Antibacterial Activity of Coffee: Relationship between Biological Activity and Chemical Markers

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The antibacterial activity of coffee depends mainly on the degree of roasting of the coffee beans. To identify easily determinable chemical markers which relate to coffee's antibacterial activity, 5-caffeoylquinic acid, caffeic acid, nicotinic acid, trigonelline, 5-(hydroxymethyl)furfuraldehyde, the content of which changed throughout the roasting process, and caffeine were considered. A new HPLC method that allows determination of these compounds simultaneously was developed. Two antibacterial activity chemical markers were found: (1) the sum of trigonelline and nicotinic acid contents and (2) the 5-caffeoylquinic acid/caffeine ratio.

Keywords: *Coffee; antibacterial activity; chemical markers*

INTRODUCTION

Roasted coffee clearly shows a degree of antibacterial activity that is not present in green coffee and depends on the degree of roasting of coffee beans (Daglia et al., 1994). This activity depends on the presence of reaction products that are formed during the roasting process. Brown melanoidins are present in the soluble fraction of roasted coffee and derive from Maillard reaction products (MRPs) and from carbohydrate caramelization together with substances from the thermal decomposition and pyrolysis of organic compounds (Belitz and Grasch, 1987). MRPs obtained from model systems, which differ in the type and concentration of the reacting sugars and amino acids, the temperature, the duration of heating, etc., show antibacterial activity against a wide range of bacteria (Einarsson et al., 1983; Stecchini et al., 1991; Daglia et al., 1992) and are considered as nontraditional food antimicrobial agents (Shibasaki, 1982).

As yet, these antibacterial compounds have not been isolated from the complex browning mixtures and identified. Therefore, it seemed worth researching easily determinable chemical indicators which may be considered as antibacterial activity markers for coffee. The aim of this study is to verify the relationship between the antibacterial activity of coffee and those compounds whose roasted coffee content closely depends on the degree of roasting and can therefore be considered as antibacterial activity indicators. We then considered the content of 5-caffeoylquinic acid (Trugo and Macrae, 1984a), the value of 5-caffeoylquinic acid/caffeine ratio (Purdon and McCamey, 1987), and the content of caffeic acid, nicotinic acid, trigonelline, and 5-(hydroxymethyl)furfuraldehyde.

The literature describes a number of HPLC methods whose purpose is to monitor the coffee roasting process by, for example, studying chlorogenic acid composition (Trugo and Macrae, 1984b) or calculating the 5-caffeoylquinic acid/caffeine ratio. Furthermore, reversed-phase HPLC is used in separate methods for the determination of, respectively, trigonelline (Trugo et al., 1983; Mazzafera, 1991) and nicotinic acid (Trugo and Macrae, 1985, 1989) in coffee and for the measurement of 5-(hydroxymethyl)furfuraldehyde in various beverages (Dauberte et al., 1990).

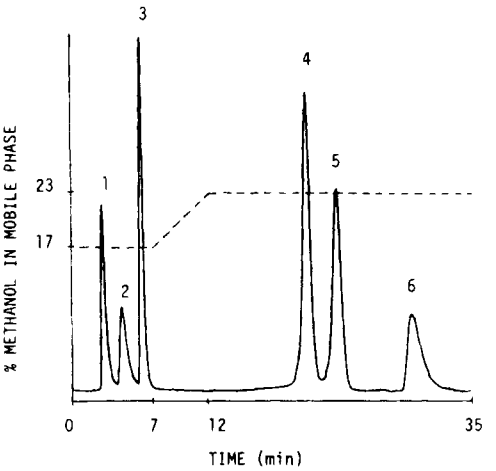

Figure 1. Chromatogram of standards (column, Lichrosorb 100 RP18; temperature, 28 ± 0.1 °C). Solvent and gradient conditions were as described in the text. The dashed line indicates variations in mobile phase composition during the analysis. Flow rate was 1 mL/min. Detection was at 260, 272, and 320 nm (au = 0.1). Peaks: (1) trigonelline (λ = 260 nm); (2) nicotinic acid (λ = 260 nm); (3) 5-(hydroxymethyl)furfuraldehyde (λ = 272 nm); (4) chlorogenic acid (λ = 320 nm); (5) caffeic acid (λ = 320 nm); (6) caffeine (λ = 272 nm).

