# Effects of *Coffea arabica* on *Streptococcus mutans* adherence to dental enamel and dentine

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Received for publication: August 11, 2007
Accepted: November 23, 2007

**Abstract**
The aim of this study was to evaluate the effects of different coffee solutions on *Streptococcus mutans* adherence to human dental enamel and dentine. Seventy-five specimens of human enamel and 75 of dentine were included in the study. Coffee solutions were prepared with two different trade marks of coffee (Mellita® and Pilão®) and two methods of preparation. The specimens were divided into ten experimental groups (n=15) according to the coffee solution tested. Each specimen was transferred to cell culture plates containing offee solution and culture medium and 0.1 ml of *Streptococcus mutans* standardized suspension was inoculated into each well. After the period of incubation, the number of bacterial cells adhered to each specimen was obtained by plating method. Results were analyzed by ANOVA and Tukey's test (5%). Results showed that *Streptococcus mutans* adherence was statistically lower in the presence of the solution obtained by boiling method and the trade mark Pilão® (p=0.00) when compared with the other groups. All the test-groups presented values of cfu/ml significantly lower in relation to the control groups (p=0.00). It could be concluded, within the conditions of the study, that the coffee solutions tested reduced significantly the adherence of *Streptococcus* to dental enamel and dentine.

**Key words:**
*Streptococcus mutans*, *Coffea arabica*, dental enamel.

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## Introduction

Caries is still considered one of the main problems of public health and many researchers in the world have been searching for alternatives to prevent the occurrence of this process. The adherence of bacterial cells to teeth surface is of great importance to the development of caries lesions and the interference on some of these mechanisms can prevent the formation of carious lesions[1].

Several previous studies demonstrated the activity of natural extracts (green, black, and oolong teas, cacao, propolis) on the dental biofilm formation and caries development[2-8]. The capacity of some extracts to affect the synthesis of extracellular polysaccharides may have an important role in the determination of their anti-cariogenic potential[7]. Limsong et al.[9] concluded that black tea (*Camellia sinensis*) followed by *Andrographis paniculata*, *Cassia alata* and *Harrisonia perforata* presented inhibitory effects on *Streptococcus mutans* (*S. mutans*) ATCC25175 adherence to hydroxyapatite.

Coffee is classified in the *Rubiaceae* family, *Coffea* genus, and the species cultivated in Brazil are *Coffea arabica* and *Coffea canephora*. Coffee grain is composed by water, mineral substances, glucides, lipids, organic acids, alkaloids, tanic acid[10], theobromine, cafein and several vitamins[11]. Few studies on the antimicrobial activity of coffee-based solutions are found in the literature. Toda et al.[12] related the effects of coffee on microbial species such as *Staphylococcus aureus*, *Salmonella thiphi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* and attributed this bactericide effect to the tanic acid. *In vitro* studies showed that extracts coffee may inhibit glucosyltransferase in several oral streptococci[13].

Daglia et al.[2] studying dark roasted coffee described that a compound originated during the roasting process had very strong antibacterial activity. This compound was effective against Gram-positive and Gram-negative reference strains. Previous studies[2,14] reported the anti-adhesive effect of coffee on the adherence of *S. mutans* to glass and saliva-coated hydroxyapatite beads, respectively.

The aim of this study was to evaluate the *in vitro* antimicrobial activity of coffee-based solutions from different origins, obtained by two distinct methods, on *S. mutans* adherence to human dental enamel and dentine.

## Material and Methods

This study was approved by Local Ethic Committee (São José dos Campos Dental School – UNESP; protocol number 091/2004-PH/CEP).

Forty human extracted teeth (premolars and molars) were included in the study. Teeth were previously cleaned up with the aid of periodontal curettes, sterilized in autoclave and maintained in physiologic solution (NaCl 0.85%). Standardized (6mm x 5 mm = 30 $mm^2$) enamel and dentine specimens (n=75, each) were prepared. The test area (16 $mm^2$) was determined with the aid of enamel for nail (Colorama, São Paulo, SP, Brazil). All the specimens were transferred to tubes containing physiologic solution and were sterilized in autoclave (121°C/20 minutes).

