JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Factors Influencing the Norharman and Harman Contents in Espresso Coffee

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Espresso coffee (EC) brews were analyzed for β-carboline [norharman (NH) and harman (H)] contents, by RP-HPLC with fluorescence detection. The influence of the coffee species (*arabica* or *robusta*), the roast degree, and the brew length was studied. The results show that the content of NH and H in EC is dependent primarily on the coffee species, followed by brew length. The roast degree has only a minor influence on the final content of NH and H in EC. When compared with other coffee brews, EC has an amount of these β-carbolines (in micrograms per liter) similar to that of mocha coffee, both being more concentrated than filter and press-pot coffees. Therefore, the consumer's preferences will determine the amount of NH and H ingested daily. For the caffeinated 30 mL of EC, the *arabica* coffees contain about 4.08 μg of NH and 1.54 μg of H. Commercial blends (usually with a maximum of 30% *robusta*) range from the cited *arabica* values to 10.37 μg of NH and 4.35 μg of H.

KEYWORDS: Norharman; harman; espresso coffee; coffee brews; β-carbolines; *arabica*; *robusta*

INTRODUCTION

Espresso coffee (EC) appeared for the first time in Italy at the beginning of the last century and has currently an increasing number of consumers worldwide. EC is prepared by a special brewing technique in which a limited amount (20−50 mL) of hot water under high pressur (9 ± 2 atm, 90 ± 5 °C) is percolated in a very short time (30 ± 5 s) through a ground coffee cake (6.5 ± 1.5 g). This process produces an individual dose of a concentrated brew, a complex mixture of the most intense flavors covered by a dense foam layer (crema), which should be tasted at the exact moment of extraction (*1*, *2*). The excellence of this beverage depends on several conditions, namely, coffee species (*Coffea arabica* and *Coffea canephora* var. *robusta*), roasting type, grinding degree, water pressure, and extraction temperature. Cid and co-workers have been working on the contribution of these parameters to the final quality of EC (*3*−*6*). Nunes et al. (*7*) analyzed also the roast degree influence in the foam properties and chemical composition in *arabica* and *robusta* ECs. Despite these possible variations, ECs together with all coffee brews contain thousands of chemicals. Theoretically, some of these compounds, according to the doses involved, may possess biological activities that could be considered either potentially adverse to health or, on the contrary, beneficial (*1*).

Herraiz (*8*, *9*) described recently the presence of the β-carbolines norharman (NH) and harman (H) in coffee brews. Values around 145 and 35 μg/L, respectively, were reported for EC. These compounds were also previously described in several plants (*Passiflora incarnata*, *Tribulus terrestris*, *Pueraria lobata*, and *Ananas comosus*) (*10*), in thermally processed foods (meat, poultry, fish, sauces, toasted bread, and cookies), in alcoholic fermentation products (beer, wine, and sake), and in the environment, but always in comparatively lower amounts (*11*, *12*). Higher levels were found in tobacco smoke, with 11.2 μg of NH and 3.6 μg of H reported per cigarette (*11*). These published results confirm that, in addition to cigarette smoking, coffee is the most important exogenous source of these compounds (*9*).

Chemically, the β-carbolines NH (9*H*-pyrido[3,4-*b*]indole) and H (1-methyl-9*H*-pyrido[3,4-*b*]indole) (**Figure 1**) belong to the heterocyclic aromatic amines group. Both are fluorescent compounds (*13*) and are also classified as alkaloids due to their natural existence in the plant kingdom (*10*), where they seem to be formed from tryptophan and pyruvate or acetate (*11*). NH and H were also detected in mammals, tryptamine being also suggested as an endogenous precursor (*11*, *14*). In thermally processed matrices (mainly in the case of cooked meat, roasted coffee, and tobacco smoke), these β-carbolines are originated through a Pictet−Spengler condensation from indolethylamines (as L-tryptophan) and carbonylic compounds (such as acetaldehyde or formaldehyde) followed by an oxidation and decarboxylation. Their formation is dependent on temperature and heating time (*12*, *15*).

An extensive range of NH and H pharmacological and psychopharmacological effects have been reported by several authors. Comutagenicity (*16*) and in vivo genotoxicity (*17*) have been suggested, as well as an involvement in addiction and alcoholism (*18*). They were able to modify the concentrations of brain neurotransmitters by interaction with several receptors

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10.1021/jf063128u CCC: $37.00
Published on Web 02/10/2007

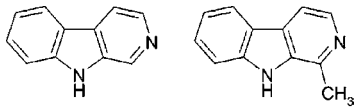

Figure 1. Chemical structures of norharman and harman (*11*).