We developed a reversed-phase HPLC method that enabled us to determine 5-caffeoylquinic acid, caffeic acid, nicotinic acid, trigonelline, caffeine, and 5-(hydroxymethyl)furfuraldehyde simultaneously.

MATERIALS AND METHODS

Apparatus. HPLC analyses were performed with a Hitachi-Merck L 6200 A chromatograph (Darmstadt, Germany) that was equipped with a Waters 490 E UV–vis detector (Millipore Corp., Bedford, MA) and a Hitachi-Merck D 2500 integrator. The column (250 × 4 mm i.d.) was a Lichrosorb 100 RP18 (5 μm, C% = 21.4) (Merck). The mobile phase was methanol (A) and 1 mM hydrochloric acid, pH 3.27 ± 0.02 (B). Mobile phase composition was 17% A and 83% B for the first 7 min; for the next 6 min, composition increased toward 23% A and 77% B, and thereafter it remained stable. Flow rate was 1 mL/min, column temperature was 28 ± 0.1 °C, and UV

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Table 1. Retention Times and Absorbance Ratios of Standard Compounds

compound	RT, min	absorbance ratio					
		210/250 nm	260/250 nm	272/250 nm	280/250 nm	290/250 nm	320/250 nm
trigonelline	2.60	17.83	1.32				
nicotinic acid	4.35	7.50	1.37				
5-(hydroxymethyl)furfuraldehyde	6.07				9.08		
chlorogenic acid	20.59					2.02	2.56
caffeic acid	23.34					2.15	1.78
caffeine	29.92			3.34			

Table 2. Regression Data and Linear Operating Ranges for Determination of the Examined Compounds

compound	regression eq	correln coeff	R^2 %	linear op range, μg/mL
trigonelline	$y = 0.14x - 0.42$	0.994641	98.93	10–45
nicotinic acid	$y = 0.22x - 0.37$	0.995281	99.06	3–15
5-(hydroxymethyl)furfuraldehyde	$y = 0.84x - 0.15$	0.991724	98.35	2–20
chlorogenic acid	$y = 0.03x - 0.04$	0.999524	99.90	30–120
caffeine	$y = 9.93x - 0.11$	0.998461	99.69	30–120

Table 3. Precision of the Results for Standard Mixture

	trigonelline	nicotinic acid	HMF[a]	chlorogenic acid	caffeine
content, μg/mL	25.95	8.59	6.33	59.97	65.35
	25.69	8.45	6.41	59.81	66.45
	25.75	8.08	6.42	59.10	65.90
	25.95	8.10	6.27	59.61	66.00
	25.75	8.60	6.41	60.10	65.05
degrees of freedom	4	4	4	4	4
mean value, μg/mL	25.82	8.36	6.37	59.72	65.75
standard deviation, μg/mL	0.12	0.26	0.07	0.39	0.55
standard error, % (P = 95%)	0.05	0.11	0.03	0.17	0.25

[a] 5-(Hydroxymethyl)furfuraldehyde.