*S. mutans* ATCC 35688 was used for the tests. Two coffee trade-marks were tested: Pilão® (Sara Lee Cafés dos Brasil LTDA, Barueri, SP, Brazil) and Melitta® (Melitta do Brasil Indústria e Comércio LTDA, Avaré, SP, Brazil). Solutions of 8% coffee power in fluoride-containing water (1 ppm) were adopted as the standard coffee solution consumed by the population[15].

Coffee solutions were obtained by two different methods: a) Simple: Coffee powder was put into a glass funnel with a paper filter (Mellita®, Avaré, SP, Brazil) and boiling water was poured through the powder; b) Boiled: coffee powder was boiled with the water for 2 min and then filtered through the paper filter (Mellita®, Avaré-SP, Brazil).

Nine milliliters of each coffee solution obtained were put into 15 tubes containing dehydrated culture medium (brain heart infusion broth, BHI – Difco, Detroit, USA and 10% sucrose) and immediately submitted to autoclave sterilization. Growth control consisted in tubes containing BHI broth supplemented with sucrose 10% and fluoride distilled water (1 ppm).

Under aseptic conditions (laminar flow chamber) each specimen (n=15; dental enamel and dentine) were transferred to cell culture plates (24-wells, Costar, NY, USA). Two milliliters of each testing solution (Coffee solution or control) were added to each well. Ten experimental groups were included in the study (Table 1).

A standardized *S. mutans* ATCC 35688 suspension containing $10^6$ cells/ml was obtained by spectrophotometry. Each well of the cell culture plate was inoculated with 0.1 ml of the bacterial suspension and incubated at 37°C/5% $CO_2$ for 24 hours. After

**Table 1** – Experimental groups (n=15) included in the study according to the trade-marks and preparation of the coffee infusions

| Groups | Trade-mark | Preparation |
|---|---|---|
| **Dental enamel** | | |
| G1 | Pilão | Simple |
| G2 | Pilão | Boiled |
| G3 | Mellita | Simple |
| G4 | Mellita | Boiled |
| G5 (Control) | Water | - |
| **Dental dentine** | | |
| G6 | Pilão | Simple |
| G7 | Pilão | Boiled |
| G8 | Mellita | Simple |
| G9 | Mellita | Boiled |
| G10 (Control) | Water | - |

the period of incubation, specimens were washed by immersion in sterile distilled water for 5 seconds and then they were transferred to tubes containing 1 ml of sterile physiologic solution and glass beads. Tubes were submitted to agitation (Phoenix AP56, São Paulo, Brazil) and the adhered cells were dispersed. From this initial suspension, dilutions of $10^{-1}$ and $10^{-2}$ were obtained in sterilized NaCl 0.85% saline solution (Labsynth, São Paulo, Brazil). Then, 0.1 ml of each suspension was plated on BHI agar and incubated at 37°C/5% $CO_2$ for 24 hours. The value of logarithm of colony forming units per specimen (log UFC) was obtained and the mean value for each group by plating method.

*Analysis of data*

The results obtained were analyzed statistically by ANOVA and Tukey's test (5%).

## Results

Mean values of CFU, standard deviation and median of adhered *S. mutans* cells in each experimental group tested for adherence to dental enamel are shown in Table 2. Statistically significant differences were observed among the groups ($p<0.05$). Group 2 showed the lowest value of CFU and this value was statistically different in relation to the other experimental groups and control group ($p=0.000$). Groups 1 (Pilão®/simple), G3 (Mellita®/simple) and G4 (Mellita®/boiled) also presented lower values of CFU in relation to control group (p=0,000).

The values obtained for the tests with dental dentine are shown in Table 3. Also, statistically significant differences were observed. Group 7 (Pilão®/boiled) showed the lowest and statistically significant values in relation to the other experimental groups and control ($p=0.000$). Groups G6 (Pilão®/simple), G8 (Mellita®/simple) and G9 (Mellita®/boiled) were similar among them ($p<0.05$) and showed CFU values statistically lower than the control group ($p=0.000$).

## Discussion

Coffee represents one of the most consumed products by the population, mainly due to the effects on the organism. Caffeine, for instance, stimulates the attention, concentration, intellectual capacity and the chlorogenic acids modulate the sense of humor and help to prevent depression and its consequences. The diary consumption of four cups of coffee is worldwide recommended for adults[11].

