APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF SOME NON-VOLATILE COFFEE COMPONENTS

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SUMMARY

High performance liquid chromatography (HPLC) was applied to the analysis of caffeine, trigonelline, nicotinic acid and sucrose in Arabica and Robusta coffee. Green and roasted coffee samples were used in this study and the degradation of sucrose and trigonelline, with the formation of nicotinic acid, was followed during roasting. Caffeine did not undergo significant degradation with only 5.4% being lost under severe roasting. Sucrose was degraded rapidly during processing with light roasting producing a 97% loss and dark roasting degrading it completely. Loss of trigonelline was strongly dependent on the degree of roasting, being higher in the Robusta coffee. Trigonelline degradation was associated with nicotinic acid formation both in the Arabica and Robusta coffees as a consequence of the roasting process. Trigonelline and sucrose were determined simultaneously by partition chromatography and detection with the mass detector. Determination of caffeine was carried out using reversed phase chromatography and nicotinic acid by ion-pair reversed phase chromatography. Detection in both cases was achieved using an ultraviolet detector at 272nm or 254nm, respectively. HPLC showed adequate precision and accuracy for routine analyses. In addition, the methods used were more rapid and simple than traditional procedures.

HPLC appears to be a suitable technique for quality control in the coffee industry, and for fundamental investigation on the mechanisms involved in the roasting process.

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INTRODUCTION

Non-volatile components of coffee are important for their participation as flavor precursors including aroma formation. This group of compounds may be divided in high and low molecular weight material, proteins and polysaccharides being examples of the former and caffeine, trigonelline, nicotinic acid, sucrose and chlorogenic acid examples of the latter. Caffeine is particularly important due to its relation to some biological effects such as stimulation of the central nervous system (1) and its implication to the delay of sleep (2). Trigonelline, a pyridine derivative, is found in coffee in significant amounts and it is largely degraded during roasting, producing important coffee flavor components. It has a bitter taste which is about one-fourth that of caffeine (3) and contributes to coffee aroma by formation of volatile degradation products (4). Gas chromatography-mass spectometry analysis of the volatile fraction obtained from trigonelline pyrolysis showed 29 compounds with nine of them being previously identified in roasted coffee, pyridines and pyrroles being some examples (4). One important non-volatile compound also formed from trigonelline degradation during roasting is nicotinic acid. Therefore, nicotinic acid formation makes coffee beverage and important source of this vitamin. Sucrose is also extensively degraded during roasting, and is largely involved in caramelization reactions to form browning pigments in roasted coffee. Glucose may be found in minor quantities in green and in roasted coffee, in which small amounts of fructose, arabinose and galactose may also be found (5). The process of pigment formation is complex probably involving the participation of chlorogenic acid during caramelization (6) and in Maillard reaction with amino and carbonyl groups (7, 8). In the present work data on caffeine, trigonelline and its transformation into nicotinic acid and sucrose in green and roasted Arabica and Robusta coffees are presented. HPLC methods for their determination are described illustrating the application of this technique to coffee analysis.

MATERIAL AND METHODS

Samples

Green Arabica (Guatemala) and green Robusta (Uganda) coffee beans were supplied by the coffee industry. One part (5 kg) of the same batch of each coffee was industrially roasted in a Probat pilot roaster at 205°C. Four different degrees of roasting were obtained according to the processing time and classified as light, medium, dark and very dark, based on their colors. All coffee samples were frozen overnight, ground in a laboratory scale mill (Brook Motors Ltd., England) and then sieved to pass through a 0.841mm sieve.

Apparatus

The liquid chromatograph consisted of a pump which was either Model 750/03-ACS (Applied Chromatography Systems Ltd, England)

or Model 110A-Altex (Beckman Ltd, England), an injection valve Model 7120 (Rheodyne Inc., USA) with a 20 µl fixed loop, a chromatographic column and a detector. Detection was by means of either an ultraviolet variable wavelength detector Model CE212 (Cecil Instruments Ltd, England) or an ACS mass detector Model 750/14.

