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HPLC/diode-array applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee

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Abstract

A simultaneous determination of trigonelline, nicotinic acid and caffeine was performed in samples of *arabica* and *robusta* coffees, before and after roasting at either different temperatures (160–240°C) or different periods of time exposures, in order to study their thermal degradation. A reverse-phase HPLC/Diode-array detector method was used. The results were compared with a model dry system roast of the compounds under study, individually and in mixture. The loss of trigonelline was strongly dependent upon the degree of roast and was associated with the formation of nicotinic acid. A slight decrease in caffeine was verified in both species. This study showed diversified behavior of the compounds when in their native form or in an artificial mixture, eliciting the chemical environmental influence. Rate constants for the chemical reactions at 240°C were determined.

Keywords: HPLC; Diode-array; Trigonelline; Nicotinic acid; Caffeine; Coffee

1. Introduction

Roasting is a time-temperature dependent process, whereby chemical changes are induced in the coffee beans. Different treatments in the roasting step modify the composition of the final product. Excessive temperature during roasting is known to cause undesirable chemical changes, especially at the bean surface requiring considerable control of the process and objective means for its quality control (Clarke, 1989; Clifford, 1985).

Several techniques have been reported for the control of the coffee roasting, namely trigonelline/nicotinic acid ratio (Stennert & Maier, 1996; Taguchi, Sakaguchi & Shimabayashi, 1985), chlorogenic acids (Trugo & Macrae, 1984), hydrocycinnamic acids (Casal et al., 1999), amino acids (Nehring & Maier, 1992), methylpyrazine (Hashim & Chaveron, 1996) and physicochemical properties (Ortolá, Londonõ, Gutiérrez & Chiralt, 1998; Nunes, Coimbra, Duarte & Delgadillo, 1997). Some of these parameters are also recognized as important features in the discrimination of coffee varieties (Andrade, Leitão, Seabra, Oliveira & Ferreira, 1997; Macrae, 1989; Martín, Pablos & González, 1996). However, there are few data available on the kinetics of the degradation process as a tool for the knowledge of the coffee roasting process.

The aim of this work was to simultaneously quantify three important nitrogenous components of coffee (trigonelline, nicotinic acid and caffeine), by a rapid and accurate HPLC method and to apply the analytical technique to the study of the influence of roasting on these products.

2. Materials and methods

2.1. Chemicals

Trigonelline hydrochloride was purchased from Fluka (Neu-Ulm, Germany), nicotinic acid and caffeine were obtained from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade (Merck, Darmstadt).

2.2. Coffee thermal treatments and sample preparation

A *Coffea arabica* sample from Brazil and another of *Coffea canephora* var. *robusta* from the Ivory Coast

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were subjected to two different protocols of roasting in a WTC blinder (Tuttlingen, Germany) stove. The first protocol was carried out at constant time (15 min) and different temperatures, ranging from 140 to 240°C and the second one was performed at constant temperature (240°C) with variation of the exposure time (5–20 min).

All samples were grounded and screened through a 25-mesh sieve immediately before sample analyses and moisture determination. Moisture was determined by drying the samples at 103°C until constant weight. A 2-g portion of grounded coffee was extracted with a total of 100 ml boiling water (Casal, Oliveira & Ferreira, 1998) and an aliquot of this solution was filtered through a 0.2 μm pore size membrane (Schleicher & Schuell, Germany) into an appropriate vial for use in the autosampler without further dilutions. All determinations were performed in duplicate.

2.3. Pyrolisis of trigonelline, nicotinic acid and caffeine in dry model systems

Trigonelline, nicotinic acid and caffeine (10.0 mg each) and a mixture which contained the proportions usually present in coffee (7.5 mg of trigonelline, 0.6 mg of nicotinic acid and 15.0 mg of caffeine) were placed in sealed silanised vials (Supelco, Bellefont, PA, USA) and heated at 240°C for 5, 8, 12, 15 and 20 min, in the same stove used for roasting the coffee. The vials were left standing to attain room temperature and the residues were dissolved in 100 ml of water. The solutions were filtered (0.2 μm) and directly analyzed by HPLC.

2.4. HPLC analysis

HPLC was carried out using two PU-980 Intelligent Pumps, a HG-980-30 Solvent Mixing Module, an AS-950 Intelligent Sampler and a MD-910 Multiwavelength Detector all from Jasco Corporation (Tokyo, Japan). A Spherisorb S5 ODS2 cartridge column (0.46 × 25.0 cm) was used coupled to a guard cartridge μBondapak C18 (10 μm particle size) from Waters Assoc. (Milford, MA, USA). The injection volume was 20 μl for all analyses performed.

