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Antiadhesive Effect of Green and Roasted Coffee on *Streptococcus mutans*' Adhesive Properties on Saliva-Coated Hydroxyapatite Beads

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Green and roasted coffees of the two most used species, *Coffea arabica* and *Coffea robusta*, several commercial coffee samples, and known coffee components were analyzed for their ability to interfere with *Streptococcus mutans'* sucrose-independent adsorption to saliva-coated hydroxyapatite (HA) beads. All coffee solutions showed high antiadhesive properties. The inhibition of *S. mutans'* adsorption to HA beads was observed both when coffee was present in the adsorption mixture and when it was used to pretreat the beads, suggesting that coffee active molecules may adsorb to a host surface, preventing the tooth receptor from interacting with any bacterial adhesions. Among the known tested coffee components, trigonelline and nicotinic and chlorogenic acids have been shown to be very active. Dialysis separation of roasted coffee components also showed that a coffee component fraction with 1000 Da < MW < 3500 Da, commonly considered as low MW coffee melanoidins, may sensibly contribute to the roasted coffee's antiadhesive properties. The obtained results showed that all coffee solutions have antiadhesive properties, which are due to both naturally occurring and roasting-induced molecules.

KEYWORDS: Antiadhesive properties; green and roasted coffees; naturally occurring coffee components; melanoidins

INTRODUCTION

Coffee brews are very widely consumed in the world due to their pleasant flavor and taste as well as for their stimulating effect on mental and physical activity. Caffeine is the most studied coffee component, and most of the literature is concerned with caffeine's pharmacological effects.

However, coffee beverages are very complex mixtures of several hundred chemicals that either occur naturally or, else, are later induced in coffee by the roasting process, that is, in the form of nicotinc acid or melanoidins. The occurrence of such a great number of chemicals in coffee has recently induced many researchers to investigate other coffee bioactivities. Coffee, in particular roasted coffee, has been found to act as a potent antioxidant and to inhibit lipid peroxidation both in chemical (*1*) and in biological systems in rat liver microsomal fractions (*2*). Roasted coffee was also shown to possess antibacterial activity against both Gram-positive and Gram-negative bacteria (*3*, *4*) including *Streptococcus mutans*, which is considered to be the major causative agent of dental caries in humans (*5*).

The emergence of pathogens resistant to conventional antibacterial agents and the need to develop new strategies for the control of infectious disease make coffee's antibacterial properties of particular interest. On the other hand, the role of dietary factors as anti-infective agents has been recently investigated, with particular emphasis on their role in inhibiting bacterial adhesion to host tissues, that being the first step of the infectious process afterward mediated by specific molecules of recognition (*6*−*10*).

The ability to adhere to the tooth surface by binding water-insoluble polysaccharides and saliva constituents of the acquired pellicle is considered to be an important virulence property of *S. mutans* (*11*), and proteins involved in this process represent primary targets of studies on dental caries prophylaxis (*12*−*15*). One of the strategies under development is the use of compounds that modify either the *S. mutans* cell wall or tooth surfaces, therefore preventing bacterial colonization and enamel demineralization (*16*−*18*). In particular, the inhibitory activity toward streptococcal coaggregation and/or adhesion to tooth surface has been recently reported for tea (*19*, *20*) and cranberry juice (*21*).

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These findings prompted us to analyze the effect of coffee on the adhesive properties of *S. mutans*. In this study green and roasted coffees of the two most used species, *Coffea arabica* and *Coffea robusta*, several commercial coffee samples, and known coffee components were analyzed for the ability to interfere with *S. mutans* sucrose-independent adsorption to saliva-coated hydroxyapatite (HA) beads.

MATERIALS AND METHODS

Reagents. Cyanocobalamin, caffeine anhydrous, trigonelline hydrochloride, nicotinic acid, and chlorogenic acid were obtained from Sigma (St. Louis, MO).