(serotonin, benzodiazepine, opioid, and imidazoline) and with cytochrome P450 enzymes (*9*). On the other hand, H induced antidepressant and antianxiety effects in rats, as well as a vasorelaxant effect (*19*, *20*), and showed anti-HIV activity by inhibiting replication in H9 lymphocyte cells (*21*). Meanwhile, it was described that H may have beneficial effects on mononeuropathic pain in rats (*22*) and on naloxone-precipitated withdrawal syndrome in morphine-dependent rats (*23*). H also increased insulin secretion from isolated human islets of Langerhans (*24*).

Elevated levels of NH and H found in the cerebrospinal fluid of Parkinson's patients is one of the factors contributing to the reported involvement of NH in the pathogenesis of idiopathic Parkinson's disease and H in tremorogenesis (*25*−*27*). However, recent epidemiological investigations also suggest that coffee intake is negatively correlated with the Parkinson's disease incidence and that habitual coffee consumption might in some way protect against the development of neurodegenerative diseases (*28*). Herraiz (*9*) showed that NH and H, isolated from MAO-inhibiting coffee, were good inhibitors of MAO A (H and NH) and MAO B (NH) isoenzymes in a competitive and reversible way.

Despite the published contents of NH and H in some coffee brews by Herraiz (*8*, *9*), including EC, no references to the possible influence of the coffee blend composition or the beverage parameters were found in literature. The aim of this work was to contribute to a better knowledge of the much appreciated espresso coffee and ascertain the influence of different factors in the final content of β-carbolines in the beverage. The contribution of the commercial coffee species and their roast degree, together with the extraction degree achieved by different brew lengths, is described. The levels of these β-carbolines are also evaluated in several commercial samples, with decaffeinated and serving included, and the values compared with other common coffee beverages.

MATERIALS AND METHODS

Reagents and Standards. Standards of harman (H) and norharman (NH) were obtained from Aldrich (Steinheim, Germany) and Sigma (St. Louis, MO), respectively. The internal standard harmaline and trifluoroacetic acid were purchased from Fluka (Neu-Ulm, Germany). Acetonitrile Chromasolv was obtained from Sigma-Aldrich (Steinheim, Germany), and sodium formate was from Merck (Darmstadt, Germany). All other chemicals were of analytical grade from several suppliers.

The HPLC water was purified with a "Seral" system (SeralPur Pro 90 CN). All coffee brews were prepared with deionized water (Amberlite MD20).

The internal standard harmaline solution was prepared with 1% of ascorbic acid and reserved in the dark and at 4 °C, being stable for 1 month.

Coffee Samples. To prepare the ECs, caffeinated ($n = 16$) and decaffeinated ($n = 3$) commercial coffee beans, as well as servings ($n = 6$), were obtained in local supermarkets and cafeterias. All samples were blends of *arabica* with unknown amounts of *robusta*, except two caffeinated brands, which were labeled as 100% *arabica*.

To prepare the other coffee brews, ground coffee of adequate grinding degrees (based on the label information) was also acquired.

Green bean samples of *Coffea arabica* ($n = 6$) and *Coffea canephora* var. *robusta* ($n = 5$), all from different geographical origins, were kindly supplied by a local industrial importer and roaster of coffee (BICAFÉ). Samples of *arabica* were from Brazil, Ethiopia, Colombia, Jamaica, Hawaii, and Costa Rica, and *robusta* samples were from Ivory Coast, Vietnam, Uganda, Cameroon, and India. All *robusta* samples were dry processed. Samples of *arabica* coffees were wet processed, except the one from Brazil, which was dry processed. All samples were roasted in a Probat L12 coffee roaster from Probat-Werke according to a standard method (210 °C, 10 min) to eliminate any variation caused by this procedure.

Additionally, two green coffee samples, one from Brazil (*arabica*) and the other from Ivory Coast (*robusta*), were separately roasted in a Probat Pré 1Z (2000) from Probat-Werke, at 210 °C, for various times of heat exposure from 7 to 11 min to achieve five different roast degrees.

