

# The Impact of Food Components and Dietary Factors on Oral Health

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## ABSTRACT

This presentation provides an overview of the impact of food components and dietary factors on oral health. The protective properties of various foods, plant extracts and plant-based polyphenolic compounds on dental caries and periodontal disease are discussed. Laboratory, human, and epidemiological studies demonstrating the multiple mechanistic actions in supporting their contribution to oral health and disease prevention are summarized. The need and development of multidisciplinary research approach toward experimental designs and strategies in clinical and epidemiological studies are also emphasized.

Key words: ionic functional foods for oral health, nutrition diet and oral health, natural products and oral health, anticariogenic foods, anticaries foods

## INTRODUCTION

Oral diseases and conditions, including dental caries, periodontal disease, orofacial disorders, and tooth loss affect more people than any other disease in the United States. Oral diseases can often result in pain and suffering, difficulty in speaking, chewing and/or swallowing, and in extreme cases, death. Next to the common cold, dental diseases are the major cause of lost work or school days. Recent research has provided evidence that oral diseases can significantly impact a person's overall health<sup>(1)</sup>. Oral bacteria may contribute to increased risk of heart attacks, strokes, and lung disease, and may be associated with premature childbirth in some women<sup>(2)</sup>.

Dental plaque has been implicated as the prime etiologic factor in both dental caries and periodontal disease. It is a complex microbial biofilm community whose colonization in the oral cavity is a continuous process, and is more resistant to killing by antibiotics or antimicrobial chemicals compared to their planktonic counterparts. The mutans group of streptococci (MS), found prominently in dental plaque, has been strongly implicated as one of the etiologic agents of dental caries in both humans and experimental animals<sup>(3)</sup>. *Streptococcus mutans* accounts for at least 90% of the isolates associated with human dental caries. Their cariogenic virulence factors include their ability to ferment sugars to produce acid (acidogenicity); to tolerate acid and thrive in a low-pH environment (aciduricity), and most importantly to utilize dietary sucrose for the synthesis of adherent glucans *via* glucosyltransferases (GTF) that facilitates the accumulation and adherence of dental plaque to tooth surfaces<sup>(4)</sup>. The acid

generated from bacterial carbohydrate metabolism leads to prolonged plaque acidification and demineralization of the tooth enamel leading to caries development. Classic approaches to dental caries prevention are mostly based on: (1) administration of fluoride, which enhances the stability of enamel and dentin to acid dissolution; (2) removal of plaque bacteria, mechanically or by anti-plaque oral hygiene products; and (3) substitution of dietary sucrose by less-cariogenic sugars. Besides dental caries, gingivitis and periodontal disease affect most of the adult population resulting in serious tooth loss before old age. Specific anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* and others can activate immunoinflammatory mechanisms within the local periodontal tissues, leading to the destruction of collagen and bone supporting the teeth<sup>(5)</sup>. These anaerobic periodontal pathogens can also produce volatile sulfur compounds (VSCs) that are associated with oral malodor (halitosis) in humans. Over the years, extensive effort has been made in search of safe and efficient antimicrobial agents that can control plaque bacteria and their virulence factors in order to reduce plaque biofilm related oral diseases.

Despite the advances made and effort spent on the identification of food components and development of food products with disease preventing and health promoting benefits, the general public seems less aware of foods that promote oral health. Much of the research has been focused on establishing the harmful relationship between foods and dental plaque bacteria and the role of antimicrobials play in this system, studies regarding the possible protective effects of foods has been limited. The

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most reported studies are those on milk and cheese that demonstrated the reduction of plaque acid and the promotion of enamel remineralization. The underlining hypothesis of our research is that selected foods or higher plants possess antimicrobial phytochemicals capable of suppressing growth and virulence factors of oral pathogens, thereby promoting oral health. Using an interdisciplinary research approach involving dentistry, oral microbiology, and natural products chemistry, we have developed methodologies in our laboratory for screening, fractionating and identifying oral antimicrobials from these sources. Many of such compounds have been isolated and identified. Those with novel structures have been fully characterized by physical and spectroscopic methods. Examples of some of the plants included: *Melaphis chinensis* (Chinese nut gall)<sup>(6)</sup>; *Zanthoxylum nitidum* ("Liang-men-zen")<sup>(7)</sup>; *Syzygium aromaticum* (Cloves)<sup>(8)</sup>; *Ceanothus americanus* ("New Jersey Tea")<sup>(9)</sup>; *Stevia rebaudiana* (Natural sweetener)<sup>(10)</sup>; *Sarcandra glabra* ("Cao-shan-hou")<sup>(11)</sup>; *Mussaenda macrophylla*<sup>(12)</sup>; *Diospyros lycioides* (Muthala-Namibian chewing sticks)<sup>(13,14)</sup>; *Salvadora persica*, ("Miswak")<sup>(15)</sup>; *H. canadensis* (Goldenseal)<sup>(16)</sup>; *Camellia sinensis*, (Tea)<sup>(17,18-20)</sup>; and *Vitis vinifera* (rasins)<sup>(21)</sup>. Their mechanistic actions against oral pathogens included inhibition of metabolism and growth; acid production; acid tolerance; plaque biofilm formation, adherence and maturation; VSC production; demineralization of dental enamel and dentin; and the suppression of virulence genes expression. We believe that selected active compounds may also find application directly as dental prophylactic/therapeutic agents or serve as lead compounds for the subsequent design and synthesis of new antiplaque agents that are even more effective than the existing ones. The following represents summary of several selected independent and collaborative research findings of the author's laboratory.