Coffee Solution Preparation. Two samples of *C. arabica* and two samples of *C. robusta* coffee beans, for a total of four different sources, were subdivided into four batches (green and light-, medium-, and dark-roasted, prepared in duplicate). The roasted batches were prepared in a pilot roaster apparatus (courtesy of Dr. Dino Ardino, La Casa del Caffè s.a.s, Florence, Italy). The roasting process is controlled according to weight loss, due to vapor formation and cell (silverskin particles) loss. The weight loss was ~9% for light-roasted samples (100−180 °C, 19−20 min), 12% for medium-roasted samples (100−190 °C, 19−20 min), and 17% for dark-roasted samples (100−210 °C, 19−20 min) (*22*). The coffee beans were then ground in a laboratory-scale mill and sieved through a no. 30 sieve. Coffee solutions were prepared using the brewed coffee procedure. Six grams of ground green and roasted coffee samples was boiled for 10 min in 100 mL of Millipore grade water. The solutions were then filtered on Millipore membranes of cellulose acetate/cellulose nitrate mixed esters (0.45 μm), and the pH value of each sample was immediately measured. The pH values ranged from 5.77 to 5.95. Commercial coffees were purchased in a local supermarket. Ground coffees were prepared as above, and instant coffees (2.5 g) were prepared using boiling Millipore grade water (100 mL). The obtained beverages were filtered as reported above.

Standard Solution Preparation. Caffeine anhydrous, trigonelline hydrochloride, nicotinic acid, and chlorogenic acid were dissolved alone or in mixture in distilled water at the concentrations (% w/v) reported in **Table 3**. The pH value of each sample was measured.

Dialysis. Dialysis was performed using a Spectra/Por Biotech cellulose ester membrane with molecular weight cutoffs of 3500 and 1000 Da. A 10 mL aliquot of coffee solution obtained from instant coffee (sample B, **Table 2**) was fractionated with a 3500 Da cutoff membrane in 1000 mL of distilled water for 24 h at 4 °C. The dialysate (MW < 3500 Da) was newly fractionated with a 1000 Da cutoff membrane in the same conditions. All of the obtained retentates and dialysates were brought to the initial volume of the coffee solution to be tested for the determination of the pH and the antiadhesive activity or to be newly submitted to dialysis. Chlorogenic acid (MW = 354.3) and cyanocobalamin (MW = 1355.4) were used as standard molecular weight markers.

Bacterial Strains, Media, and Buffers. *S. mutans* strains 9102 (*15*−*17*) and ATCC 25923 were used in this study. Bacteria were cultured in either Todd Hewitt broth (THB) or Mitis Salivarius agar (Difco Laboratories, Detroit, MI) at 37 °C in the presence of 5% CO_2. To radiolabel the bacteria, streptococci were grown in THB containing 10 μCi of [*methyl*-^{3}H]thymidine (25 Ci mmol^{-1}) mL^{-1}. Cells were harvested at midexponential phase by centrifugation (3000*g* for 10 min at 4 °C) and rinsed twice with an equal volume of 10 mM potassium phosphate buffer (PB), pH 7.0. The efficiency of cell labeling varied among strains from 300 to 1900 cells/cpm.

Evaluation of Coffee Sample Minimal Inhibitory Concentration (MIC). MIC was determined with the broth dilution method. The desired concentration was achieved through the addition of appropriate coffee sample volumes 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 ~104−105 colony-forming units (CFU) mL^{-1}. 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 milliter of the ground coffee used to prepare the coffee (6 mg mL^{-1}).

Saliva. Unstimulated whole saliva was collected from four healthy adult donors into ice-chilled cups; samples were then pooled, clarified by centrifugation (15000*g* for 15 min at 4 °C), sterilized by filtration, and stored in aliquots at −20 °C. Undiluted saliva was used in all experiments.

Adsorption to HA Beads. Fifty milligram aliquots of spheroid HA beads (Fluka Chemie, Buchs, Switzerland) (grain size = 80−200 μm) were washed three times in 1 mM PB, pH 7.0, in glass test tubes and equilibrated for 2 h in the same buffer. HA beads were allowed to settle for 30−60 s, and the supernatant solution was then removed by aspiration. HA beads were added to 500 μL of saliva, and this suspension was stirred for 1 h at 37 °C. The saliva was removed by aspiration, and the HA beads were washed with 10 mM PB, pH 7. Then a 50 mg aliquot of saliva-treated HA beads was added to 400 μL of radiolabeled bacteria diluted by adding 100 μL of saliva [(5−7) × 10^7 cells mL^{-1}] and 500 μL of coffee sample. In other experiments, saliva-coated HA beads were treated for 1 h with the coffee solution and washed again with 10 mM PB before addition of the radiolabeled bacteria as above. After incubation at 37 °C on a Mini Drum Roller (Wheaton) at 20 rpm, HA beads were washed and transferred to Pico−Fluor 15 scintillation fluid (Packard Instruments Co.,). Samples were assayed for radioactivity with an L5 1801 scintillation counter (Beckman); the efficiency of cell labeling was used to evaluate the number of bacteria adsorbed to HA. Each point of the reported result is the mean ± standard deviation from triplicate determinations of three different samples. Controls for bacteria settling due to aggregation were included; the amount of settled bacteria was always <1% of the inoculum.

