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Effect of Barley Coffee on the Adhesive Properties of Oral Streptococci

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Some beverages and foods protect tooth surfaces against *Streptococcus mutans* colonization. Adhesion of *S. mutans* is a crucial step in the initiation and development of dental caries. In this study, we showed that barley coffee (BC), a beverage made from roasted barley, interferes with *S. mutans* adsorption to hydroxyapatite (HA), and we identified its antiadhesive components. The effects of sublethal concentrations (sub-MICs) of BC on the adhesion of *S. mutans* to saliva-coated HA beads were assessed using three experimental approaches: (A) Beads were pretreated with BC before adding bacteria, (B) BC and bacteria were added to the beads simultaneously, and (C) streptococci grown in the presence of sub-MICs of BC were added to the beads. All treatments induced variable but significant inhibition of *S. mutans* sucrose-dependent and -independent adherence to HA. Similar results were obtained with other oral streptococci. BC components were fractioned by dialysis and gel filtration chromatography; the <1000 Da molecular mass (MM) fraction, which contains polyphenols, zinc, and fluoride ions, and the >1000 kDa MM fraction, which consists of a potent brown antioxidant, melanoidin, both displayed antiadhesive properties. High-MM melanoidin was not detected in unroasted barley, indicating that it forms during the roasting process. Results suggest that BC consumption may influence the colonization of tooth surfaces by cariogenic bacteria.

KEYWORDS: Barley coffee; oral streptococci; adhesion to hydroxyapatite; melanoidin; anti-adhesive properties

INTRODUCTION

It is well-established that the diet has a vital role in promoting health and preventing disease. Epidemiological and clinical studies have shown that a diet rich in fruit, vegetables, whole grains, legumes, and fish and low in saturated fats and sodium can reduce the risk of chronic diseases such as cancer and cardiovascular, coronary, and neurodegenerative pathologies and that some food components may act as immunomodulators or anti-infection agents (*1*, *2*). In rich countries, such findings have driven attempts to improve the quality of life also through diet composition by including functional foods, i.e., foods that provide health benefits beyond the nutrients that they contain (*3*−*5*). Such benefits depend on the activity of foodstuffs on the organism's physiological functions.

The oral cavity is eminently suitable for the action of functional foods. Caries is an infectious disease whose principal etiological agents in humans are held to be *Streptococcus mutans* and, to a lesser extent, *Streptococcus sobrinus*; other oral cavity bacteria (e.g., *Lactobacillus*) are involved to a greater extent in progression of the deep enamel lesion, while *Actinomyces* spp. are associated with the development of root surface caries (*6*, *7*). The virulence properties of *S. mutans* include rapid metabolism of sucrose to lactic and other organic acids, production of extracellular polysaccharides from sucrose, and adhesiveness (*7*−*9*). Attachment to tooth surfaces is a crucial step in cariogenesis that involves a variety of bacterial and host components (*9*). Bacterial surface structures (e.g., lipoteicoic acid, antigen I/II) and bridging molecules (e.g., Ca^{2+}) are responsible for the initial, sucrose-independent streptococcal interactions with enamel and the acquired pellicle. The subsequent phase of sucrose-dependent adherence is largely due to

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cell-free or cell-bound glucosyltransferase-mediated insoluble glucan synthesis. Glucan ensures plaque stability by entrapping bacteria in the matrix due to the presence of specific ligands on their surface (e.g., glucan-binding proteins).

A promising approach to the prevention of adhesion is based on inhibiting both bacterial adsorption to tooth surfaces and bacterial colonization (*10*−*12*). Several methods can achieve this goal (*10*, *12*−*15*), among which use of (i) analogues of receptors and adhesins acting as diffusible competitive inhibitors of adherence; (ii) drugs that suppress the synthesis and expression of bacterial adhesins or modify the tooth surface, thereby reducing bacterial adsorption; and (iii) active and passive immunization providing antibodies that interact with bacterial adhesins and prevent attachment.

Bacterial adhesion to both tooth surfaces and epithelial cells thus appears to be a good target for the anti-infectious properties of some functional foods. Besides antibodies that react against several pathogens, human and bovine milk also contain anti-adhesive compounds, like glycomacropeptides, which prevent caries and gingivitis, and k-casein-derived oligosaccharides, which inhibit attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* to respiratory tract epithelial cells (*16*). The high molecular weight material derived from cranberry juice effectively inhibits coaggregation of *Fusobacterium nucleatum*, a common cause of gingivitis, and other relevant oral bacteria (*17*). Hop bract polyphenols inhibit *S. mutans* cell adherence and water-insoluble glucan synthesis (*18*). Various components in green and black tea have in vitro properties that suggest an anticariogenic activity (*11*, *19*), including a direct bactericidal effect against *S. mutans* and *S. sobrinus*, prevention of bacterial adhesion to teeth, and inhibition of glucosyltransferase and human and bacterial amylases. Studies in animal models have shown that such in vitro effects can translate into the prevention of caries (*19*). A limited number of human clinical trials have also suggested that regular tea consumption reduces the incidence and severity of caries (*19*).

We have recently studied the effects of coffee on the adhesive properties of *S. mutans* (*20*). Coffee beverages are complex mixtures of several hundred chemicals, which occur naturally or are induced by the roasting process. They present interesting biological properties, including antibacterial activity, against both Gram-positive and Gram-negative bacteria (*21*, *22*). In particular, roasted coffee acts against *S. mutans* and interferes with streptococcal sucrose-independent adsorption to hydroxyapatite (HA) beads.

These data prompted us to study the ability of barley coffee (BC) to affect oral streptococci interactions with tooth surfaces. BC is obtained from roasted barley, which, interestingly, is also used as a coffee substitute, especially for children. We investigated the antibacterial activity of BC and its effects on the ability of streptococci to adhere to HA beads. The observation that it did reduce adhesion to HA beads led us to study the components that are responsible for this effect.

MATERIALS AND METHODS

Chemicals. Potassium phosphate buffer (PB), 5-*O*-caffeoyl-quinic acid (5-*O*-CQA), Folin−Ciocalteau reagent, sodium carbonate, blue dextran, bovine serum albumin (BSA), sucrose, nitric acid, and *n*-octane were purchased from Sigma-Aldrich (Milan, Italy); [methyl-^{3}H]-thymidine was purchased from Amersham Biosciences Europe GmbH (Cologno Monzese, Italy); and spheroidal HA beads were purchased from Fin-ceramica (Faenza, Italy).

