Antibacterial Activity of Coffee

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This research demonstrates that roasted coffee has antibacterial properties. This activity is evident against a wide range of bacteria. In particular, the study considered the effect on such activity of three variables: coffee variety (*Coffea arabica* and *Coffea robusta*, from a total of 10 different sources), the degree of roasting (light, medium, and dark), and, finally, differences in brewing procedure. All of the roasted coffee samples examined clearly show antibacterial activity, and this activity mainly depends on the degree of roasting. This result shows that the main causes of the antibacterial activity of coffee are substances produced by the roasting process such as Maillard reaction products, carbohydrate caramelization, and thermal decomposition products.

Keywords: *Coffee; antibacterial activity*

INTRODUCTION

The high worldwide consumption of coffee is due not only to the valuable stimulant effect it has on mental and physical activity (Arrigo, 1991) but also to the sensory properties of the beverage. The typical flavor, taste, and color of coffee result from the roasting process (Lerici and Nicoli, 1990). The brown compounds which are present in the soluble fraction of roasted coffee and which are also known as coffee melanoidins (Maneva et al., 1985; Heinrich and Baltes, 1987) are derived from Maillard reaction or from carbohydrate caramelization and thermolysis of organic compounds (Belitz and Grasch, 1987).

The literature reports the antibacterial activity of Maillard reaction products (MRPs) (Kato and Shibasaki, 1974; Leite et al., 1979; Einarsson et al., 1983; Stecchini et al., 1991) as obtained in model systems. The aim of our paper was to investigate the effect of coffee on a wide range of bacteria and to verify whether and to what extent the antibacterial activity depended on the degree of roasting of the beans.

MATERIALS AND METHODS

Roasting. Five samples of green *Coffea arabica* (A) and five of green *Coffea robusta* (R) coffee beans, from a total of 10 different sources (see Table 1), were subdivided into three roasting batches (light, medium, and dark). The batches were then roasted in a pilot roaster apparatus for the respective times of 5 (light, A_1 or R_1), 7 (medium, A_2 or R_2), and 8 min (dark, A_3 or R_3). The weight losses, due to the roasting process, were about 11% for light-roasted samples, 13.5% for medium-roasted samples, and 20% for dark-roasted samples (see Table 1).

Preparation and Brewing of the Coffee. The green and roasted coffee samples were ground in a laboratory scale mill (Moulinette S, Moulinex, France) and sieved through a no. 30 sieve.

The beverage samples were prepared with three brewing procedures:

(1) Brewed Coffee. Six grams of green or roasted and ground coffee was boiled for 10 min in 100 mL of water; 10 mL of the extract, filtered on Ruudfilter Schleicher Schuell 1573 No. 314709 diameter 190 mm, was evaporated to dryness under reduced pressure and low temperature (less than 30 °C). Since the microbiological determinations carried out on samples of coffee obtained with both lyophilization and evaporation under reduced pressure gave the same results, all of the tests were carried out using the latter method of concentration. The residue was dissolved in 1 mL of sterile water. The obtained solution was then analyzed for antibacterial activity.

(2) Italian espresso coffee and (3) mocha coffee were prepared at the same concentration as that of brewed coffee.

Italian expresso coffee was made using an expresso machine developed in Italy. Coffee was extracted quickly (15–35 s) by

Table 1. Description of Tested Coffee Samples

sample			roasting time,	
variety	origin	abbrev	min	wt loss, g %
arabica	Costa Rica	$1A_1$	5	12.8
		$1A_2$	7	17.1
		$1A_3$	8	22.3
	Colombia	$2A_1$	5	11.3
		$2A_2$	7	13.1
		$2A_3$	8	17.2
	El Salvador	$3A_1$	5	12.5
		$3A_2$	7	13.5
		$3A_3$	8	16.3
	Guatemala	$4A_1$	5	11.1
		$4A_2$	7	13.5
		$4A_3$	8	16.1
	Brazil	$5A_1$	5	11.2
		$5A_2$	7	12.5
		$5A_3$	8	18.7
robusta	Ecuador	$1R_1$	5	12.3
		$1R_2$	7	14.4
		$1R_3$	8	21.0
	Java	$2R_1$	5	10.0
		$2R_2$	7	13.9
		$2R_3$	8	22.8
	Indonesia	$3R_1$	5	10.6
		$3R_2$	7	15.1
		$3R_3$	8	21.5
	Ivory Coast	$4R_1$	5	10.6
		$4R_2$	7	11.1
		$4R_3$	8	26.9
	Zaire	$5R_1$	5	11.2
		$5R_2$	7	13.1
		$5R_3$	8	19.7