Statistical Analysis. Values represent means of three replications. Data were analyzed by analysis of variance (ANOVA) and multifactor analysis of variance (MANOVA) with the statistical package Statgraphics Plus (1998). Means were separated with the LSD method at confidence levels of 99 and 95% (ANOVA) and 95% (MANOVA).

RESULTS AND DISCUSSION

The inhibitory activity of the solution of a number of coffee samples against *S. mutans'* ability to absorb to HA beads was determined.

The coffee solution activities were expressed as a percentage of inhibitory activity (IA%) of the microrganism adsorption ability in the presence or absence of the coffee solutions. The coffee solutions having antibacterial activities were used at concentrations 10 times lower than the MIC.

Two samples of *C. arabica* and two samples of *C. robusta* from different countries were analyzed.

Each sample was analyzed at four different degrees of roasting (green and light-, medium-, and dark-roasted). In the first set of experiments bacteria and coffee samples were added simultaneously to saliva-coated HA beads treated with buffer. As reported in **Table 1**, all of the tested samples inhibited *S. mutans* adsorption and showed IA% ranging from 40.5 to 98.1%. All green coffee samples were significantly ($p < 0.01$) less active than the corresponding roasted coffees.

Similar experiments were performed by treating saliva-coated HA beads with the different coffee samples before addition of bacteria. Results reported in **Table 1** show also in this case very high IA values for all of the tested coffee solutions (IA% ranging from 42.8 to 94.7%), but different behaviors depend on coffee species and the degree of roasting. *C. arabica* green coffees gave significantly ($p < 0.01$) lower values than all of the corresponding roasted coffees. The roasted coffee IA% decreases as the degree of roasting increases, but significant differences were found only between light- and dark-roasted coffees ($p < 0.01$). *C. robusta* coffee values are generally higher than those of *C. arabica*; in particular, green coffee values were much higher. *C. robusta* roasted coffee IA values showed the same behavior as those of *C. arabica*; in fact, the IA mean values

Table 1. Inhibitory Activity Percentage (IA%) of Coffee of Different Species and Degrees of Roasting on *S. mutans'* Adsorption to Saliva-Coated HA Beads

coffee sample			*S. mutans* 9102 adsorption to HA beads			
			simultaneous addition of coffee[a]		pretreatment with coffee[b]	
species	origin	degree of roasting	*n* adsorbed bacteria ($\times 10^6$) ± SD[c]	IA%[c]	*n* adsorbed bacteria ($\times 10^6$) ± SD[c]	IA%[c]
C. arabica	Brazil	none	15.6 ± 2.30	40.5	17.4 ± 2.11	42.8
C. arabica	Brazil	light	11.8 ± 1.40	66.1	2.8 ± 0.32	90.8
C. arabica	Brazil	medium	7.3 ± 0.50	72.8	4.4+0.51	85.5
C. arabica	Brazil	dark	5.9 ± 0.40	78.1	5.8 ± 0.63	80.9
C. arabica	Colombia	none	11.0 ± 1.30	59.1	15.2 ± 2.21	50.0
C. arabica	Colombia	light	1.9 ± 0.09	92.9	3.6 ± 0.43	88.2
C. arabica	Colombia	medium	9.4 ± 0.80	65.1	5.0 ± 0.44	83.6
C. arabica	Colombia	dark	0.5 ± 0.06	98.1	6.0 ± 0.53	80.2
C. robusta	Zaire	none	10.2 ± 0.90	62.1	3.4 ± 0.42	88.8
C. robusta	Zaire	light	2.2 ± 0.08	91.8	1.8 ± 0.22	94.1
C. robusta	Zaire	medium	4.0 ± 0.05	85.1	2.0 ± 0.34	93.4
C. robusta	Zaire	dark	2.2 ± 0.30	91.8	3.6 ± 0.43	88.2
C. robusta	Ivory Coast	none	13.5 ± 1.80	49.8	1.6 ± 0.91	94.7
C. robusta	Ivory Coast	light	7.8 ± 0.60	71.0	2.6 ± 0.32	91.4
C. robusta	Ivory Coast	medium	5.4 ± 0.60	79.9	3.8 ± 0.43	87.5
C. robusta	Ivory Coast	dark	6.5 ± 0.90	75.8	4.6 ± 0.32	84.9
control			26.7 ± 3.50		30.4 ± 4.20	