The roast degree was determined by photometric analysis with infrared radiation (Colorimeter Colorette 3 from Probat-Werke) and also by the organic roast loss evaluation in dry weight (% ORL dw) (*2*, *29*). Sample moisture to calculate ORL was determined by drying at 103 ± 2 °C until constant weight. Samples were ground in a Krups GVX 2 Burr Grinder.

Brew Coffee Sample Preparation. Espresso coffees (6 ± 1 g/EC) (*1*) were prepared with an automatic espresso machine Krups (XP7220P0). For the evaluation of the extraction efficiency different volumes were extracted, ranging from 20 mL, a typical "ristretto" or "Italian", to 70 mL, the longest EC (*30*). Commercial blend servings (with or without paper) were extracted with an Espresso Professional Philips HL3854/A. Mocha coffees (25 g/200 mL) were prepared on a Delonghi EMK6 Alicia Electric Mocha Espresso Maker. To prepare filter coffees a Krups CaféPresso 10 Plus was used (27.5 g/500 mL). French press or press-pot coffees were made (27.5 g/500 mL) with a French Press Coffee Maker from Bodum (*31*, *32*). ECs were always extracted in triplicate and combined as one sample to minimize possible variations caused by the espresso machine. Mocha, press-pot, and filter coffee were prepared once for each coffee sample. Each brew was analyzed in duplicate.

***β*-Carboline Extraction.** The extraction of β-carbolines from coffee brews was achieved following a previously published procedure by Herraiz (*8*). Briefly, 5 mL of brewed coffee samples, previously diluted (1:1) with 0.1 M HCl, were spiked with 50 μL of internal standard harmaline solution (60 mg/L), with semicarbazide (0.5 mL, 10 mg/mL) to prevent artifact formation, and with ascorbic acid (0.5 mL, 50 mg/mL) as antioxidant. The aliquots were then loaded into the SPE cartridges (Bond Elut PRS, 500 mg, 3 mL; Varian) previously conditioned with methanol and 0.1 M HCl. A Visiprep SPE Vacuum Manifold 57030-U from Supelco was used to manipulate the solid-phase extraction cartridges. The columns were immediately washed with HPLC water and then rinsed with 3 mL of 0.4 M K_2HPO_4 (pH 9.1). The elution of β-carbolines was achieved with 3 mL of methanol + 0.2 M K_2HPO_4 (pH 9.1) (1:1). All washing and elution solutions contained 0.5 mL of ascorbic acid (50 mg/mL). The eluates were directly injected, in duplicate, into the HPLC system.

The procedure to determine the total amount of β-carbolines in the coffee cake, in order to study the extraction efficiency, was based on the method described by Gomes (*33*). Six grams of ground roasted coffee was extracted three times with 40 mL of 0.05 M HCl, heated almost to the boiling point (to resemble the water temperature during espresso coffee extraction), and vortex mixed (Reax 2000, Heidolph), for 2 min. After a 3 min centrifugation at 5000 rpm (Labofuge Ae, Heraeus Sepatech) the total volume of liquid extract was decanted, and aliquots of 5 mL were directly used to extract the compounds as described above.

HPLC Analysis. The chromatographic analysis was carried out in a Jasco HPLC integrated system (Tokyo, Japan) equipped with an AS-950 autosampler, with a 20 μL loop, a PU-1580 Intelligent Pump, an LG-1580-04 Quaternary Gradient Unit, a DG-1580-54 Four Line Degasser and an FP-920 programmable fluorescence detector. Data were analyzed using Borwin-PDA Controller software (JMBS).

The chromatographic separation of the compounds was achieved on a reversed phase Teknokroma Tracer Excel ODSA (5 μm; 250 × 4 mm) column (Spain), operating at ambient temperature (21−22 °C). The solvent system used was a gradient of 0.03 M formate buffer with 0.025 M trifluoroacetic acid (pH 3.0) (A) and acetonitrile (B) (*33*).

Table 1. Norharman and Harman Content Found in Espresso Coffees from Several Commercial Samples (Caffeinated and Decaffeinated)[a]

commercial sample	*n*	NH, μg/EC (30 mL) mean ± SD	NH, μg/EC (30 mL) range	H, μg/EC (30 mL) mean ± SD	H, μg/EC (30 mL) range
caffeinated blends	14	7.39 ± 1.78 a	4.20–10.37	2.82 ± 0.76 a	1.42–4.35
caffeinated 100% *arabica*	2	4.10 ± 0.66 b	3.63–4.57	1.70 ± 0.42 b	1.40–2.00
decaffeinated blends	3	6.92 ± 2.55 a	4.58–9.64	1.90 ± 0.93 c	1.17–2.95

[a] Data followed by different letters within each column are significantly different according to Student's *t* tests at $P < 0.01$. EC, espresso coffee; NH, norharman; H, harman; SD, standard deviation.

