	0.50	0.35	10.1	10.3
240	2.0	2.0	22.6	27.9	12.3	9.3	0.87	1.52	0.04	0.08	8.7	9.9

dw, dry weight.
[a] Corrected for weight loss.
[b] With previous subtraction of caffeine and trigonelline nitrogen.

3.2. Free amino acid composition

The individual free amino acid contents (D + L) are listed in Tables 2 and 3, for *arabica* and *robusta* coffee samples, respectively. Free amino acids, initially at low levels in both green samples, were highly destroyed during roasting, especially above 180 °C. At 240 °C, a temperature out of the usual roasting range, no free amino acids were detected in either species. In Portugal, roasting is performed with high temperature and long roasting times (HTLT roasting), such as 8–12 min from 180 to 220 °C (Clarke, 1987).

Generally, both unroasted samples presented the same major and minor amino acids. With the exception of glutamic acid, the amino acids determined were higher in the *robusta* coffee sample, especially at the lower temperatures assayed, as is usually described (Clifford, 1985; Illy & Viani, 1995). The three main amino acids of both *arabica* and *robusta* green coffees were Glu, followed by Asp and GABA. Orn, Pip and β-Ala, first quantified by HPLC in green coffee (Arnold et al., 1994), were also determined. These compounds were found in very small amounts and were also slightly higher in the *robusta* sample, with the exception of Pip. All amino-compounds analysed decreased with roasting, although at different rates. This fact might be related, not only to their inherent thermal stabilities, but also to the coffee matrix itself, since the same amino acids behaved differently in the *arabica* and *robusta* samples.

Table 2
Free amino acids determined in the *arabica* coffee sample

Amino acid (mg/kg dw)[a]	Unroasted	Roasted					
		140 °C	160 °C	180 °C	200 °C	220 °C	240 °C
Ala	292 ± 2.1	250 ± 0.5	119 ± 0.8	21.1 ± 0.5	4.1 ± 0.1	1.1 ± 0.1	vest
Gly	49.4 ± 0.3	45.4 ± 0.3	19.0 ± 0.4	5.7 ± 0.3	5.4 ± 0.1	2.7 ± 0.1	nd
Val	128 ± 0.7	125 ± 1.2	126 ± 2.4	58.9 ± 0.9	14.7 ± 0.8	2.4 ± 0.2	vest
Pro	303 ± 1.6	256 ± 1.2	234 ± 0.7	56.8 ± 0.5	2.0 ± 0.2	1.4 ± 0.2	nd
Ile	75.7 ± 0.1	70.9 ± 0.2	56.2 ± 0.2	20.0 ± 0.2	8.7 ± 0.4	2.0 ± 0.1	nd
Leu	73.2 ± 0.4	70.4 ± 0.2	14.5 ± 0.2	6.7 ± 0.2	1.5 ± 0.0	1.2 ± 0.2	nd
Asp	523 ± 5.3	489 ± 2.1	434 ± 3.3	369 ± 10.2	71.5 ± 8.5	6.9 ± 2.0	nd
Glu	1414 ± 11.4	1411 ± 10.2	108 ± 7.7	129 ± 2.4	30.3 ± 1.4	35.9 ± 2.3	vest
Met	18.5 ± 1.2	6.7 ± 0.4	2.4 ± 0.4	nd	nd	nd	nd
Phe	170 ± 1.5	138 ± 0.8	25.3 ± 0.3	2.8 ± 0. 1	1.4 ± 0.1	1.2 ± 0.2	vest
His	50.2 ± 1.0	49.1 ± 0.1	13.2 ± 0.1	nd	nd	nd	nd
Lys	68.3 ± 0.2	22.7 ± 0.2	9.6 ± 0.2	nd	nd	nd	nd
Orn	16.0 ± 0.2	12.1 ± 0.2	vest	vest	nd	nd	nd
Tyr	64.8 ± 1.3	39.7 ± 0.6	22.6 ± 0.4	1.5 ± 0.1	1.1 ± 0.1	vest	vest
Trp	151 ± 1.5	142 ± 1.0	27.8 ± 0.8	2.5 ± 0.1	nd	nd	nd
Pip	68.2 ± 0.0	73.0 ± 0.2	73.3 ± 0.1	16.9 ± 0.2	nd	nd	nd
GABA	397.0 ± 10.2	237 ± 2.5	220 ± 1.8	73.0 ± 1.7	17.7 ± 4.3	nd	nd
β -Ala	3.9 ± 0.1	3.6 ± 0.1	2.7 ± 0.1	0.2 ± 0.0	vest	nd	nd
Sum	3867	3443	2480	764	158	54.7	vest
%D[b]	1.4	2.6	7.1	14.5	30.1	56.0	–

[a] All values were corrected for the roast loss.
[b] Racemisation value (see Section 2).