Barley Roasting. Barley grains (var. Clarine, kindly provided by Crastan, Pontedera, Italy) were roasted in a pilot roaster apparatus. They were heated to 70−80 °C in 3 min, to 200 °C in the next 30 min, and finally to 220 °C over the subsequent 13 min; they were then cooled to 180−190 °C with water spray and finally to room temperature with air.

Preparation of the Barley Solutions. Barley grains (unroasted and roasted) were ground in a laboratory scale mill and passed through a no. 30 sieve. Two solutions were prepared according to the common Italian procedure for brewing barley, by boiling 6 g of unroasted or roasted barley powder (RBP) in 100 mL of Millipore grade water (Millipore Corp., Billerica, MA) for 10 min. Solutions were filtered through a 0.45 μm Millipore membrane of cellulose acetate/cellulose nitrate mixed esters and then freeze-dried. Dry matter was dissolved in an appropriate volume of Millipore grade water [for minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and postcontact effect (PCE) determinations] or of 1 mM PB (for adhesion experiments).

Bacterial Strains, Media, and Buffers. Used were the following streptococcal strains: *S. mutans* 9102 (*14*), *S. mutans* ATCC 25175, *S. vestibularis* ATCC 49124, *S. anginosus* ATCC 33397, *S. intermedius* ATCC 27335, *S. constellatus* ATCC 27823, *S. oralis* ATCC 10557, *S. salivarius* ATCC 13419, and *S. sanguis* ATCC 10556. Bacteria were cultured in Todd Hewitt broth (THB, Oxoid, Basingstoke, United Kingdom) at 37 °C in the presence of 5% CO_2; when sucrose-dependent adhesion was analyzed, 50 μL mL^{-1} of 2 M sucrose was added to the culture medium.

For the radiolabeling procedure, streptococci were grown in THB containing 10 μCi [methyl-^{3}H]thymidine (25 Ci mmol^{-1}) mL^{-1}. Midexponential phase cells were harvested by centrifugation (5000*g* for 15 min at 4 °C) and washed twice with an equal volume of 10 mM PB, pH 7.0. The efficiency of cell labeling varied among strains from 200 to 1400 cells/cpm.

Evaluation of MIC and MBC. Both BC and concentrated BC solutions were tested. To obtain the latter, 100 mL of BC was freeze-dried and the residue was dissolved in 10 mL of Millipore water grade. The resulting solution was filtered through a 0.22 μm Millipore membrane of cellulose acetate/cellulose nitrate mixed esters. MICs and MBCs were determined in Iso-Sensitest broth (ISB, Oxoid, Basingstoke, United Kingdom) according to Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) procedures (*23*). The MIC was the lowest BC concentration inhibiting observable growth; the MBC was the lowest BC concentration resulting in >99.9% reduction of the initial inoculum (*24*). All experiments were performed in triplicate.

Evaluation of PCE. Aliquots of bacterial cultures grown in ISB to the exponential phase (approximately 10^8 cfu mL^{-1}) were exposed to 1× and 2× MIC of BC for 1 h at 37 °C; control cultures were left untreated. Exposed and control cultures were washed twice in PB by centrifugation at 3500 rpm for 20 min and subsequently diluted in fresh broth before incubation at 37 °C. Viable counts were measured in tryptone soya agar (TSA, Oxoid, Basingstoke, United Kingdom) immediately and after 1, 3, 4, 5, and 24 h of incubation. PCE was determined using the following equation:

$$\mathrm{PCE} = t - c \quad (1)$$

where t is the time needed for a log increase in counts of treated cultures and c is the time needed for a log increase in counts of untreated cultures (*25*).

Effect of BC on Bacterial Cell Hydrophobicity. The bacterial surface hydrophobicity was evaluated according to Rosenberg (*26*). Streptococci were grown overnight in THB. After incubation, cells were harvested by centrifugation (3500 rpm, 20 min), suspended in PB, and exposed to 0.5×, 1×, and 2× MIC of BC for 1 h at 37 °C; control suspensions were left untreated. Exposed and control suspensions were then washed twice by centrifugation at 3500 rpm for 20 min, and bacteria were resuspended in PB. Aliquots (1.5 mL) of the bacterial suspensions were added to 1 mL of *n*-octane. After allowing mixing for 120 s and settling for 10 min for the separation step, absorbance of the aqueous phase was measured at 400 nm. Hydrophobicity changes in bacterial cells were expressed as percentage of absorbance variation of the PB phase of BC-treated vs control suspensions.

Bacterial Adsorption to HA Beads. Fifty milligram aliquots of spheroidal HA beads (grain size 250−875 μm) were washed three times

in 1 mM PB, pH 7.0 (*27*), in glass tubes and then equilibrated for 2 h in the same buffer. Beads were allowed to settle for 30−60 s, and the supernatant was removed by aspiration. Beads were then treated with 200 μL of saliva that was collected, clarified, and used undiluted as described previously (*14*). The effect of BC on bacterial adsorption to HA was tested as described (*14*, *15*), using three different experimental approaches (in all experiments, 1 mL final volumes were used). With treatment A, beads were pretreated with the beverage and then incubated on a Wheaton Mini Drum Roller (Wolf Laboratories Ltd., Pocklington, United Kingdom) at 20 rpm at room temperature. After 1 h, the beads were washed, the radiolabeled bacterial suspension (at a final concentration of $5-7 \times 10^7$ cells mL^{-1}) was added to the beads, and the mixture was again incubated at room temperature on the Wheaton Mini Drum Roller at 20 rpm. With approach B, BC and the radiolabeled bacterial suspensions (final concentration: $5-7 \times 10^7$ cells mL^{-1}) were added simultaneously to the beads in polypropylene microfuge tubes and incubated as described above. In treatment C, we used labeled streptococci grown in THB and supplemented with sub-MICs of BC. Control samples without BC were included in all treatments. After 1 h of incubation, beads were collected by centrifugation (200*g*, 5 min, 4 °C), washed four times with 10 mM PB to remove nonadherent bacteria, and transferred to Pico-Fluor 15 scintillation fluid (Packard Instruments Co., Meriden, CT). Radioactivity was assayed with an L5 1801 scintillation counter (Beckman Instruments, Fullerton, CA). Cell labeling was used to measure the number of bacteria adsorbed to HA. The inhibitory activity of BC (IA %) was gauged by comparing BC-treated samples with the respective untreated controls (100%). Controls for bacterial settling due to aggregation were also included; the amount of settled bacteria was always <1% of the inoculum. Further controls in which the beads were coated with BSA in order to block any bare regions of the mineral were included (*28*). To do this, beads were suspended in 1 mM PB containing BSA at the final concentration of 5 mg mL^{-1}. After 30 min of incubation, the beads were centrifuged as described above and the supernatant was removed by aspiration.