The gradient was as follows: 0 min, 85% A; 5 min, 85% A; 13 min, 70% A; 14 min, 70% A; 15.5 min, 30% A; and 16.5 min, 85%, A; performed at a constant flow rate of 1.0 mL/min.

Quantification was performed on the basis of the internal standard method using fluorescence detection. To achieve an optimum detection of the compounds, the fluorescence detector was programmed at the following wavelengths of excitation and emission: 0 min, 300 and 440 nm (NH and H); 14.6 min, 267 and 477 nm (harmaline). Gain 1 was used during the first 9 min and increased to 10 afterward to improve the sensitivity of the compound detection.

The retention times for NH, H, and harmaline were 11.9, 13.3, and 15.2 min, respectively. The detection limits were 0.6 ng/mL for NH and 0.4 ng/mL for H. The linearity ranges, with all standards subjected to the same extractive process as the samples, were 0.001−1.25 μg/mL ($r = 0.9996$) for NH and 0.001−1.20 μg/mL ($r = 0.9997$) for H.

Statistical Analysis. Data are reported as mean ± standard deviation. Data were analyzed by the one-way ANOVA. Student's *t* tests were used to discriminate between any two groups under consideration. Simple linear regression analysis was used to evaluate the relationship between the compound amount and the EC volume. All analyses were carried out with Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

Table 2. Norharman and Harman Contents in Espresso Coffees Prepared with *arabica* or *robusta* Roasted Beans[a]

coffee species and geographical origin	% ORL	color	NH, μg/EC (30 mL), mean ± SD	H, μg/EC (30 mL), mean ± SD	ratio NH/H
arabica					
Brazil	9	130	4.63 ± 0.02	1.86 ± 0.05	2.5
Ethiopia	12	128	2.84 ± 0.02	1.04 ± 0.00	2.7
Colombia	7	122	2.42 ± 0.02	1.43 ± 0.01	1.7
Jamaica	11	126	6.07 ± 0.13	1.17 ± 0.03	5.2
Hawaii	5	132	3.95 ± 0.08	1.66 ± 0.02	2.4
Costa Rica	11	127	4.57 ± 0.16	2.09 ± 0.04	2.2
av ($n = 6$)			4.08 ± 1.33 a	1.54 ± 0.40 a	2.6
robusta					
Ivory Coast	11	130	12.15 ± 0.11	4.17 ± 0.10	2.9
Vietnam	16	130	10.98 ± 0.00	3.60 ± 0.03	3.1
Uganda	9	122	10.73 ± 0.15	4.07 ± 0.10	2.6
Cameroon	16	124	9.04 ± 0.27	3.07 ± 0.04	2.9
India	10	118	10.09 ± 0.15	3.32 ± 0.06	3.0
av ($n = 5$)			10.60 ± 1.15 b	3.64 ± 0.47 b	2.9

[a] Data followed by different letters within each column are significantly different according to ANOVA at $P < 0.001$. EC, espresso coffee; ORL, organic roast loss; NH, norharman; H, harman; SD, standard deviation.

RESULTS AND DISCUSSION

Variability within Commercial Samples. A large variability in the β-carboline contents was detected in EC caffeinated samples (**Table 1**). The levels of NH ranged from 3.63 to 10.37 μg/EC, and those of H varied from 1.40 to 4.35 μg/EC, with mean values of 6.98 ± 2.01 μg/EC for NH and 2.68 ± 0.82 μg/EC for H. These values are slightly higher than the ones reported by Herraiz (*8*, *9*). NH was always found in higher quantities than H, the average ratio of NH to H being approximately 3.

Within the caffeinated ECs, a significant difference ($P < 0.05$) was observed between the blends and the varietal coffees, for both NH and H, with the 100% *arabica* samples presenting the lowest values. Despite the low number of varietal samples, the results suggest that the presence of *robusta* in the blend may contribute to a higher β-carboline content, as will be detailed below.