Reagents

All chemicals were of analytical-reagent grade unless otherwise specified. Chromatographic solvents were of HPLC grade obtained from Rathburn Chemicals Ltd (Scotland). Caffeine and sucrose standards were from BDH Laboratory Reagents (England), trigonelline was from Sigma Co (USA) and nicotinic acid from Fisons Ltd (England).

Chromatographic Phases and Columns

Microparticulate silica bonded phases Spherisorb-amino, Spherisorb-ODS and Spherisorb-ODS-2 (5 µg) were obtained from Phase Separation Ltd (Wales). Stainless steel (100, 150 or 250 mm x 5 mm i.d.) columns were packed in the laboratory using methanol as a slurry solvent for Spherisorb-amino phase and dried acetone for Spherisorb-ODS and ODS-2. Packing was achieved against gravity at a pressure of 375 bar. Sep-Pak C_{18} cartridges for sample clean-up were obtained from Waters Associates Ltd (England).

Determination of Caffeine

Ground coffee (approximately 0.5 g) was extracted with hot distilled water (100 ml at 80°C) for 10 min and transferred to a 250 ml volumetric flask. Saturated basic lead acetate (2 ml) was added, the solution made up to volume, shaken and allowed to stand for 10 min. The precipitate was removed by filtration under gravity and the extract refiltered using a Millipore filter (0.45 µm), when necessary. The filtrate was used directly for chromatography. Chromatographic separation and quantification was achieved using an isocratic system and a Spherisorb-ODS-2 column (150 x 5 mm i.d.) according to the method described by Trugo *et al.* (9).

Determination of Trigonelline and Sucrose

Ground coffee (approximately 1.25 g for green or 5 g for roasted) was shaken thoroughly with hot distilled water (5 ml at 80°C) in a 50 ml centrifuge tube. Absolute ethanol (20 ml) was added, the mixture shaken for one hour and centrifuged for 10 min (1,500 x g). The supernatant was decanted into a 250 ml evaporating flask, the procedure repeated twice more (shaking for 30 min) and the supernatants combined into the same flask. The extract was evaporated to approximately 5 ml under reduced pressure at 40°C. The residue was transferred to a 25 ml volumetric flask and made up to volume with distilled water. The extract was filtered under gravity and cleaned using a Sep-Pak C_{18} cartrige. This final extract was then used for chromatography. Chromatographic separation was achieved using a Spherisorb-amino column (250 x 5 mm i.d.) packed

in the laboratory. The mobile phase was acetonitrile/water (4:1, V/V) with a flow rate of 1.5 ml min^{-1}. Detection was achieved using the mass detector operated with the following instrumental settings: attenuation x 1; photomultiplier x 2; temperature, 60°C; air pressure, 1.5 bar. Quantification was based on peak height comparison with a calibration curve obtained from pure standards using aqueous solutions of trigonelline at concentrations of 0.125, 0.25, 0.625, 1.25 and 2 mg min^{-1}, and of 0.5, 1.0, 2.0, 3.0 and 4.0 mg min^{-1} of sucrose. Recoveries were assessed from four replicate samples with trigonelline at a level of 2.5% and sucrose at 0.5% of sample weight.

Determination of Nicotinic Acid

Boling distilled water (50 ml) was added to 5 g of ground coffee in a 125 ml conical flask. The contents were stirred throughly for 5 min and filtered using a Buchner funnel under reduced pressure. The residue was washed three more times with boiling distilled water (10 ml each time). The combined extracts were transferred to a 100 ml volumetric flask and the volume made up to the mark. The clean-up, chromatography and quantification were achieved according to the method described by Trugo, Macrae and Trugo (10).