A solvent gradient was formed with phosphate buffer 0.1 M (pH 4.0) and methanol (Casal et al., 1998). The eluents were filtered and degassed under reduced pressure in an ultrasonic bath Sonorex RK 100 (Bandelin electronic, Germany).

Quantification was carried out using calibration curves obtained with standard solutions of trigonelline, nicotinic acid and caffeine at 268, 264 and 276 nm, respectively, as recently reported (Casal et al., 1998). The determinations were performed in the linear range within 0.15–450, 0.10–500, and 0.05–500 μg ml^{-1} for trigonelline, nicotinic acid, and caffeine, respectively.

3. Results and discussion

The characteristics of the coffee samples used in this study are represented in Table 1. Fig. 1 shows a typical chromatogram obtained with a green *arabica* coffee sample (A) and after roasting at 240°C for 15 min (B). Although many interfering substances were present in the matrix, which complicated the chromatograms, the peaks were detected and identified by their UV spectra and by comparison with authentic standards.

3.1. Effect of roast temperature treatments in coffee

The results of the roasting trial conducted at constant time (15 min) and several temperatures are presented in Table 2. Trigonelline and caffeine contents were significantly different ($p < 0.99$) in the two species, both in green and roasted states. *Robusta* coffee beans contained the highest concentration of caffeine compared with *arabica*, while *arabica* was richer in trigonelline than the former, which confirms the ability of caffeine and trigonelline to discriminate these two coffee varieties. Furthermore, the values observed were in good agreement with those reported in the literature (Clifford, 1985; De Maria et al., 1995; Macrae, 1989; Martín, Pablos, Bello & González, 1997; Stennert & Maier, 1993; Stennert & Maier, 1994; Stennert & Maier, 1996; Taguchi et al., 1985; Trugo, Maria & Werneck, 1991) where they were measured by diversified methodologies. The trigonelline degradation increased after 200°C and only 5% remained in *arabica* and 15% in *robusta* at 240°C. The nicotinic acid levels increased more than 500% in both species and the caffeine levels slightly decreased.

3.2. Effect of roasting time in coffee and model dry systems

The evolution of the roast performed at constant temperature (240°C) with variation of the exposure time

Table 1
Roasting characteristics of the coffees used

Roasting temperature (°C)	Roasting time (min)	*Arabica*	*Robusta*
		Total roast loss (%)[a]	
140	15	5.0	7.6
160	15	7.8	15.8
180	15	10.0	18.0
200	15	15.5	22.7
220	15	17.7	24.1
240	5	10.8	10.1
240	8	11.9	11.8
240	12	17.3	18.9
240	15	22.6	22.8
240	20	26.1	27.9

[a] Dry matter basis.