Table 3
Free amino acids determined in the *robusta* coffee sample

Amino acid (mg/kg dw)[a]	Unroasted	Roasted					
		140 °C	160 °C	180 °C	200 °C	220 °C	240 °C
Ala	363 ± 2.1	370 ± 0.0	275 ± 1.2	158 ± 0.2	13.5 ± 0.1	2.6 ± 0.1	vest
Gly	79.6 ± 1.2	77.3 ± 0.5	56.2 ± 0.3	29.7 ± 0.1	8.4 ± 0.3	3.7 ± 0.2	vest
Val	147 ± 1.3	99.9 ± 1.1	89.9 ± 0.4	44.9 ± 0.2	5.4 ± 0.3	0.9 ± 0.2	vest
Pro	315 ± 1.2	234 ± 0.8	194 ± 0.3	81.2 ± 0.1	7.9 ± 0.1	0.4 ± 0.2	vest
Ile	88.0 ± 0.6	77.1 ± 0.3	49.3 ± 0.2	23.8 ± 0.4	2.5 ± 0.0	0.9 ± 0.2	nd
Leu	92.9 ± 0.7	75.2 ± 0.5	58.6 ± 0.3	20.2 ± 0.1	1.9 ± 0.1	0.7 ± 0.2	vest
Asp	602 ± 1.5	501 ± 0.9	345 ± 0.1	106 ± 0.3	5.6 ± 0.2	3.8 ± 0.2	nd
Glu	1165 ± 12.3	1191 ± 7.9	1028 ± 1.7	229 ± 0.1	83.1 ± 0.0	30.3 ± 0.1	vest
Met	29.9 ± 1.3	27.2 ± 0.5	21.9 ± 0.0	7.7 ± 0.1	nd	nd	nd
Phe	251 ± 0.3	273 ± 1.0	174 ± 0.2	55.2 ± 0.3	4.4 ± 0.3	6.4 ± 0.3	vest
His	90.6 ± 2.2	92.7 ± 0.2	50.2 ± 2.7	24.8 ± 0.6	nd	nd	nd
Lys	87.9 ± 0.8	87.7 ± 0.8	67.4 ± 1.2	18.6 ± 0.6	nd	nd	nd
Orn	45.7 ± 1.7	22.4 ± 0.4	13.1 ± 0.3	nd	nd	nd	nd
Tyr	111 ± 0.6	98.4 ± 0.2	66.4 ± 0.4	27.2 ± 0.3	1.7 ± 0.0	vest	vest
Trp	431 ± 1.0	375 ± 1.4	256 ± 0.8	84.0 ± 2.3	3.4 ± 0.1	nd	nd
Pip	17.8 ± 0.4	19.0 ± 0.2	33.4 ± 0.7	15.9 ± 0.3	4.3 ± 0.2	0.6 ± 0.2	nd
GABA	668 ± 7.0	287 ± 3.2	199 ± 2.8	91.4 ± 0.5	37.6 ± 2.4	12.5 ± 0.8	nd
β-Ala	5.7 ± 0.3	5.1 ± 0.5	5.8 ± 0.9	3.0 ± 0.1	vest	nd	nd
Sum	4590	3913	2982	1020	179.7	62.8	vest
%D[b]	1.6	3.0	8.8	15.5	41.5	68.3	–

[a] All values were corrected for the roast loss.
[b] Racemisation value (see Section 2).

For D-amino acids, the mean racemisation values (included in Tables 2 and 3) describe a similar behaviour in both samples, consisting of a gradual increase with the roasting temperature, although with higher values for the *robusta* coffee sample. The racemisation observed in the green coffee samples is in accordance with the values described for plants in general (Brückner & Westhauser, 2003). For a more detailed illustration, Figs. 1 and 2 present the individual racemisation values for the six main isomers, for *arabica* and *robusta* coffees, respectively.