[a] Saliva-coated HA beads simultaneously treated with coffee solution. [b] Saliva-coated HA beads pretreated with coffee solution. [c] Results represent mean values of three different experiments.

decrease as the degree of roasting increases, but no significant differences among the IA values given by the *C. robusta* green and/or roasted samples were found.

The multifactor analysis of variance was used to evaluate the contribution due to factors such as coffee species, degree of roasting, and type of experiment (three classification levels) reflected in all of the IA values given by all of the samples in the two different sets of experiment. Both species and type of experiment were statistically significant factors (**Figure 1**), whereas degree of roasting had a statistically significant effect on IA% only when green and roasted coffees were considered (**Figure 1**).

Successively the investigations were extended to three commercial products submitted to different treatments (ground coffee, caffeine-free ground coffee, instant coffee, and caffeine-free instant coffee) (**Table 2**). Three samples of ground coffee, one of which consisted of 100% *C. arabica* (A) and the other ones of a mixture of coffees of the two species (B and C), were analyzed. The experiments were carried out by pretreating the saliva-coated HA beads with the coffee solutions. For these samples, also, very high IA values were registered (range = 83.4−95.7%). The 100% *C. arabica* ground sample showed an IA value statistically lower ($p < 0.05$) than those of the other ground samples, and this is consistent with the previously reported data. The obtained results showed that technological treatments have positive effects on the IA% of coffee and seem to indicate that caffeine is not involved in the antiadhesive properties of coffee solutions.

To achieve any information about the compounds responsible of the tested activity of coffee solutions, the IA% of standard solutions of known coffee components (chlorogenic acid, trigonelline, caffeine, and nicotinic acid) were evaluated. These components were tested alone or in mixtures at concentrations (**Table 3**) that mimic *C. arabica* green and dark-roasted coffee brews and *C. robusta* dark-roasted coffee (*4*), which have features close to those of the Mediterranean coffee brews. The

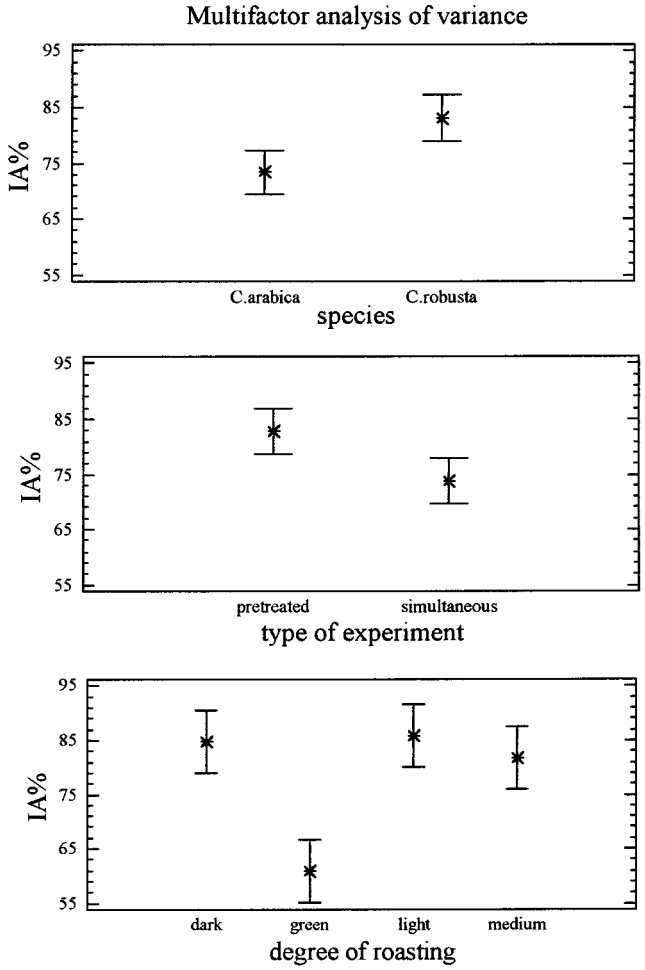

Figure 1. Multifactor analysis of variance: contribution due to coffee species, type of experiment, and degree of roasting (three classification levels) on IA% given by all of the tested coffee samples.