Bacterial Detachment from HA Beads. The ability of BC to detach bacteria from HA beads was determined by the procedure described by Tarsi et al. (*15*). All of the experiments were run in triplicate. The radiolabeled bacterial suspension (1 mL) was added to saliva-coated HA beads; after 1 h of incubation, the beads were collected by centrifugation (200*g*, 5 min, 4 °C) and washed four times with 10 mM PB to remove nonadherent bacteria. A separate sample was used to assess total HA-bound radioactivity. BC was then added to bacteria-treated beads; the mixtures were centrifuged as described above, at time zero and after 1 and 2 h of incubation at room temperature on the Wheaton Mini Drum Roller at 20 rpm. Labeled bacteria in the supernatants were counted. Control samples without BC were also included. The ratio of detached to total bead-bound bacteria was then determined.

Dialysis. Dialysis was performed using Spectra/Por Biotech cellulose ester membranes (Spectrum Europe B.V., Breda, NL) with molecular mass cut-offs of 1000 and 300000 Da. A 10 mL aliquot of each barley solution was fractioned by dialysis in 1000 mL Millipore grade water for 6 h at 4 °C. Dialysates and retentates were freeze-dried, and the residue was dissolved in 1 mL of Millipore grade water (MIC assays) or 10−20 mL of Millipore grade water (adsorption to and the detachment from HA beads). Recovered 5-*O*-CQA (>95%) was used as a standard molecular mass marker. Dialysates and retentates were only tested on *S. mutans*, *S. intermedius*, and *S. constellatus*.

Fluoride Ion Determination. An EDT DR359 direct concentration read-out specific ion meter (EDTdirectION, Dover, United Kingdom) used in conjunction with a QSE333 combination ion-selective electrode was employed for all fluoride determinations. To check electrode performance, we first ascertained that the mV/decade slope lay within the theoretical value of 54−60 mV/decade.

Zinc Ion Determination. Zinc contained in the BC solutions was analyzed in a Perkin-Elmer Analyst 600 AA spectrophotometer (Perkin-Elmer, Wellesley, MA). Instrument calibration and data calculation were performed on a PC using the AA WinLab software (Technology Center of New Jersey, North Brunswick, NJ). To eliminate organic matter, samples were mineralized in a CEM-MDS 81D microwave oven (Scientific Support, Inc., Hayward, CA) and then dissolved in 30% nitric acid. The calibration curve was linear for the 0.25−0.5−1 ppm absolute concentration ranges.

Total Phenol Content. The total phenol content was measured according to the Folin−Ciocalteau method described by Yew Tang et al. (*29*), with some modifications. The method involves reduction of Folin−Ciocalteau reagent by phenol electrons. In brief, 100 μL of the <1000 Da molecular mass (MM) fraction was mixed with 5 mL of Millipore grade water, 500 μL of 2 N Folin−Ciocalteau reagent, and 2 mL of Na_2CO_3 (15%, w/v). The reaction mixture was then brought to a final volume of 10 mL with Millipore grade water. A Beckman DU 640 spectrophotometer (LabX, Midland, ON, Canada) was used to measure absorbance at 750 nm for 2 h after incubation at room temperature. 5-*O*-CQA was used as the standard. The total polyphenol content, determined in the <1000 Da MM dialysis fraction, was expressed as milligram equivalents of 5-*O*-CQA per milliliter of BC. The equation of the standard curve was $y = 0.001x - 0.0019$, $R^2 = 0.9979$, where y = absorbance at 750 nm and x = polyphenol concentration.

Gel Filtration Chromatography (GFC). We used a 655A-11 Merck-Hitachi liquid chromatograph with a UV wavelength monitor (SMI-LabHut Ltd., Maisemore, United Kingdom); chromatograms were recorded at 210 nm. GFC separation of the >300000 Da MM roasted barley fraction was performed using a Superformance Universal glass-cartridge system (300 mm × 10 mm) (Merck). The stationary phase was TSK gel Toyopearl HW-75F (exclusion limits 1000−10000 kDa) (Tosoh Biosep GmbH, Stuttgart, Germany). The mobile phase was Millipore grade water (flow rate, 0.4 mL min^{-1}). Blue dextran (MM = 2000 kDa) was used as the standard. The eluates corresponding to the three chromatographic peaks obtained were concentrated under vacuum and freeze-dried, and the residues were restored to the dialyzed BC volume with Millipore grade water. GFC fractions were only tested on *S. mutans*, *S. intermedius*, and *S. constellatus*.

Statistical Analysis. Student's t and χ^2 tests were applied to assess the difference in adsorption efficiency of BC-treated and untreated samples, which were analyzed on the same day.

RESULTS AND DISCUSSION

Antibacterial Activity of BC toward Oral Streptococci. To evaluate the effects of the barley beverage on oral streptococci interactions with saliva-coated HA beads, we first determined its antimicrobial properties against different streptococcal strains. Because the original BC solution (60 mg RBP mL^{-1}) showed no antibacterial activity, we tested a concentrated solution (600 mg RBP mL^{-1}) and obtained MIC, MBC, and PCE values reflecting weak activity. MIC values corresponding to 240 mg RBP mL^{-1} and MBCs corresponding to 480 mg RBP mL^{-1} were found for both S. *constellatus* ATCC 27823 and *S. intermedius* ATCC 27335. Higher MICs and MBCs were obtained for the other bacteria studied, whereas PCE tests yielded no significant difference in recovery of normal growth between bacteria exposed to 1× and 2× MIC of BC and untreated controls, irrespective of strain. Accordingly, two BC solutions corresponding to sublethal concentrations of 60 and 30 mg RBP mL^{-1} were used in the adhesion experiments described below.