In *arabica* coffee, the racemisation values remained below 15% until 160 °C, after which they began to increase at different rates. This temperature has been associated with the actual roasting start, when the beans reach about 5% moisture content and the process becomes exothermic (Clarke, 1987). Asp, Glu and Phe achieved racemisation values greater than 50% at 220

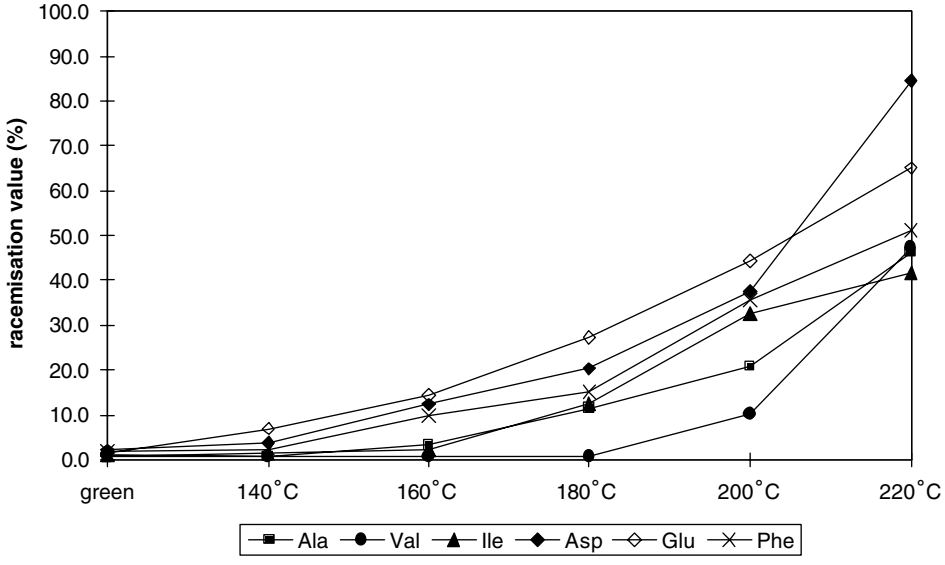

Fig. 1. Evolution of free amino acid racemisation values in the *arabica* coffee sample.

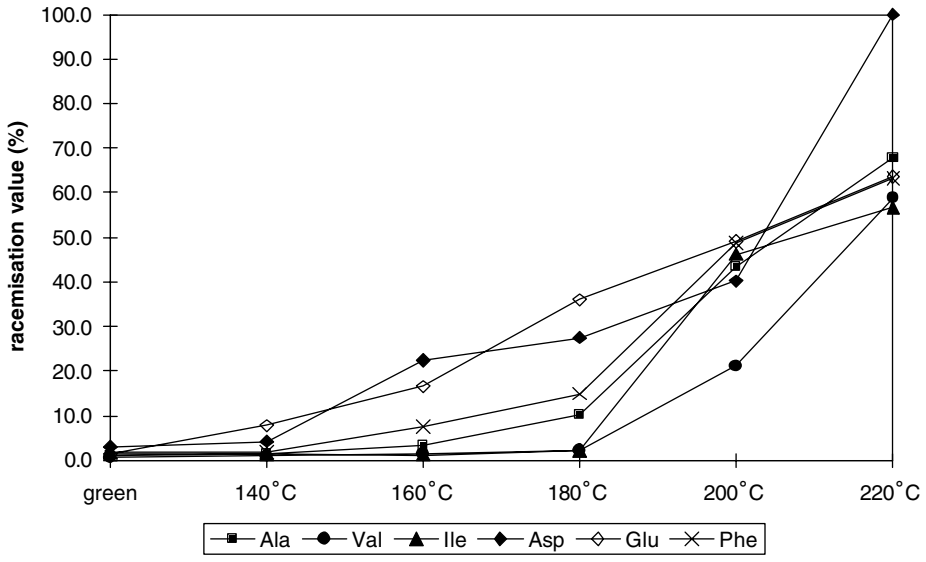

Fig. 2. Evolution of free amino acid racemisation values in the *robusta* coffee sample.

°C, before being completely lost at 240 °C, as presented in Table 2. On the other hand, Val was the more stable amino acid (racemisation began to increase only after 180 °C).

The racemisation order of amino acids in *robusta* showed almost the same trend as that of *arabica* but the process was more intense. At 160 °C, Asp and Glu already had values above 15%. At 220 °C, all amino acid racemisation values were above 55%, indicating a higher D-enantiomer amount than the L-counterpart. Asp registered the highest racemisation value, achieving 100% at 220 °C. Once again, Val was the most stable amino acid.

These observations might indicate that the *arabica* coffee matrix is more protected from the temperature effects, especially at the lower temperatures. The higher *arabica* lipid content (see Table 1) expelled to the surface during roasting could act as a physical barrier, protecting the inner coffee matrix (Clifford, 1985). It would be interesting to determine the D% at the surface of the beans and compare with the inside amounts.