Table 2. Inhibitory Activity Percentage (IA%) of Different Treated Commercial Coffees on *S. mutans'* Adsorption to HA Beads

			S. mutans 9102 adsorption to HA beads with pretreatment with coffee[a]	
sample	coffee species	technological treatment	*n* adsorbed bacteria ($\times 10^6$) ± SD[b]	IA%
A	*C. arabica*	ground	4.6 ± 0.5	83.4
		caffeine-free ground	2.6 ± 0.1	90.6
B	*C. arabica, C. robusta*	ground	3.7+ 0.1	86.6
		caffeine-free ground	2.8 ± 0.3	90.6
		instant	1.2 ± 0.1	95.7
C	*C. arabica, C. robusta*	ground	2.9 ± 0.4	89.5
		caffeine-free instant	2.4 ± 0.1	91.3
		instant	1.9 ± 0.2	93.1
	control		27.7 ± 2.2	

[a] Saliva-coated HA beads pretreated with coffee solution. [b] Results represent mean values of three different experiments.

results obtained showed that all of the considered compounds are active, with caffeine giving the lowest values, as had been expected considering the IA% values given by the commercial caffeine-free samples. These data confirm no detectable caffeine involvement in the IA% of coffee. These data also indicate that trigonelline is a very effective inhibitor of *S. mutans* adsorption, with IA% ranging from 70.7 to 75.6%, and suggest that this compound may have the major responsibility for coffee antiadhesive activity. In fact, because high trigonelline IA values were found at even the lower concentration tested, its activity

Table 3. Inhibitory Activity Percentage (IA%) of Coffee Components Present in either Green or Roasted Coffee on *S. mutans'* Adsorption to HA Beads

			S. mutans 9102 adsorption to HA beads with pretreatment with coffee[a]	
coffee compound and mixture	concn % (w/v)	pH	*n* adsorbed bacteria ($\times 10^6$) ± SD[b]	IA%
chlorogenic acid	2.400	3.05	15.4 ± 1.8	46.3
	0.720	3.99	18.9 ± 2.0	34.1
trigonelline	0.900	3.39	8.4 ± 0.9	70.7
	0.420	3.52	7.0 ± 0.6	75.6
caffeine	0.900	6.42	25.2 ± 1.9	12.2
nicotinic acid	0.015	4.26	18.2 ± 1.7	36.6
mixture A[c]			11.2 ± 1.3	60.1
chlorogenic acid	2.400	3.25		
trigonelline	0.900			
caffeine	0.900			
mixture B[d]			9.1 ± 0.8	68.3
chlorogenic acid	0.720	4.02		
trigonelline	0.420			
caffeine	0.900			
nicotinic acid	0.015			
mixture C[e]			7.0 ± 0.8	75.6
chlorogenic acid	2.400	3.41		
trigonelline	0.900			
caffeine	0.900			
nicotinic acid	0.015			
control			28.7 ± 3.1	

[a] Saliva-coated HA beads pretreated with coffee solution. [b] Results represent mean values of three different experiments. [c] Mixture A mimics a green *C. arabica* sample. [d] Mixture B mimics a dark roasted *C. arabica* sample. [e] Mixture C mimics a dark roasted *C. robusta* sample.

may also be important in roasted coffee, in which trigonelline is in part destroyed to give nicotinic acid. It must be pointed out that trigonelline when alone showed an activity similar to or even higher than that of the whole tested mixtures, suggesting negative interactions among the tested compounds. Nicotinic acid, which showed an IA% of 36.6, seems to be a very highly active component considering the very low concentration at which it was tested in comparison to the other compounds. The highest activities, shown by the mixtures corresponding to dark-roasted coffees, indicated that nicotinic acid may give a significant contribution to the roasted coffee activity. The lower values given by the mixtures in comparison to coffee solutions seem to indicate that other compounds in coffee possibly contribute to its IA%.