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Table 2. Influence of Coffee Brewing Procedure on MIC Value[a]

brewing procedure	antibacterial activity[b] MIC, mg/mL
brewed coffee	3
Italian espresso coffee	3
mocha coffee	3

[a] Coffee was obtained by dark roasting *C. robusta* from Zaire. [b] The antibacterial activity was assayed against *S. aureus* ATCC 25923.

superheated deionized water, and filtration was accelerated by steam at elevated pressure (4–5 bar). Mocha coffee was prepared using a mocha machine made of three perfectly fitting parts. The lower part is a container in which water is brought to the boiling point, the central part is a metallic filter that contains the powdered coffee, and the upper part is a receptacle that collects the beverage. The water is forced through the coffee by the slight pressure produced by the steam and remains in contact with the coffee for about 1 min.

Bacterial Cultures. The antibacterial activity of the coffee samples was assayed against both Gram-negative and Gram-positive bacteria. The tested bacteria were *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 23355, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, *Streptococcus faecalis* ATCC 6057, and *Bacillus subtilis* ATCC 6633.

Strains were maintained on tryptic soy agar slopes (TSA, Difco) at 4 °C and transferred weekly.

Inocula were cultivated overnight in tryptic soy broth (TSA, Difco) at 37 °C.

Antibacterial Activity. Minimum inhibitory concentration (MIC) was determined with the broth dilution method. Desired concentrations were achieved by the addition of appropriate volumes of the coffee samples to 1 mL of Iso-Sensitest broth (ISB, Oxoid) in 15 × 150 mm test tubes. Overnight cultures, diluted with sterile broth, were added to the test tubes to bring inoculum size to about 10^4–10^5 CFU/mL. The MIC value was evaluated, after 18 h of incubation at 37 °C, as the lowest concentration that completely inhibited the formation of visible growth. MIC was expressed as milligrams per milliliter of the roasted ground coffee used to prepare coffee (3–60 mg/mL).

Dialysis. Dialysis was performed in Spectra/Por 6 membrane tubes (Spectrum) with a molecular weight cutoff at 1000. Ten milliliters of coffee extract was fractionated by dialysis in 1000 mL of heat-sterilized water for 24 h (dialysis marker: chlorogenic acid).

The unfractioned coffee extract, the retentate, and the dialysate were evaporated to dryness under reduced pressure and low temperature; the residues were dissolved in 1 mL of sterile water. The obtained solutions were analyzed for antibacterial activity.

Statistical Analysis. All analyses were performed in duplicate. Data were analyzed by multifactor analysis of variance (MANOVA) with the statistical analysis package Statgraphics (1991). Means were separated with the least significant differences test at a confidence level of 95%.

RESULTS AND DISCUSSION

We first showed that there was total lack of antibacterial activity of all 10 green coffee batches, when the MIC test was carried out with *S. aureus* (Gram-positive) and *E. coli* (Gram-negative).

We then verified the relationship between roasted coffee antibacterial activity and brewing procedure. Antibacterial activity was assayed against *S. aureus*, as it had been in a previous study that investigated the MRP antibacterial activity from a model system (Daglia et al., 1992). The results, reported in Table 2, show that antibacterial activity does not depend on the brewing procedure. For this reason, all subsequent analyses were performed on brewed coffee alone, since this is the simplest and most reproducible procedure.

Table 3. MICs for Different Brewed Coffee Samples

bacterium	antibacterial activity MIC, mg/mL					
	$5A_1$	$5A_2$	$5A_3$	$5R_1$	$5R_2$	$5R_3$
Gram-positive						
S. aureus, ATCC 25923	17	11	6	12	4	3
B. subtilis, ATCC 6633	54	24	3	6	<3	<3
S. pyogenes, ATCC 19615	<3	<3	<3	<3	<3	<3
S. faecalis, ATCC 6057	60	60	12	60	18	12
Gram-negative						
E. coli, ATCC 25922	57	52	35	57	46	23
E. cloacae, ATCC 23355	18	18	6	18	12	6
P. vulgaris, ATCC 13315	12	12	<3	12	<3	<3
P. aeruginosa, ATCC 27853	24	24	6	18	12	6
S. typhimurium, ATCC 14028	>60	60	12	60	24	12