RESULTS AND DISCUSSION

Caffeine

The variation in caffeine composition of coffee is mainly due to genetic differences found in varieties of coffee beans, and also to agronomic factors. Differences in analytical techniques, however, may also be responsible for variability in analytical data found in the literature. Routine methods are based on the measurement of UV absorption of coffee extracts (11, 12), and they are subject to interference from substances present in the extract and quantified as caffeine. More specific and rapid HPLC techniques have been increasingly used for caffeine determination in a wide range of food products (13). In this work, reversed phase chromatography using Spherisorb ODS-2, was applied to caffeine determination in green and roasted coffee at different roasting degrees. The comparison between ODS-2, a fully capped reversed phase material, and the conventional ODS has been previously reported for purine alkaloid analysis (9). The Robusta green coffee studied showed higher caffeine content (2.4%, dry matter) in comparison with the Arabica coffee (1.2%) which is in agreement with literature values (14). The values found in the roasted samples were slightly higher both for the Arabica and Robusta when compared with their respective green beans and these values increased with the degree of roasting. This supports the findings of Merritt and Proctor (15) when they studied seven different varieties of coffees submitted to the roasting process at different temperatures. Nevertheless, if the weight loss during roasting is considered, the results actually show an opposite effect, as Table 1 illustrates. Small amounts of caffeine are in fact lost during roasting at 205°C. Although

TABLE 1

CAFFEINE CONTENT OF COFFEES AND PERCENTAGE LOSSES AT DIFFERENT DEGREES OF ROASTING

Coffee	Roasting time (min)	Caffeine contents a	b	Caffeine[c] loss (%)
Arabica	7	1.18	1.14	5.0
(Guatemala)	10	1.28	1.23	−2.5[d]
	13	1.32	1.19	0.8
	19	1.27	1.15	4.2
Robusta	5	2.45	2.39	0.4
(Uganda)	7	2.49	2.39	0.4
	14	2.48	2.27	5.4
	16	2.46	2.27	5.4

a Results expressed in g % dry matter, are average of determination in duplicate.
b Results expressed on dry green bean basis (g % caffeine x % recovery of coffee beans after roasting/100).
c Caffeine content in the green bean: Arabica, 1.20; Robusta 2.40 g % dry matter.
d Caffeine content was 2.5% higher than in the original green bean.

the temperature of sublimation of caffeine is around the threshold of pyrolysis, that is about 185°C (16), internal pressures created within the bean may raise its sublimation point and also make the liberation of caffeine vapor more difficult. Consequently, much loss of caffeine by sublimation is not expected, as has been confirmed by the results found. Coefficient of variation of the method was 2.4% indicating a good precision. The detection limit was estimated to be 0.5 $\mu g\ ml^{-1}$ which correspond to a level of 50-100 mg % dry food, but this limit could be greatly reduced by changing the detector attenuation. Linearity was assessed constructing a calibration curve and a good correlation was obtained (0.99997). Recovery was examined by the method of standard addition to the sample showing an average of 100%.

Trigonelline

Many methods have been described in the literature for the determination of trigonelline in coffee products. They may be simply based on titration of a trigonelline-iodine complex (17) or by colorimetry such as the procedure of Huff (18) involving the use of a highly toxic reagent (cyanogen bromide). However, these procedures lack precision and are subject to interference. More accurate results may be obtained by the measurement of UV absorption of trigonelline (19), provided the extract is sufficiently free of interferences. Chromatographic techniques appear to be more specific and in this case HPLC is preferred since it provides better separation in a shorter time (9). In the study herein described, the

possibility of using partition chromatography with amino-columns was investigated and an HPLC method was developed using a mass detector as the detection system. The mass detector is based on the detection of solute molecules by light scattering after nebulization and evaporation of the chromatographic solvent. The theoretical basis of the instrument has been elucidated by Charlesworth (20) and it has been applied to the determination of sugars (21) and lipids (22, 23) by HPLC. Using this chromatographic system it was possible to determine trigonelline and sucrose simultaneously by isocratic elution. A calibration plot obtained with aqueous trigonelline solutions (0.125-2.0 mg ml^{-1}) showed linearity only above 0.25 mg ml^{-1} but this was a function of the detector. The same calibration curve shapes were obtained when six different sugars were studied under the same conditions. Consequently, quantification was achieved by comparison of peak heights from the sample extracts, with the calibration curve. Recovery was adequate showing an average of 98o/o with a coefficient of variation of 4o/o. The Arabica and Robusta green coffees studied showed similar levels of trigonelline. The loss of trigonelline after roasting showed a similar pattern to that reported by other authors using different methods (24, 25) and this loss was strongly dependent on the degree of roasting, being higher in the Robusta coffee (Table 2). Trigonelline is partially converted to nicotinic acid during the roasting process by demethylation and its progressive loss was closely associated with an increase in nicotinic acid in both Arabica and Robusta coffees (Figures 1a and 1b). This is consistent with the results obtained by Hughes and Smith (24) using titration and colorimetric methods to determine trigonelline and nicotinic acid, respectively. The HPLC method was