To verify the possibility that coffee melanoidins, which are generated during the roasting process, contribute to coffee IA%, the coffee solution components were fractionated using a dialysis membrane with cutoffs of 3500 and 1000 Da (**Figure 2**). The obtained results (**Table 4**) showed that strong activity is due to the MW < 1000 Da components (IA% = 55.3%), which include the standard compounds tested. Nevertheless, considerable activity is presented also by the components with 1000 < MW < 3500 Da (IA% = 41.3%), which are commonly considered as lower MW coffee melanoidins. Higher MW coffee melanoidins (MW > 3500 Da) were shown to be scarcely active as expected by the lower IA% of the dark-roasted coffee samples.

In conclusion, the results presented in this paper show antiadhesive properties of coffee solutions that possess high IA% against the ability of *S. mutans* to adsorb to saliva-coated HA beads, at least in the applied experimental conditions. Most of such activity may be due to small molecules occurring naturally in coffee (chlorogenic acid and trigonelline) or induced by roasting (nicotinic acid). The results of the assays carried out

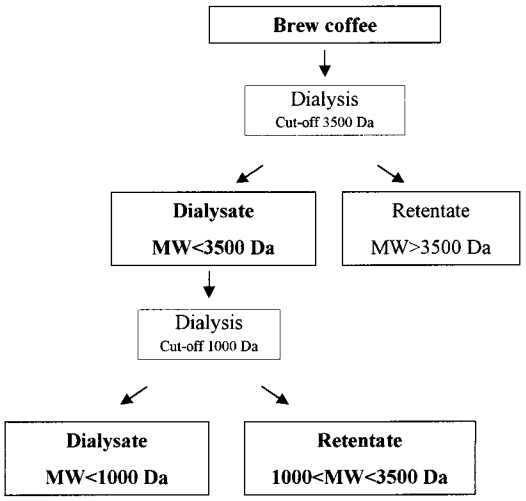

Figure 2. Scheme of isolation of the antiadhesive coffee components.

Table 4. Inhibitory Activity Percentage (IA%) of Coffee Fractions Obtained by Dialysis on *S. mutans'* Adsorption to HA Beads

			S. mutans 9102 adsorption to HA beads with pretreatment with coffee[b]	
sample	pH	mg/mL[a]	*n* adsorbed bacteria ($\times 10^6$) ± SD[c]	IA%[d]
brewed coffee	5.16	11.70	2.1 ± 0.9	93.0
retentate (MW > 3500 Da)	5.02	2.80	22.3 ± 2.0	25.7
dialysate (MW < 3500 Da)	6.01	8.35	9.8 ± 1.0	67.3
retentate (3500 < MW < 1000 Da)	5.69	4.98	17.6 ± 1.6	41.3
dialysate (MW < 1000 Da)	7.26	3.16	13.4 ± 1.1	55.3
control			30.0 ± 3.2	

[a] Milligrams of dry residue per milliliter of coffee solution. [b] Saliva-coated HA beads pretreated with coffee solution. [c] Results represent mean values of three different experiments. [d] Percentage of inhibitory activity.

on standards, alone or in mixtures as occurring in green and dark-roasted coffee, and the data obtained from the dialysis given by roasted coffee fractions with lower and higher MW than 1000 Da also seem to indicate that the lower MW coffee melanoidins give a significant contribution to the activity of the coffee solutions.

Although the mechanism by which coffee compounds inhibit *S. mutans'* adsorption to HA beads was not investigated, the results obtained with coffee-pretreated HA beads suggest that coffee active molecules may adsorb to host surfaces, preventing the tooth receptor from interacting with bacterial adhesins. In this study coffee effects on *S. mutans'* sucrose-independent adsorption was analyzed; preliminary results show that the same compounds are also active on sucrose-dependent interactions, which suggests that coffee may act in preventing both reversible and irreversible *S. mutans* adherence to solid surfaces.

In the absence of animal model data, caution is advised in the interpretation of the in vivo significance of our present findings. Nevertheless, we can hypothesise that due to both antibacterial (*4*, *5*) and antiadhesive activity, coffee could minimize *S. mutans'* colonization of tooth surface and might be effective in preventing *S. mutans*-induced tooth decay.

LITERATURE CITED

(1) Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Lerici, C. R. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett.* **1997**, *114*, 71−74.