Table 4. MICs for the 30 Brewed Coffee Samples

sample	antibacterial activity MIC, mg/mL		sample	antibacterial activity MIC, mg/mL	
	S. aureus	*E. coli*		*S. aureus*	*E. coli*
$1A_1$	11	41	$1R_1$	8	49
$1A_2$	4	41	$1R_2$	4	34
$1A_3$	3	28	$1R_3$	3	29
$2A_1$	12	40	$2R_1$	6	46
$2A_2$	11	40	$2R_2$	4	29
$2A_3$	6	40	$2R_3$	3	23
$3A_1$	15	51	$3R_1$	6	57
$3A_2$	10	50	$3R_2$	4	35
$3A_3$	6	40	$3R_3$	3	23
$4A_1$	11	40	$4R_1$	11	57
$4A_2$	6	34	$4R_2$	6	42
$4A_3$	6	35	$4R_3$	4	23
$5A_1$	17	57	$5R_1$	11	57
$5A_2$	11	52	$5R_2$	4	46
$5A_3$	6	35	$5R_3$	3	23

We then evaluated the antibacterial activity of two coffee varieties, *C. arabica* ($5A_1$, $5A_2$, $5A_3$) and *C. robusta* ($5R_1$, $5R_2$, $5R_3$), against several bacteria. The results (see Table 3) show that Gram-positive bacteria were generally more sensitive to coffee than were Gram-negative bacteria, with the exception of *S. faecalis*. It should be noted that the *S. pyogenes* was the most sensitive to coffee, whereas *S. typhimurium* proved to be the most resistant.

The antibacterial activity of coffee proved to be consistent with that described in other investigations of antibacterial activity of MRPs obtained in model systems, notwithstanding the widely varying reagents and reaction conditions (Einarsson et al., 1983; Stecchini et al., 1991; Daglia et al., 1992).

Moreover, the results showed that dark-roasted coffee was more active than medium- and/or light-roasted coffee and that *C. robusta* seemed to have a greater effect than *C. arabica*. It was not clear how coffee variety influenced coffee antibacterial activity.

Accordingly, we assayed 30 sample batches (*C. robusta* × 5 sources + *C. arabica* × 5 sources, the sum × 3 degrees of roasting) against *S. aureus* and *E. coli* (see Table 4).

We then used multifactor analysis of variance to analyze the effect of coffee variety and degree of roasting on MIC values. The results, reported in Table 5, show that the antibacterial activity is affected by coffee variety only when the relevant data pertain to bacteria which are more sensitive to coffee, whereas it is invariably affected by degree of roasting.

Table 5. Multifactor Analysis of Variance

response variable	classification factor	sig level[a]	response variable	classification factor	sig level[a]
MIC (*S. aureus*)	A, variety	0.0010	MIC (*E. coli*)	A, variety	0.1388
	B, degree of roasting	0.0000		B, degree of roasting	0.0000
	AB, interaction	0.3827		AB, interaction	0.0060

[a] Significance level of less than 0.05 indicates that the response variable differs significantly across the level of the classification factor.

Table 6. Antibacterial Activity of Coffee Extracts Fractionated by Dialysis

		antibacterial activity on *S. aureus*[a] MIC, mg/mL		
source of coffee	roasting	unfractionated coffee extract	retentate	dialysate
C. arabica $1A_1$	light	11	5	>100
$1A_2$	medium	4	8	3
$1A_3$	dark	3	10	3
C. robusta $2R_1$	light	6	4	>100
$2R_2$	medium	4	5	2
$2R_3$	dark	3	6	2
commercial blend of *C. arabica* and *C. robusta*	medium-strong	3	4–5	2

[a] The results are the mean values of two determinations.

To achieve preliminary information about the substances with antibacterial activity present in the coffee, we fractionated by dialysis three extracts of *C. arabica* (1A) and three extracts of *C. robusta* (2R), using, respectively, extracts of light-, medium-, and dark-roasted beans and a commercial blend of *C. arabica* and *C. robusta*. We then evaluated the antibacterial activity of the unfractionated coffee extracts, the retentates, and the dialysates on *S. aureus*.

The results, reported in Table 6, show that the high molecular weight fractions of light-roasted coffees have antimicrobial activity unlike the dialysates. This result confirms the hypothesis that polymers obtained from Maillard reaction are responsible for the antibacterial activity of light-roasted coffee. On the contrary, stronger roasting conditions produce an increase of the antibacterial activity in the low molecular weight fractions. These fractions contain secondary products of the thermolysis of mixtures of protein and carbohydrate mixtures (heterocyclic amines, furans, phenolic substances, etc.) and degradation substances obtained from the polymers.

The results showing that the antibacterial activity of coffee is caused by the roasting process are (1) the lack of this activity in green coffee and (2) the relationship between coffee antibacterial activity and degree of roasting.

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