The decaffeination process might be responsible for a decrease of precursors in green beans. Considering that β-carbolines are mostly formed during the roasting stage, lower amounts of NH and H would be expected in decaffeinated ECs. A significant difference ($P < 0.05$) was, actually, observed between caffeinated and decaffeinated ECs, with regard to H (**Table 1**). However, no statistical difference ($P > 0.05$) was found for NH between the two mentioned groups. To better ascertain the decaffeination process influence, it would be necessary to analyze a higher number of decaffeinated samples, preferably from the same original batch of caffeinated green beans.

Servings are single doses of ground and compacted coffee coated with paper, produced to make espresso coffee in a rapid, simple, and clean way in adapted machines. To investigate the influence of the paper in the final amount of β-carbolines in the brew, espresso coffees were prepared with servings of commercial blends (composition not known) and with the respective decoated servings. The mean values found for ECs prepared with coated ($n = 6$) and decoated ($n = 6$) servings were, respectively, 8.54 ± 1.21 and 8.64 ± 1.29 μg/30 mL for NH and 2.70 ± 0.52 and 2.67 ± 0.57 μg/30 mL for H. No significant differences ($P > 0.05$) were found between the two analyzed groups, for both compounds. Therefore, it can be concluded that the compounds are not retained by the coating.

Several factors can be responsible for the large variability described between the analyzed commercial ECs (**Table 1**). By using an espresso automatic machine it was possible to control some parameters: the grinding degree, the weight of ground coffee used, the pressure applied to make the coffee cake, and the water temperature and pressure. Therefore, the consumer's preference for different EC lengths, the proportion of *arabica* and *robusta* beans of the blend, and the roasting procedure used by industries appears to be the more important parameter that may influence the EC composition, as will be detailed below.

Influence of Coffee Species: *arabica* and *robusta*. The NH and H levels vary significantly within each coffee species (*arabica* and *robusta*), as can be observed in **Table 2**. In the *arabica* samples, the NH values ranged from 2.42 to 6.07 μg/EC. In the *robusta* ECs, NH amounts varied from 9.04 to 12.15 μg/EC. H was always detected in smaller amounts than NH, with values ranging from 1.04 to 2.09 μg/EC in *arabica* ECs and from 3.07 to 4.17 μg/EC in *robusta* ones.

All samples were submitted to the same roast procedure (210 °C, 10 min), although different % ORLs and colors can be observed within each species (**Table 2**). Besides, the *robusta*

Table 3. Norharman and Harman Contents at Different Roast Degrees[a]

roasting time at 210 °C (min)	% ORL	color	μg/EC (30 mL)		μg/cake (6 g)		% of extraction	
			NH	H	NH	H	NH	H*
arabica								
7	8	190	5.73 ± 0.02 b	2.29 ± 0.00 b	14.41 ± 0.02 a	6.96 ± 0.01 a	40	33
8	10	189	6.18 ± 0.00 c	2.62 ± 0.00 c	16.03 ± 0.12 b	7.32 ± 0.10 b	39	36
9	11	145	6.30 ± 0.02 d	2.68 ± 0.02 d	17.44 ± 0.02 d	7.77 ± 0.01 c	36	35
10	12	124	4.98 ± 0.04 a	2.08 ± 0.06 a	17.14 ± 0.10 cd	6.94 ± 0.09 ab	29	30
11	14	119	5.70 ± 0.02 b	1.96 ± 0.03 a	16.92 ± 0.04 c	6.83 ± 0.05 a	34	29
robusta								
7	4	184	15.21 ± 0.04 e	5.21 ± 0.01 g	40.99 ± 0.25 e	14.57 ± 0.10 gf	37	36
8	8	161	17.87 ± 0.19 g	5.77 ± 0.09 h	41.00 ± 0.26 e	14.75 ± 0.08 g	44	39
9	9	141	16.51 ± 0.06 f	5.17 ± 0.02 g	42.01 ± 0.33 ef	13.85 ± 0.12 e	39	37
10	12	125	15.13 ± 0.05 e	4.71 ± 0.08 f	42.80 ± 0.33 f	14.11 ± 0.12 ef	35	33
11	14	113	15.30 ± 0.04 e	4.31 ± 0.09 e	44.41 ± 0.02 g	13.10 ± 0.02 d	34	33

[a] Mean value ± standard deviation, $n = 1$, for each roasting time within each species. Data followed by different letters within each column are significantly different according to Student's tests at $P < 0.05$; *, significant differences between *arabica* and *robusta* data ($P < 0.05$). EC, espresso coffee; ORL, organic roast loss; NH, norharman; H, harman.

samples achieved higher roast losses. Those variations are due to both chemical and physical intrinsic characteristics of the beans. Nevertheless, no correlation was observed between the compound contents and the roast degree.