Effect of BC on Adsorption of Bacteria to and Detachment from HA Beads. Three experimental approaches were used to evaluate the effects of sub-MICs of BC on streptococcal adhesion to saliva-coated HA beads: Beads were pretreated with BC before adding the bacteria (A); BC and bacteria were added to HA beads simultaneously (B); and streptococci grown in the presence of sub-MICs of BC were added to beads (C). The results obtained with two *S. mutans* strains are reported in **Table 1**, while those obtained with the other oral streptococci are shown in **Table 2**. In all cases, the effect of BC was tested on both sucrose-independent and sucrose-dependent adsorption. All BC treatments inhibited bacterial adsorption to saliva-coated

Table 1. IA (%) of Sublethal RBP Concentrations toward *S. mutans* Adsorption to Saliva-Coated HA Beads as Compared with RBP-Untreated Controls[a]

		adsorption to HA beads × 10^6 bacteria/50 mg HA ± SD				
S. mutans strain	BC treatment	30 mg RBP mL^{-1}	IA %	60 mg RBP mL^{-1}	IA %	control
		sucrose-independent				
9102	A[b]	12.4 ± 1.1	29	9.8 ± 1.1	44	17.5 ± 1.2
	B[c]	6.7 ± 0.9	64	4.4 ± 0.5	76	18.4 ± 2.0
	C[d]	8.6 ± 0.9	45	7.2 ± 0.8	54	15.6 ± 1.3
ATCC 25175	A[b]	7.8 ± 0.6	10	5.6 ± 0.7	36	8.7 ± 0.6
	B[c]	7.7 ± 0.5	15	5.2 ± 0.5	43	9.1 ± 1.1
	C[d]	8.2 ± 0.9	12	5.8 ± 0.6	38	9.3 ± 0.8
		sucrose-dependent				
9102	A[b]	17.0 ± 0.2	49	14.4 ± 1.1	57	33.4 ± 3.9
	B[c]	8.0 ± 0.7	75	3.8 ± 0.3	88	32.0 ± 1.0
	C[d]	16.0 ± 2.2	46	9.8 ± 1.9	67	29.7 ± 3.1
ATCC 25175	A[b]	6.7 ± 0.5	50	5.9 ± 0.6	56	13.4 ± 1.6
	B[c]	4.9 ± 0.6	60	2.8 ± 0.1	77	12.2 ± 2.0
	C[d]	7.9 ± 0.8	44	4.9 ± 0.5	65	14.1 ± 1.8

[a] Results are the means of three different experiments ± SD. [b] BC-pretreated saliva-coated HA beads. [c] Saliva-coated HA beads simultaneously treated with BC and bacteria. [d] Bacteria grown in the presence of sub-MICs of BC. Differences between BC-treated and the respective control samples are significant ($p \leq 0.05$). Differences between the two RBP concentrations are significant ($p \leq 0.1$).

beads irrespective of the presence of sucrose, the degree of inhibition depending on beverage concentration, and bacterial strain. In *S. mutans* strains, significantly greater inhibition was observed in the presence than in the absence of sucrose ($p \leq 0.05$), whereas the opposite was generally true of the other oral streptococci ($p \leq 0.1$).

In general, inhibition was greater in experiments where BC and bacteria were added to HA simultaneously (approach B) than with the other two approaches ($p \leq 0.01$).

As a further control of BC inhibition of bacterial binding to salivary molecules, some beads were coated with BSA to cover any regions of the material that might be available for bacterial attachment, as described previously (*28*). BC reduced binding to a degree comparable to that reported above for the BSA-uncoated samples (data not shown).

We then studied the effect of bacterial detachment from HA beads using the original BC solution (60 mg mL^{-1}), the diluted solution (30 mg mL^{-1}), and a 2-fold concentration (120 mg mL^{-1}). BC was added after radiolabeled bacteria had been allowed to adsorb to the beads in the presence and absence of sucrose; the proportion of detached cells was determined by measuring radioactivity in the supernatant. Values were similar to those obtained in control samples with all three concentrations after 1 or 2 h of incubation (data not shown).

Surface Hydrophobicity after Exposure to BC-Supplemented Medium. To establish whether the effects of BC on attachment to HA beads could be attributed to changes in surface hydrophobicity, we evaluated the affinity of bacteria exposed to BC toward *n*-octane and found a marked, dose-dependent influence on bacterial surface hydrophobicity for all bacteria from a BC concentration of 0.5× MIC, the sole exceptions being *S. intermedius* ATCC 27335 and *S. vestibularius* ATCC 49124, whose surface hydrophobicity was unaffected (**Table 3**). No change was observed at the lower concentrations used in adherence experiments.

BC Fractions Affecting *S. mutans*, *S. intermedius*, and *S. constellatus* Interactions with HA Beads. In order to identify the compounds responsible for the antiadhesive activity of the BC beverage and obtain preliminary indications about their MM, BC was subjected to membrane dialysis (cutoff, 1000 Da). This allowed us to separate low-MM components (i.e., metallic cations, anions, monomeric, and oligomeric polyphenols) from polymeric components (e.g., proteins, polysaccharides, and high-MM melanoidins) that form with heat treatment. Both dialysates and retentates were tested. Restoration of the original volume subjected to dialysis with Millipore grade water (see Materials and Methods) enabled testing for the antibacterial and antiadhesive properties of all BC constituents at the same concentrations at which they are found in the original BC solution.

Table 2. IA (%) of Sublethal RBP Concentrations toward Sucrose-Independent (First Section) and Sucrose-Dependent (Second Section) Adsorption of Oral Streptococci to Saliva-Coated HA Beads as Compared with RBP-Untreated Controls[a]