Table 4. Precision of the Results for Examined Compounds Found in the *C. arabica* Brewed Coffee ($1A_1$)

	trigonelline	nicotinic acid	HMF	chlorogenic acid	caffeine
content, μg/mL	42.90	5.28	3.36	59.46	66.98
	42.18	5.94	3.66	59.10	66.66
	43.20	6.09	3.24	59.40	67.68
	42.60	6.24	3.60	59.76	66.58
	42.06	6.32	3.48	59.28	66.30
degrees of freedom	4	4	4	4	4
mean value, μg/mL	42.59	5.97	3.47	59.40	66.84
standard deviation, μg/mL	0.48	0.41	0.17	0.24	0.53
standard error, % (P = 95%)	0.21	0.18	0.08	0.11	0.24

Table 5. Precision of the Results for Examined Compounds Found in the *C. robusta* Brewed Coffee ($1R_1$)

	trigonelline	nicotinic acid	HMF	chlorogenic acid	caffeine
content, μg/mL	31.26	2.70	1.68	114.78	96.36
	31.50	2.94	1.91	117.12	95.70
	31.14	2.94	2.04	117.12	97.26
	31.08	2.88	1.80	116.58	96.84
	31.20	2.76	1.86	117.72	97.17
degrees of freedom	4	4	4	4	4
mean value, μg/mL	31.24	2.84	1.86	116.66	96.67
standard deviation, μg/mL	0.16	0.11	0.13	1.13	0.64
standard error, % (P = 95%)	0.07	0.05	0.06	0.50	0.29

detection was at 260, 272, and 320 nm. Samples were injected via a Rheodyne 7125 injection valve with a 20 μL loop.

Reagents. Caffeic acid, nicotinic acid, trigonelline hydrochloride, caffeine anhydrous, and 5-(hydroxymethyl)furfuraldehyde (HMF) were obtained from Fluka (Buchs, Switzerland); chlorogenic acid (5-caffeoylquinic acid) was obtained from Sigma (St. Louis, MO).

Methanol and hydrochloric acid (1 mM) were obtained from E. Merck (Darmstadt, Germany). All solvents were of HPLC grade and were filtered and degassed.

Coffee Batches. We examined 30 coffee batches obtained from 5 samples of green *Coffea arabica* and 5 samples of green *Coffea robusta* coffee beans from different sources, subdivided into three roasting batches (light, medium, and dark). The sources of green *C. arabica* were (1A) Costa Rica, (2A) Colombia, (3A) El Salvador, (4A) Guatemala, and (5A) Brazil. The sources of green *C. robusta* were (1R) Ecuador, (2R) Java, (3R) Indonesia, (4R) Ivory Coast, and (5R) Zaire.

As described in the previous paper (Daglia et al., 1994), the batches were roasted for 5 (light, A_1 or R_1), 7 (medium, A_2 or R_2), and 8 min (dark, A_3 or R_3). Six grams of green and roasted coffee samples was ground and boiled for 10 min in 100 mL of water; 10 mL of extract was evaporated to dryness under reduced pressure and low temperature (less than 30 °C). The residue was dissolved in 1 mL of sterile water, and the solution was analyzed for antibacterial activity.

Sample Preparation for HPLC Analyses. Ten milliliters of the filtered brewed coffee was diluted to 100 mL and filtered through a 0.45 μm filter (Millipore Corp.).

Antibacterial Activity. Antibacterial activity was assayed against *Staphylococcus aureus* ATCC 25923. The minimum inhibitory concentration (MIC) values, already reported in the previous paper (Daglia et al., 1994), were determined with the broth dilution method in Iso-Sensitest broth (ISB, Oxoid). MIC was evaluated as the lowest concentration that inhibited the formation of visible microbial growth and expressed as mil-

Table 6. Contents of Examined Compounds in Brewed Coffee Obtained from Light-, Medium-, and Dark-Roasted Coffee Beans[a]