3.3. Amino acid composition after hydrolysis

Tables 4 and 5 show the individual amino acid obtained after hydrolysis, including both D- and L-

Table 4
Arabica amino acids content after protein hydrolysis

Amino acid (g/100 g dw)[a]	Unroasted	Roasted					
		140 °C	160 °C	180 °C	200 °C	220 °C	240 °C
Ala	0.43 ± 0.02	0.43 ± 0.03	0.44 ± 0.03	0.43 ± 0.02	0.44 ± 0.02	0.44 ± 0.01	0.40 ± 0.03
Gly	0.46 ± 0.03	0.47 ± 0.04	0.48 ± 0.03	0.49 ± 0.03	0.49 ± 0.02	0.47 ± 0.01	0.21 ± 0.03
Val	0.30 ± 0.01	0.30 ± 0.04	0.29 ± 0.03	0.27 ± 0.03	0.26 ± 0.02	0.26 ± 0.02	0.28 ± 0.03
Pro	0.80 ± 0.03	0.82 ± 0.06	0.81 ± 0.03	0.80 ± 0.03	0.82 ± 0.03	0.83 ± 0.00	0.78 ± 0.03
Ile	0.41 ± 0.03	0.40 ± 0.03	0.40 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.36 ± 0.00	0.35 ± 0.02
Leu	0.70 ± 0.02	0.71 ± 0.05	0.73 ± 0.03	0.73 ± 0.01	0.70 ± 0.03	0.73 ± 0.00	0.67 ± 0.03
Asp	0.84 ± 0.01	0.77 ± 0.01	0.69 ± 0.01	0.56 ± 0.00	0.46 ± 0.01	0.44 ± 0.01	0.39 ± 0.01
Glu	1.67 ± 0.05	1.55 ± 0.01	1.49 ± 0.02	1.47 ± 0.02	1.45 ± 0.11	1.36 ± 0.01	0.73 ± 0.03
Met	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.05 ± 0.01	0.02 ± 0.00
Phe	0.37 ± 0.00	0.38 ± 0.00	0.39 ± 0.00	0.40 ± 0.00	0.40 ± 0.00	0.38 ± 0.01	0.36 ± 0.00
His	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.01	0.09 ± 0.00	0.06 ± 0.00	0.04 ± 0.00
Lys	0.58 ± 0.01	0.56 ± 0.01	0.49 ± 0.00	0.43 ± 0.02	0.19 ± 0.00	0.17 ± 0.01	0.12 ± 0.01
Tyr	0.30 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.28 ± 0.00	0.30 ± 0.00	0.29 ± 0.01
OH-Pro	0.61 ± 0.04	0.56 ± 0.05	0.54 ± 0.03	0.57 ± 0.03	0.53 ± 0.03	0.50 ± 0.02	0.19 ± 0.02
Sum	7.6	7.3	7.1	7.0	6.5	6.3	4.8
%D[b]	1.1	1.1	1.3	2.1	3.2	5.3	11.9

[a] All values were corrected for the roast loss.
[b] Racemisation value (see Section 2).