		adsorption to HA beads × 10^6 bacteria/50 mg HA ± SD				
strain	BC treatment	30 mg RBP mL^{-1}	IA %	60 mg RBP mL^{-1}	IA %	control
		sucrose-independent				
S. anginosus	A[b]	11.3 ± 1.2	13	7.9 ± 0.9	39	13.0 ± 0.9
ATCC 33397	B[c]	5.6 ± 0.6	33	3.2 ± 0.2	62	8.4 ± 0.7
	C[d]	7.9 ± 0.9	20	4.5 ± 0.6	55	9.9 ± 0.7
S. constellatus	A[b]	5.3 ± 0.4	47	2.1 ± 0.3	79	10.0 ± 0.9
ATCC 27823	B[c]	1.9 ± 0.1	74	7.4 ± 0.5	92	7.2 ± 0.8
	C[d]	4.9 ± 0.6	45	2.6 ± 0.4	71	8.9 ± 1.0
S. intermedius	A[b]	13.2 ± 1.1	20	6.1 ± 0.4	63	16.5 ± 2.0
ATCC 27335	B[c]	6.1 ± 0.4	58	4.2 ± 0.6	71	14.5 ± 0.9
	C[d]	7.5 ± 0.8	40	4.8 ± 0.6	62	12.5 ± 1.6
S. oralis	A[b]	15.0 ± 1.3	32	11.9 ± 1.1	46	21.5 ± 2.4
ATCC 10557	B[c]	9.7 ± 0.8	56	9.1 ± 0.7	59	22.1 ± 2.0
	C[d]	15.3 ± 1.2	40	14.4 ± 1.8	45	25.5 ± 3.0
S. salivarius	A[b]	9.2 ± 0.6	32	8.9 ± 0.7	34	13.5 ± 1.8
ATCC 13419	B[c]	4.5 ± 0.3	52	4.0 ± 0.2	57	9.4 ± 0.8
	C[d]	8.1 ± 0.9	30	6.7 ± 0.9	42	11.5 ± 1.8
S. sanguis	A[b]	5.9 ± 0.6	45	5.1 ± 0.5	53	10.8 ± 1.7
ATCC 10556	B[c]	7.2 ± 0.6	50	4.9 ± 0.3	66	14.3 ± 1.2
	C[d]	8.4 ± 0.9	33	5.6 ± 0.3	54	12.5 ± 1.9
S. vestibularius	A[b]	6.7 ± 0.3	33	5.7 ± 0.3	43	10.0 ± 1.5
ATCC 49124	B[c]	4.1 ± 0.2	45	3.2 ± 0.2	57	7.4 ± 0.7
	C[d]	6.0 ± 0.7	29	4.3 ± 0.5	49	8.5 ± 0.5
		sucrose-dependent				
S. anginosus	A[b]	11.3 ± 1.0	13	9.5 ± 0.6	27	13.0 ± 1.0
ATCC 33397	B[c]	6.5 ± 0.7	24	5.2 ± 0.4	40	8.6 ± 0.7
	C[d]	8.8 ± 0.7	20	7.8 ± 0.7	29	11.0 ± 1.2
S. constellatus	A[b]	11.4 ± 1.2	10*	5.3 ± 0.4	58	12.7 ± 1.5
ATCC 27823	B[c]	7.4 ± 0.5	33	3.4 ± 0.4	69	11.1 ± 0.9
	C[d]	9.7 ± 0.9	9*	5.4 ± 0.7	50	10.7 ± 1.3
S. intermedius	A[b]	13.7 ± 0.9	28	11.2 ± 1.1	41	19.0 ± 1.0
ATCC 27335	B[c]	10.2 ± 1.2	42	6.0 ± 0.5	65	17.2 ± 1.0
	C[d]	12.7 ± 1.7	30	10.1 ± 0.9	44	18.1 ± 0.2
S. oralis	A[b]	13.2 ± 1.2	39	8.2 ± 0.5	62	21.7 ± 1.9
ATCC 10557	B[c]	13.3 ± 1.4	41	5.5 ± 0.9	75	22.2 ± 1.5
	C[d]	14.3 ± 1.7	28	10.9 ± 1.1	45	19.8 ± 2.3
S. salivarius	A[b]	13.9 ± 1.6	4*	11.7 ± 1.4	23	14.5 ± 1.0
ATCC 13419	B[c]	4.3 ± 0.3	58	3.1 ± 0.1	70	10.3 ± 0.9
	C[d]	10.7 ± 0.9	20	4.9 ± 0.6	63	13.4 ± 1.5
S. sanguis	A[b]	5.6 ± 0.7	43	4.1 ± 0.2	58	9.8 ± 0.9
ATCC 10556	B[c]	5.2 ± 0.3	57	4.5 ± 0.6	62	12.0 ± 0.9
	C[d]	7.3 ± 0.8	44	6.3 ± 0.9	52	13.1 ± 1.9
S. vestibularius	A[b]	8.4 ± 0.6	3	7.5 ± 0.6	14	8.7 ± 0.8
ATCC 49124	B[c]	3.7 ± 0.5	26	2.8 ± 0.1	46	5.0 ± 0.6
	C[d]	5.8 ± 0.8	19	4.5 ± 0.7	38	7.2 ± 0.8

[a] Results are the means of three different experiments ± SD. [b] BC-pretreated saliva-coated HA beads. [c] Saliva-coated HA beads simultaneously treated with BC and bacteria. [d] Bacteria grown in the presence of sub-MICs of BC. Differences between BC-treated and the respective control samples are significant ($p \leq 0.05$) with the exception of samples*. Differences between the two RBP concentrations are significant ($p \leq 0.1$).

Table 3. Effect of RBP Exposure on Bacterial Cell Surface Hydrophobicity

bacteria	loss of hydrophobicity (%)		
	0.5 × MIC	1 × MIC	2 × MIC
	120 mg RBP mL^{-1}	240 mg RBP mL^{-1}	480 mg RBP mL^{-1}
S. constellatus ATCC 27823	65.90 ± 1.10	80.75 ± 1.06	94.44 ± 2.62
S. intermedius ATCC 27335	NA[a]	5.00 ± 0.50	14.00 ± 0.60
	150 mg RBP mL^{-1}	300 mg RBP mL^{-1}	600 mg RBP mL^{-1}
S. mutans 9102	86.10 ± 2.50	118.45 ± 5.30	155.85 ± 15.34
S. mutans ATCC 25175	50.80 ± 5.30	73.90 ± 8.70	100.00 ± 11.23
S. oralis ATCC 10557	132.10 ± 10.12	163.45 ± 19.02	179.84 ± 22.00
S. salivarius ATCC 13419	14.70 ± 9.80	79.30 ± 17.96	313.85 ± 16.68
S. sanguis ATCC 10556	23.10 ± 3.10	59.65 ± 0.49	126.10 ± 5.51
S. anginosus ATCC 33397	59.10 ± 4.64	121.26 ± 12.92	236.36 ± 29.32
S. vestibularis ATCC 49124	NA[a]	7.20 ± 2.26	14.35 ± 4.60

[a] No activity.