sample	5-caffeoylquinic acid, g/100 g	nicotinic acid, mg/100 g	trigonelline, g/100 g	caffeine, g/100 g	HMF, mg/100 g
1A green	3.975	–[b]	1.760	1.456	–
$1A_1$	2.039	51	1.184	1.228	73
$1A_2$	0.580	29	0.531	1.017	5
$1A_3$	0.140	11	0.225	1.058	–
2A green	3.915	–	2.088	1.210	–
$2A_1$	2.149	119	1.175	1.227	35
$2A_2$	1.465	76	0.831	0.995	5
$2A_3$	0.551	20	0.719	0.884	4
3A green	3.240	–	2.105	1.128	–
$3A_1$	1.681	22	1.173	1.137	7
$3A_2$	0.754	20	0.884	1.115	3
$3A_3$	0.441	–	0.732	1.084	4
4A green	4.234	–	2.060	1.195	–
$4A_1$	2.174	40	1.114	1.160	7
$4A_2$	1.208	22	0.890	1.098	4
$4A_3$	0.659	–	0.635	0.974	3
5A green	3.945	–	2.185	1.035	–
$5A_1$	2.029	45	1.202	1.064	10
$5A_2$	1.729	10	0.990	0.963	6
$5A_3$	0.323	–	0.466	1.000	–
1R green	3.910	–	1.380	2.025	–
$1R_1$	1.853	23	0.751	1.956	27
$1R_2$	0.817	13	0.539	1.713	6
$1R_3$	0.153	–	0.171	1.567	3
2R green	4.056	–	1.508	2.215	–
$2R_1$	2.286	47	0.847	2.109	23
$2R_2$	1.227	22	0.644	2.019	8
$2R_3$	0.258	–	0.159	1.957	–
3R green	4.200	–	1.468	1.840	–
$3R_1$	1.955	44	0.856	1.650	6
$3R_2$	0.712	33	0.581	1.770	6
$3R_3$	0.081	22	0.279	1.733	4
4R green	4.910	–	1.715	2.410	–
$4R_1$	3.039	40	0.991	2.195	7
$4R_2$	1.935	39	0.938	2.148	5
$4R_3$	0.131	10	0.080	1.766	–
5R green	5.430	–	1.148	2.992	–
$5R_1$	3.036	54	0.971	1.799	54
$5R_2$	1.688	11	0.870	1.767	3
$5R_3$	0.508	13	0.446	1.707	4

[a] Reported data pertain to dry matter and are the average of at least duplicate determinations. [b] Concentration less than sensitivity level.

ligrams per milliliter of the roasted ground coffee used to prepare coffee.

Statistical Analysis. We performed all calculations using the Statgraphics (1991) statistical analysis package. To adopt the most appropriate chromatographic method, we used the two-level full factorial design. To determine the relationship between coffee's antibacterial activity and the given chemical markers, we used the simple regression analysis procedure with an exponential model, at confidence and prediction limits of 95%.

RESULTS AND DISCUSSION

Experimental Design. We used the two-level full factorial design to examine the following process factors: methanol concentration in mobile phase (%), flow rate (mL/min), and column temperature (°C). The selected response variables were the selectivity factor (α) calculated for the couple nicotinic acid and 5-(hydroxymethyl)furfuraldehyde and the retention time (RT) of the last peak eluted (caffeine). The accrued data were fitted to a second-order model which was used to generate the response surface contour plots. From these we calculated the chromatographic conditions, which gave the selectivity factor as $\alpha = 1.5$ and the retention

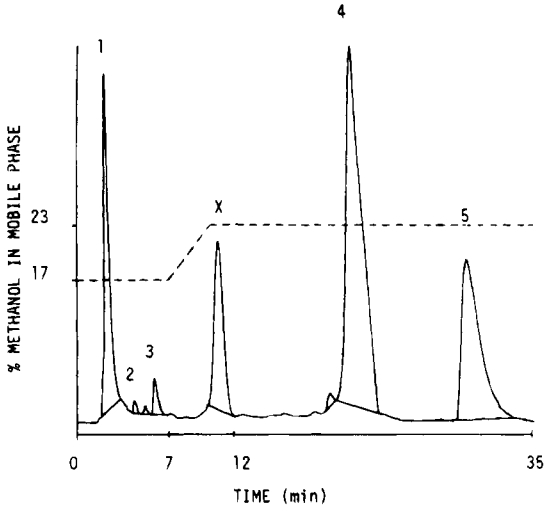

Figure 2. Chromatogram of brewed coffee obtained from *C. arabica* ($1A_1$). Chromatographic conditions were as described in the text. Peaks: (1) trigonelline (λ = 260 nm); (2) nicotinic acid (λ = 260 nm); (3) 5-(hydroxymethyl)furfuraldehyde (λ = 272 nm); (4) chlorogenic acid (λ = 320 nm); (5) caffeine (λ = 272 nm); (X) 3-caffeoylquinic acid (λ = 320 nm).