Data obtained in this study suggest that coffee, as observed for different types of tea, may be related to caries prevention, reducing the adherence of bacteria to dental surface. The antimicrobial and anti-caries activity of tea has been demonstrated by several studies[4,8]. However, only recently the anti-caries activity of coffee was cited in the literature[14,16-17].

In the present study, the solutions of Pilão® and Mellita® coffees, obtained by both simple and boiled methods, reduced significantly the adherence of *S. mutans* to dental surfaces (enamel and dentine) demonstrating potential anti-caries activity. These results are in accordance with the studies of Daglia et al.[17], who verified anti-adhesive effects of coffee on saliva-coated hydroxiapatite specimens. In a previous study of our group[16], the same coffee solutions were able to reduce the adherence of *S. mutans* to the surface of glass.

Considering the methods of coffee solutions preparation (simple and boiled), both in dental enamel and in dentine tests, boiled solutions showed the best performance. These results suggest that the complex chemical composition of coffee may still be altered during the solution preparation, preserving or transforming certain substances that favor anti-adhesive activity.

Coffee anti-caries potential is related to its capacity of altering the biosynthesis of extracellular polysacharides (mainly *mutans*), avoiding the adhesion of streptococci[13]. Paolino et al.[18] observed that tanic acid inhibited glucosytransferase enzyme of *S. mutans* strains and similar inhibition was observed on *S. sanguis* strains. Kashket et al.[10] observed reduction of *S. mutans* adhesion to hydroxyapatite by tanic acid. Several *in vitro* experiments showed that cacao[19], tea[4] and coffee[17] inhibited glucosyltransferase of several oral streptococci, however some cacao extracts and coffee did

**Table 2** – Mean values (± standard deviation) of *Streptococcus mutans* colony-forming units/specimen (log 10) and median obtained for each experimental group with dental enamel specimens

| Groups | Trademark/Method | Mean ± Standard deviation | Median |
|---|---|---|---|
| G1 | Pilão / Simple | 2.45 ± 0.23 $^{A}$ | 2.50 |
| G2 | Pilão / Boiled | 1.82 ± 0.35 $^{B}$ | 1.84 |
| G3 | Mellita / Simple | 2.62 ± 0.36 $^{A}$ | 2.71 |
| G4 | Mellita / Boiled | 2.25 ± 0.13 $^{A}$ | 2.25 |
| G5 | Control | 4.38 ± 0.10 $^{C}$ | 4.40 |

**different letters indicate statistically significant differences (p<0,05).*

**Table 3** – Mean values (± standard deviation) of *Streptococcus mutans* colony-forming units/specimen (log 10) and median obtained for each experimental group with dental dentine specimens

| Groups | Trademark/Method | Mean ± Standard deviation | Median |
|---|---|---|---|
| G6 | Pilão/ Simple | 1.95 ± 0.82 $^{D}$ | 2.27 |
| G7 | Pilão/ Bioled | 1.18 ± 0.67 $^{E}$ | 1.47 |
| G8 | Mellita/ Simple | 2.29 ± 0.24 $^{D}$ | 2.27 |
| G9 | Mellita/ Boiled | 1.78 ± 0.72 $^{D}$ | 2.04 |
| G10 | Control | 4.10 ± 0.11 $^{F}$ | 4.15 |

**different letters indicate statistically significant differences (p<0,05).*

not loose that characteristic even without tanic acid and caffeine, suggesting the role of other substances in this inhibition[10].

The reduced *S. mutans* adherence observed in this study may be associated to the combination of tanic acid and trigonelin with other coffee compounds[14,16-17]. Considering that coffee is constituted by several substances such as water, mineral substances, glucides, lipids, organic acids, alkaloids, tanic acids, theobromine, cafein and several vitamins, the isolated evaluation of each compound may clarify the specific agent related to its anti-adhesive effect. More studies, particularly *in vivo* and *in situ*, are necessary to clarify the effectiveness under the dynamic characteristics of oral milieu and the clinical applications of these findings. It could be concluded that the different coffee solutions tested were able to reduce significantly *S. mutans* adherence to dental enamel and dentine. Pilão®/boiled group showed the best results.

**Acknowledgements**

The authors gratefully acknowledge the financial support from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

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