Table 4. IA (%) of BC Dialysis and GFC Fractions toward *S. mutans* 9102 Adsorption to Saliva-Coated HA Beads[a]

BC fraction	treatment	adsorption to HA beads × 10^6 bacteria/50 mg HA ± SD: sucrose-indpendent	IA %	sucrose-dependent	IA %
MM < 1000 Da	A[b]	11.9 ± 2.2	30	16.3 ± 1.9	46
	B[c]	10.7 ± 1.4	35	15.1 ± 1.7	49
MM > 1000 Da	A[b]	9.6 ± 1.1	44	15.7 ± 1.6	48
	B[c]	9.0 ± 0.8	45	13.1 ± 3.3	56
MM 1000 < Da < 300000	A[b]	19.2 ± 3.1	NA[d]	32.2 ± 5.1	NA[d]
	B[c]	20.2 ± 2.2	NA[d]	31.1 ± 3.3	NA[d]
MM > 300000 Da	A[b]	8.9 ± 0.7	48	18.1 ± 2.2	40
	B[c]	7.2 ± 0.9	56	16.9 ± 1.7	43
GFC1	A[b]	11.1 ± 2.0	35	18.7 ± 3.2	38
	B[c]	8.7 ± 0.6	47	17.2 ± 1.4	42
GFC2	A[b]	18.3 ± 1.2	NA[d]	31.2 ± 2.8	NA[d]
	B[c]	17.4 ± 2.3	NA[d]	30.5 ± 2.9	NA[d]
GFC3	A[b]	20.0 ± 2.6	NA[d]	32.4 ± 3.9	NA[d]
	B[c]	17.9 ± 1.9	NA[d]	33.5 ± 2.0	NA[d]
control A[e]		17.1 ± 2.2		30.2 ± 3.8	
control B[e]		16.4 ± 1.7		29.7 ± 2.7	

[a] Results are the means of three different experiments ± SD. Similar results were obtained with *S. mutans* ATCC 25175. Each BC fraction was freeze-dried, resuspended in distilled water, and restored to the same concentration as the original beverage. [b] Saliva-coated HA beads pretreated with BC. [c] Saliva-coated HA beads simultaneously treated with BC and bacteria. [d] No activity (no inhibition or not statistically significant inhibition). [e] Control values of experiments A and B.

Results showed that the dialysate alone was responsible for the antibacterial activity, since it had the same MIC value as the BC beverage for all three bacteria. With regard to the antiadhesion activity (**Tables 4**−**6**), which was evaluated using approaches A and B, both dialysates and retentates were active toward the three strains and exhibited similar values, with significant differences between BC-treated and control samples ($p \leq 0.05$).

Table 5. IA (%) of BC Dialysis and GFC Fractions toward *S. constellatus* ATCC 27823 Adsorption to Saliva-Coated HA Beads[a]

BC fraction	treatment	adsorption to HA beads × 10^6 bacteria/50 mg HA ± SD: sucrose-independent	IA %	sucrose-dependent	IA %
MM < 1000 Da	A[b]	6.1 ± 0.7	28	6.8 ± 0.5	41
	B[c]	5.5 ± 0.5	31	5.7 ± 0.7	44
MM > 1000 Da	A[b]	4.6 ± 0.5	46	5.2 ± 0.7	55
	B[c]	4.1 ± 0.6	49	4.4 ± 0.2	57
MM 1000 < Da < 300000	A[b]	8.6 ± 0.6	NA[d]	12.0 ± 1.8	NA[d]
	B[c]	8.4 ± 0.6	NA[d]	11.3 ± 0.0	NA[d]
MM > 300000 Da	A[b]	4.8 ± 0.2	44	4.9 ± 0.2	57
	B[c]	3.7 ± 0.2	54	4.4 ± 0.5	57
GFC1	A[b]	5.6 ± 0.7	34	6.1 ± 0.5	47
	B[c]	4.8 ± 0.5	40	5.2 ± 0.6	49
GFC2	A[b]	9.0 ± 1.2	NA[d]	12.4 ± 1.1	NA[d]
	B[c]	8.7 ± 0.9	NA[d]	12.5 ± 0.9	NA[d]
GFC3	A[b]	8.9 ± 1.1	NA[d]	12.3 ± 1.1	NA[d]
	B[c]	8.3 ± 0.5	NA[d]	13.1 ± 2.1	NA[d]
control A[e]		8.5 ± 0.7		11.5 ± 1.9	
control B[e]		8.0 ± 0.9		10.2 ± 0.9	

[a] Results represent the means of three different experiments ± SD. Each BC fraction was freeze-dried, resuspended in distilled water, and restored to the same concentration as the original beverage. [b] Saliva-coated HA beads pretreated with BC. [c] Saliva-coated HA beads simultaneously treated with BC and bacteria. [d] No activity (no inhibition or not statistically significant inhibition). [e] Control values of in experiments A and B.

Table 6. IA (%) of BC Dialysis and GFC Fractions toward *S. intermedius* ATCC 27335 Adsorption to Saliva-Coated HA Beads[a]

BC fraction	treatment	adsorption to HA beads × 10^6 bacteria/50 mg HA ± SD: sucrose-independent	IA %	sucrose-dependent	IA %
MM < 1000 Da	A[b]	8.1 ± 0.1	40	10.4 ± 1.2	47
	B[c]	7.5 ± 0.8	42	9.2 ± 0.8	48
MM > 1000 Da	A[b]	7.4 ± 0.9	46	9.4 ± 0.8	52
	B[c]	7.0 ± 0.9	46	8.3 ± 0.9	53
MM 1000 < Da < 300000	A[b]	14.6 ± 1.9	NA[d]	19.9 ± 2.1	NA
	B[c]	13.9 ± 2.7	NA[d]	20.5 ± 1.9	NA
MM > 300000 Da	A[b]	7.6 ± 0.5	44	9.8 ± 1.0	50
	B[c]	7.1 ± 0.6	45	8.5 ± 0.6	52
GFC1	A[b]	8.0 ± 0.5	41	10.2 ± 1.5	48
	B[c]	7.5 ± 0.5	42	9.6 ± 0.9	46
GFC2	A[b]	13.5 ± 0.7	NA[d]	19.3 ± 1.7	NA
	B[c]	13.8 ± 1.7	NA[d]	20.0 ± 2.2	NA
GFC3	A[b]	14.1 ± 1.9	NA[d]	20.5 ± 2.2	NA
	B[c]	14.6 ± 2.1	NA[d]	21.1 ± 2.2	NA
control A[e]		13.6 ± 1.7		19.5 ± 1.1	
control B[e]		12.9 ± 1.5		17.7 ± 1.4	

[a] Results represent the mean values of three different experiments ± SD. Each BC fraction was freeze-dried, resuspended in distilled water, and restored to the same concentration as the original beverage. [b] Saliva-coated HA beads pretreated with BC. [c] Saliva-coated HA beads simultaneously treated with BC and bacteria. [d] No activity (no inhibition or not statistically significant inhibition). [e] Control values of in experiment A and B.