TABLE 2

TRIGONELLINE CONTENT OF COFFEES AND PERCENTAGE LOSSES AT DIFFERENT DEGREES OF ROASTING

Type of coffe	Roasting time (min)	Trigonelline contents a	b	Trigonelline[c] loss (o/o)
Arabica	7	0.89	0.86	21.1
(Guatemala)	10	0.73	0.70	35.8
	13	0.39	0.35	67.9
	19	0.18	0.16	85.3
Robusta	5	0.73	0.71	34.9
(Uganda)	7	0.60	0.58	46.8
	14	0.33	0.32	70.6
	16	0.12	0.11	89.9

a Results expressed in g o/o dry matter, are average of determinations in duplicate.

b Results expressed on dry green bean basis (g o/o trigonelline x o/o recovery of coffee beans after roasting/100).

c Trigonelline content in the green bean: Arabica, 1.09; Robusta, 1.09 (g o/o, dry matter).

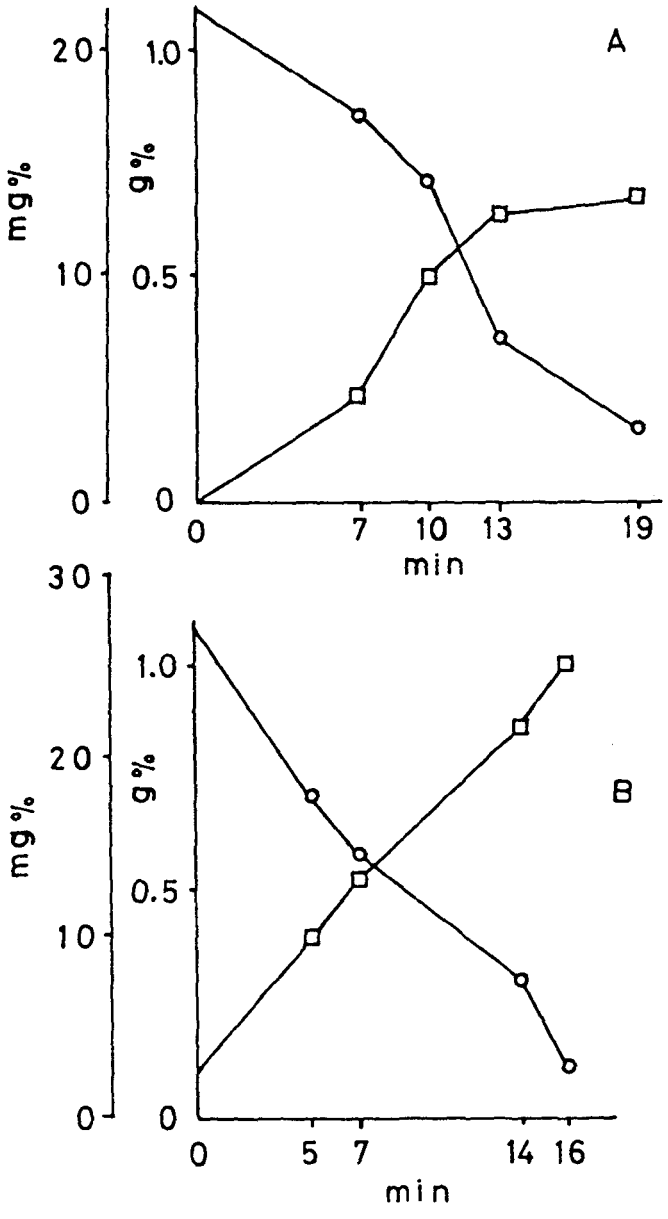

FIGURE 1

Degradation of trigonelline and formation of nicotinic acid during coffee roasting. The samples were roasted at different degrees (times) and analyzed by HPLC according to the method described. (A) Arabica coffee; (B) Robusta Coffee; (o) Trigonelline expressed in g o/o; (□) Nicotinic acid expressed in mg o/o