The mean values of both NH and H were statistically higher in the *robusta* samples ($P < 0.001$), corresponding to more than double the *arabica* amounts (**Table 2**). The NH/H ratio found in *robusta* ECs ranged from 2.6 to 3.0, similar to those found in the *arabica* ones (2.2 and 2.7), with two exceptions found (1.7 and 5.2) in this last group. Statistically, no significant differences ($P > 0.05$) were observed between the *arabica* and *robusta* ratios.

It would be interesting to understand the factors behind the higher contents consistently found in the *robusta* ECs. The amount of precursor, namely, free tryptophan, might be a factor of great influence, as suggested by Gomes (*33*), because the tryptophan levels are considerably higher in *robusta* green coffee beans than in *arabica* ones (*34*).

The postharvest treatment used in the green coffees could also be a plausible justification, because all the *robusta* beans were dry processed, whereas the wet processing method was used for the majority of the *arabica* coffees. This last procedure can be responsible for a decrease in the concentration of some precursors. However, the Brazilian *arabica* coffee (dry processed) showed no statistical difference ($P > 0.05$) in the NH and H contents when compared with the other *arabica* samples, remaining statistically different from the *robusta* group ($P < 0.001$). To ascertain the influence of the postharvest treatment on the final amount of NH and H in coffee brews, it would be necessary to study a higher number of wet- and dry-processed samples, preferably from the same original batch of green beans.

A study with a higher number of samples within each geographical origin would also be valuable to analyze the influence of this parameter on the amount of β-carbolines in the brew.

The differences observed between the two coffee species justify the variation found between the previously analyzed ECs of commercial samples (**Table 1**), because distinct proportions of *arabica* and *robusta* beans are used by industries to prepare specific blends. In Portugal, the addition of some *robusta* coffee to the blends (usually no more than 30%) is a common procedure to increase body and improve the crema, together with some cost reduction. Indeed, the commercial ECs (**Table 1**) with the lowest levels of NH and H were labeled as being 100% *arabica* coffee, and the values per EC are within those of **Table 2** for *arabica* beans. An addition of *robusta* beans to the blend is responsible for higher amounts of β-carbolines in the final coffee brew. This can also justify the fact that our caffeinated blend ECs (**Table 1**) show comparatively higher levels (246 μg/L for NH and 94 μg/L for H) than those reported by Herraiz (*8*, *9*) (around 125−145 and 32−35 μg/L for NH and H, respectively). Despite the presence of *robusta* in the coffee blends being mentioned by the author, these results suggest that our blends should have more *robusta*, as is typical in Portugal. Herraiz's results are more in accordance with the *arabica* samples analyzed in this study (135 and 50 μg/L).

Roast Degree Influence. Values of around 0.10 ± 0.03 and 0.02 ± 0.01 μg/g, respectively, for NH and H were reported in green beans against 1.11 ± 0.43 and 0.28 ± 0.09 μg/g for the same compounds in beverages extracted from roasted beans, suggesting that roasting is responsible for NH and H formation (*8*). In a more detailed study, Gomes (*33*) reported, for coffee beans, an increase in their β-carboline contents with the roasting temperature.

The results presented in **Table 3** show that, for the same temperature, slight variations of the roasting time have some influence on the final contents of NH and H in EC. Their levels initially enhance with the roasting time, but then decrease with the rise of the roast length. For NH, no significant differences ($P > 0.05$) were found between the amounts extracted to EC in the lightest and in the highest roast degrees, for both *arabica* and *robusta*, but for H, a significant reduction ($P < 0.05$) was verified.

To better understand this behavior, the β-carboline total amount in the coffee cake (6 g) was also evaluated. In a general way, an effective increase with the roasting time was verified for only NH ($P < 0.05$). The results for H show an initial increase followed by a slight decrease. In *robusta*, an effective loss of H was verified during the roasting procedure with smaller amounts in the higher roast degree, when compared with those of the first roast. Therefore, it is possible that H might play a role as an intermediate of other compounds formed during the roasting procedure (NH, for instance).