Study of the dialysate's chemical composition revealed components that affect oral health, i.e., fluoride, zinc ions, and polyphenols. Fluoride, which increases the tooth's resistance to acidic attack by promoting fluorapatite formation, was measured using a fluoride-selective electrode. Its concentration was 37 ng mL^{-1} in BC beverage. Zn(II) ion, which effectively inhibits plaque formation and gingivitis (*30*), was found at a

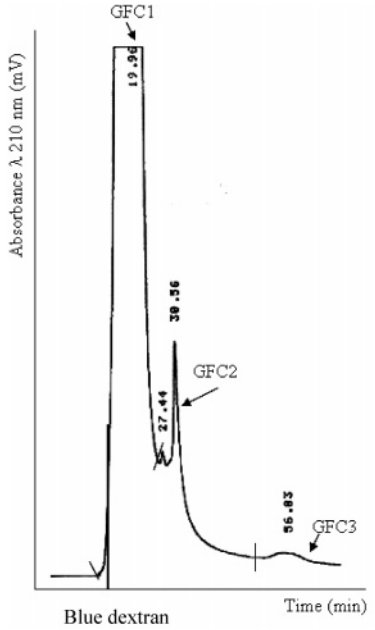

Figure 1. GFC chromatogram of roasted barley >300000 Da MM fraction obtained with resin HW-75F. Procedures are described in the text. Blue dextran: MM = 2000 kDa.

concentration of 0.24 μg mL^{-1}. The total polyphenol content was again assessed with the Folin−Ciocalteau assay. A number of studies have shown that many polyphenol derivatives actively reduce caries development in animals and humans (*31*−*35*), because they decrease the cell surface hydrophobicity of *S. mutans* and reduce both the production of acidic compounds and the ability of streptococci to synthesize adherent water-insoluble glucan from sucrose. Results showed that these components occur in BC at a concentration of 784 μg mL^{-1}. All of these compounds are capable of influencing the adhesion properties of oral streptococci.

The >1000 Da MM retentate was further fractioned by membrane dialysis with a cutoff of 300000 Da, which demonstrated high antiadhesive activity toward all three strains, while the dialysate (1000−300000 Da MM components) showed no activity (**Tables 4**−**6**).

The >300000 Da MM retentate was further resolved with the GFC technique, yielding three chromatographic peaks (GFC1, GFC2, and GFC3; **Figure 1**). The first peak showed activity in adhesion experiments (**Tables 4**−**6**); it corresponded to the GFC1 eluate, with R_t 19.96 min, which was higher than the one for blue dextran (R_t 18.03 min), used as the standard (MM = 2000 kDa). The GFC2 and GFC3 fractions displayed no antiadhesive activity. The GFC1 eluate possessed marked antiadhesive properties. In a previous study of the antioxidant properties of BC (*36*), spectrophotometric and electrophoretic analyses showed this fraction to consist of a single brown component with MM >1000 kDa. The component can thus be considered either as an antioxidant and as an antiadhesive agent. The fact that it was not found in the chromatogram of the unroasted barley solution suggests that this component, which is responsible for most of the antiadhesive and antioxidant activity of BC, forms during the roasting process.

In conclusion, BC possesses weak antibacterial and bactericidal activity as well as high antiadhesive properties. With regard to the former, sublethal BC concentrations impaired adsorption of *S. mutans* and other oral streptococci to saliva-coated HA. The effects seem to depend on masking of saliva-treated HA receptors for bacterial ligands as well as on masking and/or modification of bacterial adhesins. Indeed, HA adsorption was reduced when the beverage was present in the adhesion mixture and when it was used for bead pretreatment. In addition, there was a significant reduction in adsorption after bacterial growth in the presence of sub-MICs of BC, suggesting that a modification in bacterial surface ligand synthesis and/or expression may have occurred too. These effects do not seem to depend on a change in surface hydrophobicity, which is usually connected to adhesion ability. In fact, BC concentrations higher than those showing activity in the adhesion experiments affected affinity toward *n*-octane.

The antiadhesive properties of BC are attributable both to a low-MM fraction and to a high-MM component. The activity of the former could be due to the protective action of fluoride, zinc ions, and polyphenol derivatives. As for the high-MM antiadhesive component, this was not found in unroasted barley solution, which exerts very weak antiadhesive activity. It may thus be concluded that a compound generated during the roasting process is largely responsible for the antiadhesive properties of the BC beverage. It can be considered as a Maillard reaction end product of the melanoidin family. Such compounds, which have a variable but as yet unclearly defined chemical structure, commonly occur in foodstuffs subjected to heat treatment. In a previous work (*36*), the compound was also found to be a potent antioxidant both in chemical and in biological systems.

Although the elucidation of the mechanisms of action of BC is beyond the scope of the present study, our data indicate that its interactions with bacteria are complex and depend on microorganism surface characteristics and the combined action of a number of beverage components.

ABBREVIATIONS USED

BC, barley coffee; HA, hydroxyapatite; 5-*O*-CQA, 5-*O*-caffeoyl-quinic acid; THB, Todd Hewitt broth; PB, potassium phosphate buffer; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; ISB, Iso-Sensitest broth; PCE, postcontact effect; TSA, tryptone soya agar; IA, inhibitory activity; BSA, bovine serum albumin; MM, molecular mass; RBP, roasted barley powder; GFC, gel filtration chromatography.

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LITERATURE CITED

(1) Mandel, I. D. Caries prevention: current strategies, new directions. *J. Am. Dent. Assoc.* **1996**, *127*, 477−488.
(2) Russel, M. W.; Hajishengallis, G.; Chikders, N. K.; Michalek, S. M. Secretory immunity in defence against cariogenic mutans streptococci. *Caries Res.* **1999**, *33*, 4−15.
(3) Chandan, R. Functional foods. In *Dairy-Based Ingredients*; American Association of Cereal Chemists: St. Paul, MN, 1997; pp 96−99.
(4) Hasler, C. M. Functional food: The Western perspective. *Nutr. Rev.* **1996**, *54*, S6−S10.
(5) Huggett, A. C.; Verschuren, P. M. The safety assurance of functional foods. *Nutr. Rev.* **1996**, *54*, S132−S140.
(6) Hardie, J. M.; Whiley, R. A. Plaque microbiology of crown caries. In *Dental Plaque Revisited*; Newman, H. N., Wilson, M., Eds.; Bioline: Cardiff, 1999; pp 283−294.
(7) Loesche, W. J. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev.* **1986**, *50*, 353−380.
(8) Jenkinson, H. F. Adherence and accumulation of oral streptococci. *Trends Microbiol.* **1994**, *2*, 209−212.