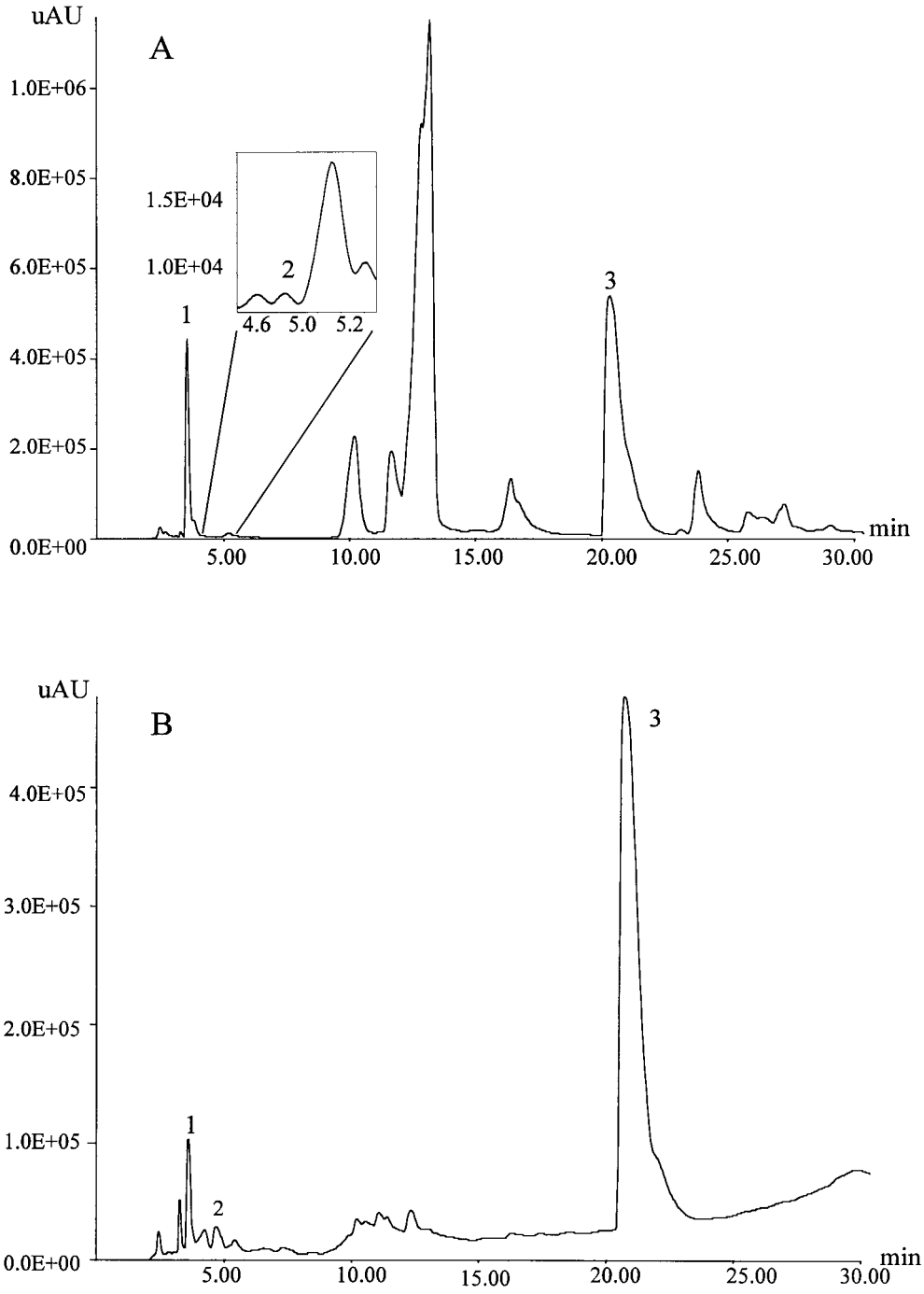

Fig. 1. Chromatograms of green *arabica* coffee (A) before roast and (B) after roast at 240°C for 15 min (recorded at 265 nm). Peaks: 1 = trigonelline; 2 = nicotinic acid; 3 = caffeine.

is reported simultaneously with the dry model system roasts of the pure standards (individually or in mixture). Results are reported as percentage of the initial content after being corrected for a dry green bean basis.

Native trigonelline (Fig. 2) was almost completely degraded in both varieties although with a higher degradation rate in *arabica* than in *robusta*. When a mixture of standards was treated under the same conditions this compound presented a similar behavior to that of its native form in coffee, although the degradation was initiated immediately. Finally, isolated trigonelline showed a similar degradation pattern but with a smaller rate.

The nicotinic acid content increased after 8 min of heating and continued up to 15 min. For an exposure

Table 2
Results (mean ± sd in mg/kg dmb) for the roast program performed at different temperatures and constant time (15 min)

	Trigonelline		Nicotinic acid		Caffeine	
	Arabica	*Robusta*	*Arabica*	*Robusta*	*Arabica*	*Robusta*
Green	8.91	6.32	0.03	0.02	12.36	20.84
140°C	8.47	6.37	0.06	0.06	14.37	22.12
160°C	8.31	5.86	0.08	0.05	15.18	21.71
180°C	8.29	5.78	0.06	0.04	13.57	19.81
200°C	7.80	5.43	0.07	0.06	13.87	19.93
220°C	5.57	4.20	0.13	0.06	12.95	19.88
240°C	0.49	0.97	0.17	0.13	10.96	19.25

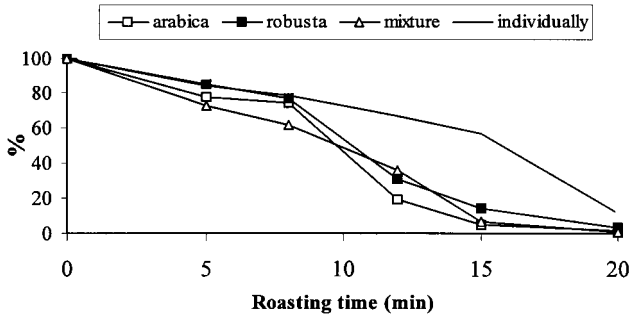

Fig. 2. Experimental influence of the time of roast (at 240°C) on the concentrations of trigonelline in coffee and model systems.