shown to be useful in following the transformation of trigonelline during coffee roasting, and in addition it allowed the simultaneous determination of sucrose (Figure 2).

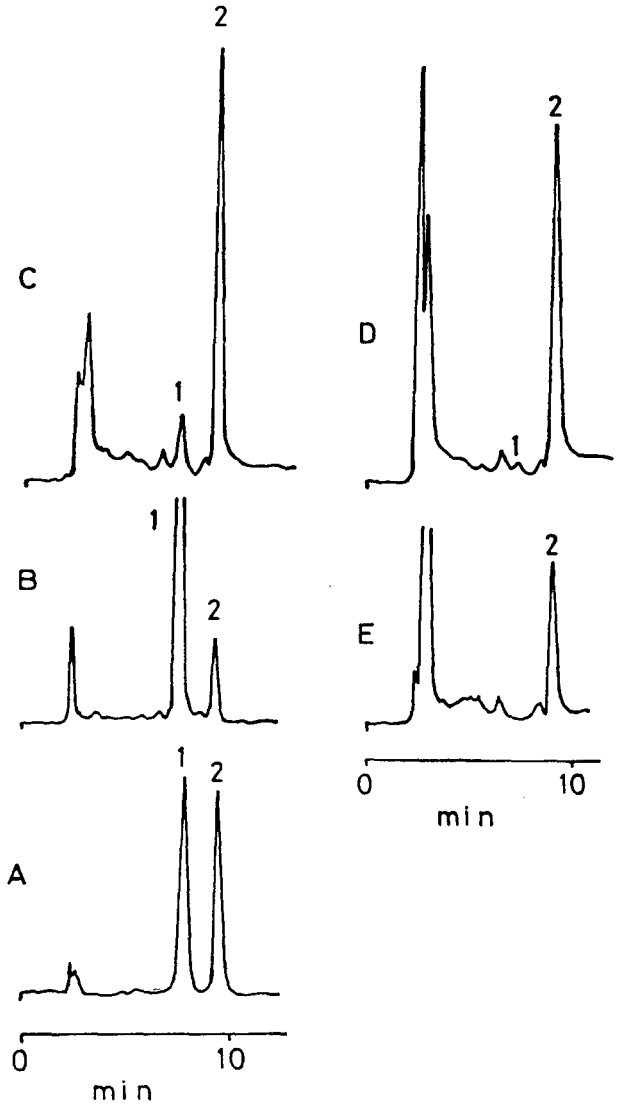

FIGURE 2

Separation of sucrose and trigonelline. Column: Spherisorb amino (250 x 5 mm i.d.); Solvent: acetonitrile/water 80:20, V/V, at 1.5 ml min^{-1}; Detection: mass detector set at attenuation x1, photomultiplier x 2 and evaporation temperature of 60°C. (A) standard solution; (B) green Arabica coffee; (C) light roasted; (D) medium roasted; (E) dark roasted. (1) sucrose; (2) trigonelline

Sucrose

Determination of sucrose was achieved simultaneously with trigonelline by using the mass detector as the detection system. Recovery was examined by four replicate additions of green coffee samples at a level of 5o/o of sample weight with results averaging 96o/o. Similarly to trigonelline, linearity of the mass detector was only attained above 0.5 mg ml^{-1} and quantification was then achieved by comparison of peak height values with the calibration curve. Sucrose contents in the green coffees were 8.5o/o for Arabica and 7.1o/o for Robusta. The Robusta coffee showed a higher sucrose value compared to previous reports (14). This, however, could be interpreted as further evidence of the wide variation that might be expected in the composition of coffee from different origins. Sucrose was rapidly degraded during roasting and the rate of degradation was very similar for both Arabica and Robusta coffee. The light roast produced about 97o/o loss, increasing to 99o/o in the medium roast and in the dark and very dark roast sucrose eventually disappeared. The occurrence of glucose and fructose (26) and also small amounts of arabinose (5) have been reported in some roasted coffees. Nevertheless, in the present work no other free sugars could be detected and, if they were present, their levels would have been below 0.01o/o. Additional data may be found in a previous report (27).