(9) Whittaker, C. J.; Klier, M.; Kolenbrander, P. E. Mechanisms of adhesion by oral bacteria. *Annu. Rev. Microbiol.* **1996**, *50*, 513−552.
(10) Hajishengallis, G.; Michalek, S. M. Current status of a mucosal vaccine against dental caries. *Oral Microbiol. Immunol.* **1999**, *14*, 1−20.
(11) Matsumoto, M.; Minami, T.; Sasaki, H.; Sobue, S.; Hamada, S.; Ooshima, T. Inhibitory effects of oolong tea extract on caries-inducing properties of mutans streptococci. *Caries Res.* **1999**, *33*, 441−445.
(12) Mandel, I. D. Caries prevention: current strategies, new directions. *J. Am. Dent. Assoc.* **1996**, *127*, 1477−1488.
(13) Olsson, J.; Carlén, A.; Holmberg, K. Modulation of bacterial binding to salivary pellicle by treatment with hydrophilizing compounds. *Arch. Oral Biol.* **1990**, *35*, 137S−140S.
(14) Tarsi, R.; Muzzarelli, R. A. A.; Guzmàn, C. A.; Pruzzo, C. Inhibition of *Streptococcus mutans* adsorption to hydroxyapatite by low molecular weight chitosans. *J. Dent. Res.* **1997**, *76*, 665−672.
(15) 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. Ecol.* **1998**, *13*, 217−224.
(16) Aniansson, G.; Andersson, B.; Lindstedt, R.; Svanborg, C. Anti-adhesive activity of human casein against *Streptococcus pneumoniae* and *Haemophilus influenzae*. *Microb. Pathog*. **1990**, *8*, 315−323.
(17) 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.
(18) Tagashira, M.; Uchiyama, K.; Yoshimura, T.; Shirota, M.; Uemitsu, M. Inhibition of hop bract polyphenols of cellular adherence and water-insoluble glucan synthesis of *Streptococcus mutans*. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 332−335.
(19) Hamilton-Miller, J. M. Anti-cariogenic properties of tea (Camellia sinensis). *J. Med. Microbiol.* **2001**, *50*, 299−302.
(20) Daglia, M.; Tarsi, R.; Papetti, A.; Grisoli, P.; Dacarro, C.; Pruzzo, C.; Gazzani, G. Anti-adhesive effect of green and roasted coffee on *Streptococcus mutans* adhesive properties on saliva coated hydroxyapatite beads. *J. Agric. Food Chem*. **2002**, *50*, 1225−1229.
(21) Daglia, M.; Cuzzoni, M. T.; Dacarro, C. Antibacterial activity of coffee. *J. Agric. Food Chem.* **1994**, *42*, 2270−2272.
(22) 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.
(23) NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *Approved Standard M7-A6*, 6th ed.; Wayne, PA, 2003.
(24) NCCLS. Methods for determining bactericidal activity on antimicrobial agents. *Approved Guideline*; Wayne, PA, 1999; Vol. 19.
(25) Craig, W. A; Gudmundsson, S. Postantibiotic effect. In *Antibiotics in Laboratory Medicine*, 3rd ed.; Lorian, V., Eds.; Williams & Wilkins: Baltimore, MD, 1991; pp 403−431.
(26) Rosenberg, M. Bacterial adherence to hydrocarbons: A useful technique for studying cell surface hydrophobicity. *FEMS Microbiol. Lett.* **1984**, *22*, 289−295.
(27) Osawa, K.; Miyazaki, K.; Shimura, S.; Okuda, J.; Matsumoto, M.; Ooshima, T. Identification of cariostatic substances in the cacao bean hush: their anti-glucosyltransferase and antibacterial activities. *J. Dent. Res.* **2001**, *80*, 2000−2004.
(28) Gibbons, R. J.; Etherden, I. Albumin as a blocking agent in studies of streptococcal adsorption to experimental salivary pellicles. *Infect. Immun.* **1985**, *50*, 592−594.
(29) Yew Tang, S.; Whiteman, M.; Feng Peng, Z.; Jenner, A.; Leong Yong, E.; Halliwell, B. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. *Free Radical Biol. Med.* **2004**, *36*, 1575−1587.
(30) Ooshima, T.; Minami, T.; Aono, W.; Hamada, S. Reduction of dental plaque deposition in humans by oolong tea extract. *Caries Res.* **1994**, *28*, 146−149.
(31) Otake, S.; Makimura, M.; Nishihara, Y.; Hirasawa, M. Anticaries effects of polyphenolic compounds from Japanese green tea. *Caries Res.* **1991**, *25*, 438−443.
(32) Shouji, N.; Fukushima, K.; Hirasawa, M. Anticaries effect of a component from Shiitake (mushroom). *Caries Res.* **2000**, *34*, 94−98.
(33) Sanagida, A.; Canda, T.; Oliveira, Cordeiro, J. G. Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of *S. mutans. J. Agric. Food Chem.* **2000**, *48*, 5666−5671.
(34) Ooshima, T.; Osaka, Y.; Sasaki, H.; Osawa, K.; Yasuda, H.; Matsumoto, M. Cariostatic activity of cacao mass extract. *Arch. Oral Boil.* **2000**, *45*, 805−808.
(35) Osawa, K.; Miyazaki, K.; Okuda, J.; Matsumoto, M.; Ooshima, T. Identification of cariostatic substances in the cacao bean husk: Their anti-GTF and antibacterial activities. *J. Dent. Res.* **2001**, *80*, 2000−2004.
(36) Papetti, A.; Daglia, M.; Aceti, C.; Quaglia, M.; Gregotti, C.; Gazzani, G. Isolation of an in vitro and ex vivo antiradical melanoidin from roasted barley. *J. Agric. Food Chem.* **2006**, *54*, 1209−1216.

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