Table 7. Coffee Antibacterial Activity and Degree of Roasting Chemical Markers

sample	antibacterial activity[a] MIC, mg/mL[b]	sum of trigonelline and nicotinic acid, g/100 g	5-caffeoylquinic acid/caffeine ratio
$1A_1$	11	1.235	1.660
$1A_2$	4	0.731	0.570
$1A_3$	3	0.425	0.132
$2A_1$	12	1.294	1.751
$2A_2$	11	0.966	1.472
$2A_3$	6	0.739	0.623
$3A_1$	15	1.195	1.478
$3A_2$	10	0.904	0.676
$3A_3$	6	0.732	0.465
$4A_1$	11	1.154	1.874
$4A_2$	6	0.912	1.100
$4A_3$	6	0.635	0.677
$5A_1$	17	1.247	1.907
$5A_2$	11	0.990	1.795
$5A_3$	6	0.466	0.323
$1R_1$	8	0.774	0.947
$1R_2$	4	0.552	0.477
$1R_3$	3	0.171	0.098
$2R_1$	6	0.887	1.084
$2R_2$	4	0.666	0.608
$2R_3$	3	0.159	0.132
$3R_1$	6	0.900	1.123
$3R_2$	4	0.613	0.462
$3R_3$	3	0.301	0.088
$4R_1$	11	1.031	1.384
$4R_2$	6	0.887	0.901
$4R_3$	4	0.180	0.074
$5R_1$	11	1.025	1.688
$5R_2$	4	0.721	0.955
$5R_3$	3	0.283	0.298

[a] Antibacterial activity was assayed against *S. aureus* ATCC 25293. [b] MIC was expressed as mg/mL of the roast ground coffee used to prepare coffee beverage.

Table 8. Regression Models for Prediction of MIC Value as a Function of Chemical Markers

independent variable	regression model, $y = \exp(a + bx)$	t value	R^2	standard error of estimation
sum of trigonelline and nicotinic acid	$a = 0.735609$	6.61142	80.41	0.240884
	$b = 1.443350$	10.72130		
5-caffeoylquinic acid/caffeine ratio	$a = 1.124620$	13.6619	79.10	0.2488909
	$b = 0.790469$	10.2949		

time of caffeine as about 30 min. These conditions, applied throughout the analyses, are reported under Materials and Methods.

Identification and Quantification. Table 1 lists all of the compounds used as standards, as well as retention times and absorbance ratios.

Figure 1 shows the elution pattern of standards. Figures 2 and 3 show the chromatograms obtained from the analysis of samples respectively prepared with *C. arabica* ($1A_1$) and *C. robusta* ($1R_1$). Caffeic acid was not found in either variety. The peak marked "X" was identified as a chlorogenic acid isomer (neochlorogenic acid = 3-caffeoylquinic acid).

We calculated concentrations for 5-caffeoylquinic acid, nicotinic acid, trigonelline, caffeine, and 5-(hydroxymethyl)furfuraldehyde using the external standard method. The linearity of calibration was supported by the regression data reported in Table 2.

Tables 3–5 report the precision of the method for each examined compound, respectively, for standard mixture and for brewed coffees obtained from light-roasted *C. arabica* from Costa Rica ($1A_1$) and from light-roasted *C. robusta* from Zaire ($1R_1$).