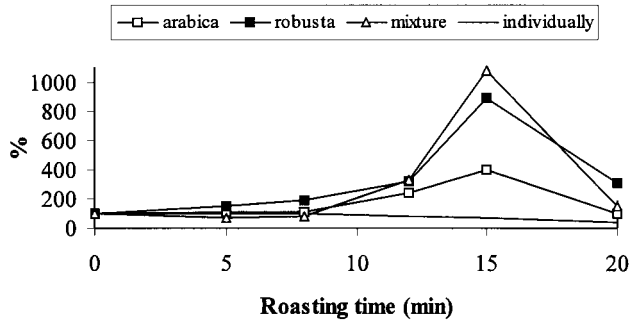

Fig. 3. Experimental influence of the time of roast (at 240°C) on the concentrations of nicotinic acid in coffee and model systems.

time greater than 15 min a gradual decrease of the nicotinic acid content was observed. The formation of nicotinic acid in the mixture of standards corresponded to a tenfold increase of its initial content. This fact clearly demonstrates that other compounds exist in coffee matrix, which avoid demethylation of trigonelline into nicotinic acid and probably allow the production of other compounds. Moreover the observation that this thermodegradation reaction took place upon 8 min of heat exposure (or after 200°C) indicates that some time is needed for the coffee bean to reach the convenient

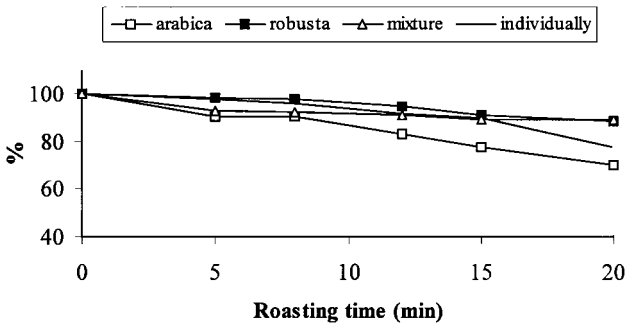

Fig. 4. Experimental influence of the time of roast (at 240°C) on the concentrations of caffeine in coffee and model systems.

Table 3
Rate constants (g/kg/min) for the thermochemical reactions in roasted coffee at 240°C

	Arabica		*Robusta*	
	K	R^2	*K*	R^2
Trigonelline	−0.344	0.995	−0.262	0.996
Nicotinic acid	0.186	0.998	0.214	0.928
Caffeine	−0.231	0.997	−0.161	0.974

energy for starting the chemical degradation reactions. According to Macrae (1989) the roasting procedure of coffee involves an initial step during which the contained water in beans is removed. After such step the real roasting is initiated only when an energetic level of about 60–100 kcal per kilo of coffee is attained, which occurs at about 200°C (activation time). Because industrial roasting is made by revolving the beans in a container with circulation of hot gases, the time necessary to achieve this temperature is shorter than in our laboratorial trials. Caffeine (Fig. 4), the third nitrogenic compound included in our study, showed a slight decrease after 10 min of roasting. It would be expected that caffeine sublimation might have occurred to a higher extent when its sublimation temperature was reached (185°C). As Macrae (1989) concluded, this observation should be related with porosity and the internal pressure created within the beans that may cause some difficulties for caffeine sublimation. Nevertheless, in the standard mixture, where caffeine is probably free of chemical and physical linkages, a similar gradual decrease of its content was also observed.

By applying linear regression analyses on the concentration (C-C0 in g kg^{-1}) in coffee versus time data (5–20 min), the constant rates at 240°C were calculated from the slope of linear regression analyses and were assumed to be first-order for trigonelline and nicotinic and zero-order for caffeine. The results are reported in Table 3.

4. Conclusions

The results indicate that the technique proposed herein allows a simultaneous study of the roasting evolution (trigonelline and nicotinic acid contents) and discrimination of varieties (trigonelline and caffeine contents).

The study showed diversified behaviour of these compounds when found in their native form or in an artificial mixture, eliciting the chemical environmental influence.

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