Table 5
Robusta amino acids content after protein hydrolysis

Amino acid (g/100 g dw)[a]	Unroasted	Roasted					
		140 °C	160 °C	180 °C	200 °C	220 °C	240 °C
Ala	0.49 ± 0.01	0.50 ± 0.04	0.49 ± 0.03	0.51 ± 0.01	0.48 ± 0.01	0.45 ± 0.00	0.41 ± 0.04
Gly	0.63 ± 0.01	0.62 ± 0.04	0.61 ± 0.03	0.62 ± 0.06	0.64 ± 0.01	0.46 ± 0.00	0.32 ± 0.01
Val	0.39 ± 0.01	0.41 ± 0.04	0.43 ± 0.01	0.40 ± 0.04	0.39 ± 0.02	0.36 ± 0.03	0.35 ± 0.00
Pro	0.83 ± 0.01	0.73 ± 0.06	0.76 ± 0.07	0.72 ± 0.06	0.75 ± 0.02	0.76 ± 0.02	0.73 ± 0.05
Ile	0.44 ± 0.00	0.46 ± 0.04	0.45 ± 0.03	0.45 ± 0.03	0.38 ± 0.01	0.38 ± 0.01	0.35 ± 0.00
Leu	0.91 ± 0.02	0.90 ± 0.04	0.88 ± 0.06	0.87 ± 0.09	0.78 ± 0.01	0.66 ± 0.03	0.48 ± 0.00
Asp	0.85 ± 0.03	0.84 ± 0.02	0.57 ± 0.02	0.41 ± 0.01	0.34 ± 0.00	0.29 ± 0.00	0.30 ± 0.00
Glu	1.50 ± 0.03	1.36 ± 0.04	1.10 ± 0.09	1.04 ± 0.05	1.08 ± 0.04	0.86 ± 0.01	0.83 ± 0.00
Met	0.16 ± 0.01	0.17 ± 0.00	0.17 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.03 ± 0.00
Phe	0.52 ± 0.00	0.53 ± 0.00	0.46 ± 0.01	0.48 ± 0.01	0.67 ± 0.00	0.33 ± 0.00	0.39 ± 0.00
His	0.14 ± 0.01	0.11 ± 0.00	0.11 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Lys	0.66 ± 0.01	0.65 ± 0.01	0.55 ± 0.02	0.38 ± 0.01	0.16 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
Tyr	0.40 ± 0.00	0.37 ± 0.00	0.36 ± 0.01	0.34 ± 0.00	0.29 ± 0.00	0.36 ± 0.00	0.30 ± 0.01
OH-Pro	0.39 ± 0.04	0.41 ± 0.04	0.41 ± 0.03	0.38 ± 0.03	0.38 ± 0.02	0.32 ± 0.02	0.23 ± 0.02
Sum	8.3	8.0	7.3	6.8	6.5	5.5	4.8
%D[b]	1.0	1.1	1.4	1.9	3.3	5.2	11.1

[a] All values were corrected for the roast loss.
[b] Racemisation value (see Section 2).

enantiomers, all corrected for the mass loss. The sum of the individual amino acids is lower then the corrected protein content described in Table 1. The fact that this methodology is unable to determine some amino acids (Casal et al., 2000a, 2000b) could be an explanation, but it also confirms that some of the nitrogen accounted as protein in the roasted coffee has other sources than amino acids, e.g., caffeine or trigonelline.

Generally, all the amino acid contents were similar in both species, although slightly higher in *robusta*, as already observed for the free amino acids. Their behaviour was quite similar in both samples and occurred according to the amino acid thermal stabilities (Baxter, 1996). The more stable amino acids, such as Tyr, Val, Leu, Phe, or Ala, remained almost constant for all the temperatures assayed. Lys, Met and His were the most degraded amino acids, again in both samples. Comparing the results at 240 °C, *robusta* coffee samples had a slightly higher total amino acid loss. Again, temperature seems to have a higher effect on this matrix. The results were similar to others described in the literature (Clarke, 1987; Clifford, 1985), except for OH-Pro for which no published data were found. This amino acid was described as in high content in the cell wall proteins (arabinogalactan), which seem to account for approximately one-third of the green bean protein (Clifford, 1985).

In order to better understand the temperature influence on the amino acids, racemisation values were also calculated. No differences were observed in the mean racemisation values presented in Tables 4 and 5, for *arabica* and *robusta* samples, respectively. Comparing with the free amino acids, the racemisation was less intense and no differences were found in the individual racemisation values between the two samples (data not shown). As expected, the free amino acids racemise more easily than peptide-bound amino acids.

Taking into account that a typical "expresso" coffee contains about 7.5 mg/ml of protein-like matter (6.5 g ground coffee; 30 ml) (Illy & Viani, 1995), and considering the results obtained for the D-amino acid contents, it can be calculated that about 10 mg of D-amino acids are ingested per cup. These amounts, as in other foods, are considered low values, with probably no pejorative effects. Nevertheless, problems could arise when the D-amino acid oxidase is reduced or inactive. While waiting for further insights into the D-amino acid role and function in the vertebrates, their ingestion should probably be kept as low as possible.

One can speculate that the *robusta* coffee matrix seems more prone to racemisation than the *arabica* one, although further samples would have to be analysed to corroborate these results. Less dark roasts should be favoured and roasting processes inducing less racemisation should be achieved. It would be interesting to compare the results obtained here for traditional roasts with other roasting procedures, namely the recent "high-yield" or fast roasting process (Illy & Viani, 1995).

Given the steady increase of some D-amino acids with roast, this parameter might be important for roasting control, as already suggested by Nehring and Maier (1992). Nevertheless, in their study, the amino acids were analysed after hydrolysis, which requires a long analysis time. From the reported results, it is believed that the free amino acids may be used as well, reducing the total analysis time and thus meeting industry requirements.

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