Table 6 reports content values for the examined compounds that are present in all 30 coffee batches. The great difference between *C. arabica* and *C. robusta* as to compound levels agrees perfectly with the data reported in the literature: (a) *C. arabica* shows lower levels of caffeine and 5-caffeoylquinic acid and greater levels of trigonelline than *C. robusta*. (b) As expected, we found very small amounts of nicotinic acid and only traces of 5-(hydroxymethyl)furfuraldehyde; both compounds are generally present only in light- and medium-roasted coffee.

The HPLC method, suggested for the simultaneous identification of six, and the determination of five, brewed coffee compounds, offers the advantage of a substantial reduction in analysis time with proven accuracy.

Relationship between Antibacterial Activity and Chemical Markers. We considered the following to be coffee's antibacterial activity indicators: (1) The sum of trigonelline and nicotinic acid contents depends strongly on the degree of roasting (for trigonelline content), and nicotinic acid formation is associated with trigonelline degradation in the first phase of the roasting process. (2) The 5-caffeoylquinic acid/caffeine ratio was proposed by Purdon and McCamey (1987) to be a

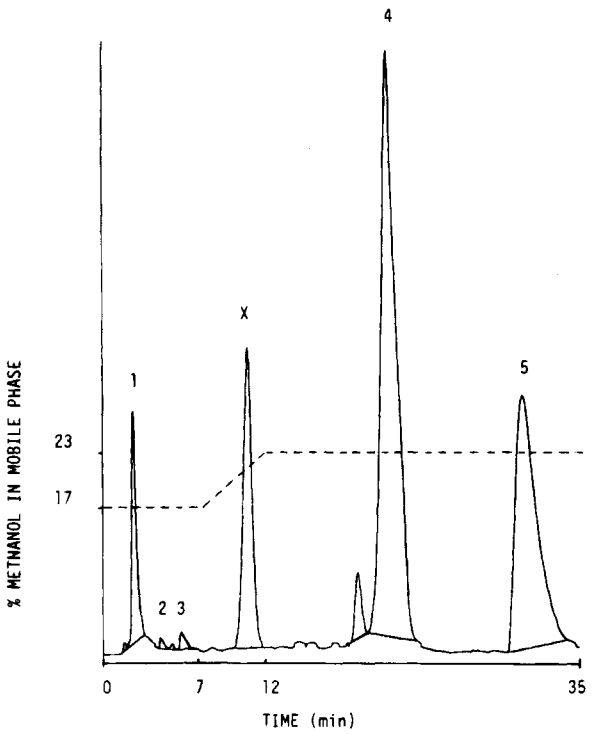

Figure 3. Chromatogram of brewed coffee obtained from *C. robusta* ($1R_1$). Chromatographic conditions were as described in the text, and peaks are as described in Figure 2.

quick and efficient measurement of the degree of roasting. Both variables depend on coffee bean variety (see Table 7).

The simple regression analysis results, reported in Table 8, show that both independent variables provide useful predictive information regarding the antibacterial activity against bacteria, such as *S. aureus*, which are very sensitive to coffee MRPs. The R^2 indicates that the amount of variance accounted for by both regression models is about 80%; moreover, the standard error of estimation is lower than 0.25.

As both variables depend on coffee bean variety, the suggested regression models, obtained by fitted data pertaining to *C. arabica* (15 samples) and *C. robusta* (15 samples), allow accurate predictions of the antibacterial activity for those coffees brewed as in southern Europe and the United Kingdom. In these countries, coffee is obtained from similar blends of *C. arabica* and *C. robusta*. A regression model, obtained from data pertaining only to *C. arabica*, could be used for the prediction of the antibacterial activity of coffee brewed from *C. arabica* alone or from coffee blends in which *C. arabica* is very high, as found in the United States and Scandinavian countries.

The regression model obtained from data pertaining to chemical markers may replace the assay for antibacterial activity. Moreover, it should be noted that the sum of trigonelline and nicotinic acid contents, like the 5-caffeoylquinic acid/caffeine ratio, depends on the degree of roasting of coffee beans and may be used as an efficient measurement for monitoring the roasting process.

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