(2) Daglia, M.; Papetti, A.; Gregotti, C.; Bertè, F.; Gazzani, G. In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *J. Agric. Food Chem*. **2000**, *48*, 1449−1454.
(3) Daglia, M.; Cuzzoni, M. T.; Dacarro, C. Antibacterial activity of coffee: relationship between biological activity and chemical markers. *J. Agric. Food Chem*. **1994**, *42*, 2273−2277.
(4) Daglia, M.; Cuzzoni, M. T.; Dacarro, C. Antibacterial activity of coffee. *J. Agric. Food Chem.* **1994**, *42*, 2270−2272.
(5) Dacarro, C.; Daglia, M.; Cuzzoni, M. T.; Bonferoni B. Antibacterial activity of coffee against *Streptococcus mutans. Igiene Moderna* **1995**, *104*, 379−387.
(6) Andersson, B. O.; Ponnas, O.; Hanson, L. A.; Lagergard, T.; Svanborg-Eden, C. Inhibition of attachment of *Streptococcus pneumoniae* and *Hemophylus influenzae* by human milk and receptor oligosaccharides. *J. Infect. Dis.* **1986**, *153*, 232−237.
(7) Beachey, E. H. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J. Infect. Dis*. **1981**, *143*, 325−344.
(8) Coppa, G. V.; Gabrielli, O.; Giorni, P.; Montanari, M. P.; Varaldo, P. Preliminary study of breastfeeding and bacterial adhesion to uroepithelial cells. *Lancet* **1990**, *335*, 569−571.
(9) Guzmàn, C. A.; Piatti, G.; Pronzato, C.; Crippa, A.; Pruzzo, C. Inhibition of bacterial colonization of the gut by human and bovine casein. *Microecol. Ther.* **1995**, *25*, 236−239.
(10) Ofek, I.; Kahane, I.; Sharon, N. Toward anti-adhesion therapy for microbial diseases. *Trends Microbiol.* **1996**, *4* (8), 297−299.
(11) Loesche, W. J. Role of *Streptococcus mutans* in human dental decay. *Micobiol. Rev.* **1986**, *50*, 353−380.
(12) Hajishengallis, G.; Michalek, S. M. Current status of a mucosal vaccine against dental caries. *Oral Microbiol. Immunol*. **1999**, *14*, 1−20.
(13) Mandel, I. D. Caries prevention: current strategies, new directions. *J. Am. Dent. Assoc*. **1996**, *127*, 1477−1488.
(14) Russel, M. W.; Hajishengallis, G.; Childers, N. K.; Michalek, S. M. Secretory immunity in defense against cariogenic mutans streptococci. *Caries Res.* **1999**, *33* (1), 4−15.
(15) De Leo, C.; Coppola, R. C.; Blasi, G.; Eftimiadi, C.; Salvarani, M.; Molina, A. M. Prevalence of *Streptococcus mutans* and dental decay in schoolchildren living in Genoa (Italy). *Eur. J. Epidemiol*. **1990**, *6*, 166−174.
(16) Olsson, J.; Carlén, A.; Holmberg, K. Modulation of bacterial binding to salivary pellicle by treatment with hydrophilizing compunds. *Arch. Oral Biol.* **1990**, *35*, 137S−140S.
(17) Tarsi, R.; Muzzarelli, R. A. A.; Guzmàn, C. A.; Pruzzo, C. Inhibition of adsorption to hydroxyapatite by low molecular weight chitosans. *J. Dent. Res*. **1997**, *76* (2), 665−672.
(18) Tarsi, R.; Corbin, B.; Pruzzo, C.; Muzzarelli, R. A. A. Effect of low molecular weight chitosans on the adhesive properties of oral streptococci. *Oral Microbiol. Immunol*. **1998**, *13*, 217−224.
(19) Hamilton-Miller, J. M. Anti-cariogenic properties of tea (*Camellia sinensis*). *J. Med. Microbiol.* **2001**, *50*, 299−302.
(20) Matsumoto, M.; Minami, T.; Sasaki, H.; Sobue, S.; Hamada, S.; Ooshima, T. Inhibitory effects of olong tea extract on caries-inducing properties of mutans streptococci. *Caries Res*. **1999**, *33*, 441−445.
(21) Weiss, E. I.; Lev-Dor, R.; Kashamn, Y.; Goldhar, J.; Sharon, N.; Ofek, I. Inhibiting interspecies coaggregation of plaque bacteria with a cranberry juice constituent. *J. Am. Dent. Ass*. **1999**, *129*, 1719−1723.
(22) Belitz, H. D.; Grosh, W. Coffee, tea, cocoa. In *Food Chemistry*; Springer Verlag: Berlin, Germany, 1999; pp 874−883.

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