When extraction percentages were compared, significant differences ($P < 0.05$) were verified only for H, with higher extraction percentages achieved in the *robusta* samples. Besides, the amount of NH extracted into the ECs does not enhance with the roast degree, despite its increased levels in the coffee powder. Nevertheless, the values extracted into the 30 mL ECs cor-

Table 4. Norharman and Harman Content Variation with the Espresso Coffee Length[a]

	EC volume (mL)							
	20	30	40	50	60	70	*r*	S
				arabica ($n = 4$)				
NH (μg/EC)	2.99 ± 0.57	3.74 ± 1.27	4.33 ± 1.23	4.91 ± 1.62	5.70 ± 2.76	6.34 ± 2.82	0.6452	ns
H (μg/EC)	1.15 ± 0.31	1.45 ± 0.58	1.75 ± 0.61	1.89 ± 0.73	2.28 ± 1.11	2.15 ± 0.65	0.5862	ns
% of extraction	29 ± 5	35 ± 2	42 ± 4	46 ± 4	53 ± 3	56 ± 6	0.9185	*
				robusta ($n = 4$)				
NH (μg/EC)	8.85 ± 0.00	11.56 ± 0.83	13.63 ± 0.27	14.94 ± 0.73	17.36 ± 1.10	17.91 ± 0.67	0.9749	*
H (μg/EC)	2.93 ± 0.15	3.88 ± 0.40	4.83 ± 0.90	5.03 ± 0.14	6.22 ± 0.04	6.44 ± 0.27	0.9540	*
% of extraction	33 ± 1	43 ± 2	52 ± 4	56 ± 3	67 ± 4	69 ± 3	0.9617	*

[a] Mean value ± standard deviation. S, statistical significance by row, according to ANOVA: *, $P < 0.001$; ns, not significant ($P > 0.05$). *r*, correlation factor; EC, espresso coffee; NH, norharman; H, harman.

Table 5. Norharman and Harman Contents in Different Coffee Brews[a]

		commercial blend					
		A		B		C	
brew type		NH	H	NH	H	NH	H
espresso	μg/L	328 ± 6 a	155 ± 2 a	235 ± 1 a	86 ± 1 a	147 ± 6 a	57 ± 2 a
mocha	μg/L	342 ± 0 a	137 ± 0 b	292 ± 1 a	99 ± 1 b	165 ± 1 a	52 ± 0 a
press-pot	μg/L	182 ± 1 b	79 ± 0 c	157 ± 7 b	56 ± 3 c	87 ± 3 b	31 ± 0 b
filter	μg/L	188 ± 7 b	85 ± 2 c	174 ± 7 b	62 ± 3 c	91 ± 0 b	29 ± 0 b
espresso	μg/30 mL	9.84 ± 0.16 f	4.66 ± 0.05 f	7.06 ± 0.03 f	2.59 ± 0.03 f	4.42 ± 0.11 e	1.71 ± 0.05 e
mocha	μg/60 mL	20.55 ± 0.02 d	8.24 ± 0.01 d	17.54 ± 0.07 d	5.93 ± 0.06 d	9.93 ± 0.08 d	3.11 ± 0.02 d
press-pot	μg/60 mL	10.94 ± 0.06 e	4.74 ± 0.01 e	9.45 ± 0.42 e	3.38 ± 0.18 e	5.21 ± 0.10 e	1.84 ± 0.02 e
filter	μg/120 mL	22.54 ± 0.80 d	10.25 ± 0.26 d	20.83 ± 0.96 d	7.36 ± 0.46 d	10.9 ± 0.09 d	3.48 ± 0.03 d

[a] Mean value ± standard deviation. Data followed by different letters within each column are significantly different according to Student's tests at $P < 0.001$. NH, norharman; H, harman.

respond, in a general way, to around 30% of the total amounts existent in the coffee cake.

The lower extractability at higher roast degrees verified in ECs could be explained by the formation of oil droplets that, by dissolving NH and H, could trap them in the spent grounds. The use of hot 0.5 M HCl to totally quantify the compounds increases their water extractability.

To reach a more accurate conclusion, it would be interesting to study a higher number of samples from the two species.

Influence of EC Length. Generally, an EC is prepared with a 30−40 mL volume, but it can also be prepared as a "ristretto" (20 mL or less) or as a "lungo" (50 mL or more) espresso (*30*). This characteristic will depend on the preferences of each consumer, and the total amount of NH and H will certainly vary with the final volume of the brew.