Nicotinic Acid

Nicotinic acid determination in coffee is nutritionally important since it is formed during roasting from trigonelline. Nicotinic acid is present in the coffee beverage as a free vitamin in contrast to cereals and other biological tissues in which it largely appears in the form of niacinamide, as a constituent of the coenzymes niacin-adenine dinucleotide and niacin-adenine dinucleotide phosphate (28). A small amount of nicotinic acid was found in green Robusta coffee (1.9 mg o/o, dry matter) but it was not quantified in Arabica coffee (i.e. below 0.5 mg o/o). Nicotinic acid content of roasted coffees was strongly dependent on the degree of roasting, with higher levels being found in the Robusta sample. Trigonelline is extensively degraded during roasting with losses of 20-90o/o, depending on the roasting degree. It was determined, however, that only a small fraction is actually converted to nicotinic acid with ranges of conversion of 0.4-1.2o/o in the Arabica, and 0.9-2.3o/o in the Robusta. The rate of nicotinic acid formation throughout the roasting process was approximately the same for the Robusta coffee, but for the Arabica it increased steadily up to the dark roasting stage and then slowed down (Figure 1). The chromatograms showed very complex patterns, particularly for the roasted samples (Figure 3). Several peaks were detected, some of them increasing and others decreasing on roasting; however, they were not identified in the present work.

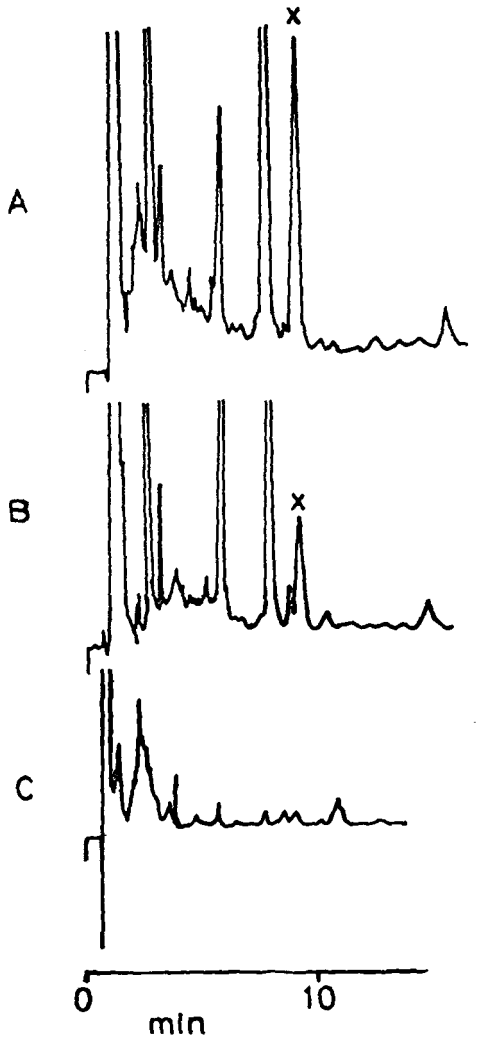

FIGURE 3

Separation of nicotinic acid in roasted coffee. Column: Spherisorb ODS-2 (150 x 5 mm i.d.); Solvent: 0.01 M tripotassium citrate containing 8% methanol, V/V and 0.05 M tetrabutylammonium hydroxide at pH 7; Detection: 254 nm. (A) dark roast; (B) light roast; (C) green Arabica coffee; (x) nicotinic acid

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