The influence of the water volume in the extraction of the β-carbolines NH and H from the coffee cake (in EC preparation) was studied in both *arabica* and *robusta* coffees. The mean results are reported in **Table 4**. As expected, it can be observed that an increase in the water volume that goes through the coffee cake causes a higher extraction of the compounds. In both *robusta* and *arabica*, the 70 mL EC had approximately double amounts of β-carbolines per brew than those observed in the 20 mL EC.

Again, the *robusta* ECs presented values of NH and H approximately 3 times higher than *arabica* for all analyzed volumes, and in both species the ratio NH/H for each volume was kept around 3.

With the volume increase, the individual samples (*arabica* and *robusta*) described a correlation factor (*r*) superior to 0.9, for both compounds, with significant differences ($P < 0.001$) between the distinct volumes.

However, when we group the samples according to the respective species (*arabica* or *robusta*), this statistical significance ($P < 0.001$) is only maintained by the *robusta* group. Low correlations were verified in the *arabica* group ($r = 0.6$ for NH and H) due to the high variability among the *arabica* samples analyzed. This justifies the higher standard deviations reported for the *arabica* group (**Table 4**).

The NH and H extraction percentages, within each species, can be considered together, because they seem to be independent of the compound, increasing with the brew length. Nevertheless, even the longer ECs are only able to extract about 50−70% of the total β-carboline amounts of the coffee cake. Differences with a higher significance level ($P < 0.001$) were found between the species, the β-carbolines being more efficiently extracted in the *robusta* brews (**Table 4**).

NH and H Stability after EC Extraction. EC is an extemporary beverage that should be consumed immediately after extraction—the consumer waits for the espresso, not the opposite (*2*). However, occasionally, some time may pass between the extraction and consumption. The β-carboline amount was studied at 0, 5, 10, 15, 30, 45, and 60 min after EC preparations. No significant differences ($P > 0.05$) were found between the contents at the different times for either NH or H, showing the stability of these β-carbolines in the brew.

Comparison of EC with Other Coffee Brews. Coffee brew preparation is possible by many different ways. Decoction methods (boiled, Turkish, vacuum, and percolator coffee), infusion processes (filter and napoletana coffee), and pressure methods (press-pot or French press, mocha, and espresso) are described in the literature (*1*). Certainly, for the same coffee blend, the procedure, as well as the amounts of adequately ground coffee used, will influence the final level of β-carbolines in the brew. In **Table 5**, it is possible to compare ECs with other coffee beverages. Generally, when the results are expressed in micrograms per liter, the four analyzed beverages can be grouped into two groups ($P < 0.001$), for both NH and H:

espresso/mocha against press-pot/filter, with higher amounts in the former. Considering the three samples together, the same grouping is evident but with a lower level of significance ($P < 0.05$). The lower concentrations achieved in the filter and press-pot coffees is dependent not only on the method itself but also on the lower ratio of "ground coffee weight/volume of water" used to prepare the brews.

The β-carboline content per coffee brew was also analyzed. The EC is, generally, a very short beverage (20−40 mL). However, higher volumes per cup are usually used to prepare the other coffee beverages, especially filter coffee, because it is considered a light one. A cup of filter coffee may achieve 200 mL, whereas a mocha usually does not surpass 120 mL. Obviously, these volumes might vary according to cultural and personal preferences. In **Table 5** the results are described in micrograms per brew, calculated for the mean volumes generally consumed (*32*). When the three samples are considered together, the formation of two different groups is evident ($P < 0.05$): espresso (30 mL)/press-pot (60 mL), with lower amounts, against filter (120 mL)/mocha (60 mL), for both NH and H. In this case, the beverage volume is the preponderant factor.

In summary, the exposure of humans to this exogenous source of β-carbolines will certainly vary with the method of brew extraction and also with the strength of the brew prepared, the coffee species used, and their roast degree. Also, the brew volume and number of cups ingested daily are important factors. In a general way, when an EC is consumed, it may have distinct values of NH and H according to the final volume of the brew, ranging approximately between 30 and 70% of the total amount of β-carbolines found in the beans. Despite being one of the most concentrated coffee brews, the amount of compounds ingested per EC cup is generally lower, when compared with coffee other brews, due to its small volume per cup (20−70 mL).

ACKNOWLEDGMENT

We thank BICAFÉ for providing the coffee samples and Krups for the automatic coffee machine.

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Received for review October 31, 2006. Revised manuscript received January 3, 2007. Accepted January 4, 2007. R.C.A. is grateful to Fundação para a Ciência e a Tecnologia for a Ph.D. grant (SFRH/BD/22449/2005).

JF063128U