### I. Tea (*Camellia sinensis*)

Despite the animal data supporting the positive relation between tea and dental caries prevention, it was not until the past decade that researchers paid attention to the benefits of tea and its relevance to oral health. Tea and its polyphenols have been reported to inhibit *in vitro* growth, acid production, and GTF enzyme activity of mutans streptococci<sup>(22)</sup>. Polyphenols in black and oolong teas, inhibited *in vitro* growth of selected cariogenic and periodontal pathogens<sup>(23-26)</sup>. The monomeric polyphenols extracted from oolong tea extract (OTE) were found to bind proteins and exerted synergistic antibacterial activity against oral streptococci, including *S. mutans* and *Streptococcus sobrinus*<sup>(27)</sup>. OTE was also effective in reducing the rate of acid production by mutans streptococci<sup>(28)</sup>. Many of the available *in vitro* antimicrobial studies of green and oolong tea polyphenols have demonstrated a positive inhibitory effect on GTF enzyme activity and sucrose-dependent cell adherence<sup>(29,30)</sup>. However, most of these studies

investigated the planktonic cells of *S. mutans*, and fewer data are available on their effects on the highly resistant biofilm cells.

In our laboratory, tea and its polyphenols have been demonstrated to inhibit growth, viability, acid production, metabolism, and glucosyltransferase enzyme activity of *S. mutans*. We have reported that EGCG inhibited growth and viability of *S. mutans* planktonic cells (MIC, 31.25 µg/mL; MBC, 62.50 µg/mL) and biofilm formation (15.6 µg/mL). It reduced viability of the more resistant pre-formed biofilm at much higher concentration (Sessile MIC80, 625 µg/mL). The acidogenicity and acidity of *S. mutans* was suppressed at sub-MIC levels of EGCG (1.95 - 15.60 µg/mL). At both the enzymatic and transcriptional levels, EGCG significantly suppressed the *ldh*, *eno*, *atpD*, and *aguD* genes of *S. mutans* compared to the non-treated controls. Enzymatic activity of F<sub>1</sub>F<sub>0</sub>-ATPase and lactate dehydrogenase was also inhibited (IC<sub>50</sub> of 15.60 µg/mL and 31.25 µg/mL)<sup>(31)</sup>. Although previous researchers have reported the inhibitory activity of selected tea catechins on *S. mutans* adherence and GTF activity, few have performed mechanistic studies of EGCG on biofilm attachment and *gtfs* expression. We have recently found that EGCG exhibited dosage-dependent inhibition (7.80 - 31.25 µg/mL) on the initial attachment of *S. mutans* UA159 cells to surfaces. The effect of EGCG on the transcriptional expression of *gtf* B, C, D genes in *S. mutans* UA159 quantified by real-time PCR revealed that EGCG at sub-lethal level (15.60 µg/mL) significantly suppressed these virulence genes that may have led to the inhibition of initial attachment and biofilm formation of *S. mutans* cells without necessarily suppressing the resident oral bacteria<sup>(32)</sup>. In summary, EGCG represents a natural anti-cariogenic agent with antimicrobial activity against *S. mutans* and suppresses specific virulence factors associated with its cariogenicity. EGCG also reduced the production of the malodorous volatile sulfur compounds (VSCs) by periodontal pathogens associated with halitosis, e.g. *P. gingivalis* and *F. nucleatum*. At sub-lethal levels EGCG suppressed *mgl* gene, the major determinant for the production of methyl mercaptan (CH<sub>3</sub>SH) by *P. gingivalis*<sup>(33)</sup>.

A controlled clinical study in adult humans demonstrated that *in vivo* rinsing with tea extracts affected dental plaque regrowth/metabolism, composition of cariogenic microflora, and pH/fluoride of saliva and plaque. Short-term frequent rinses with tea inhibited the subsequent regrowth and glycolysis of human supragingival plaque bacteria compared to the water rinse group. It was suggested that black tea and its polyphenols may benefit human oral health by inhibition of dental plaque, its acidity and its cariogenic microbiota<sup>(34,35)</sup>.

In addition to inhibition of GTF activity, tea has been shown to inhibit salivary amylase activity<sup>(36,37)</sup>. Starch-containing foods may serve as slow-release sources of fermentable carbohydrate when hydrolyzed by salivary amylase in the oral cavity. Tea consumption may potentially reduce the cariogenic potential of these foods. Based on the above-summarized *in vitro* studies, it is ap-

parent that tea polyphenols exert an anticariogenic effect, at least in part, *via* an antimicrobial mode of action rather than by an action relying primarily on the de- or remineralization of tooth enamel. We believe that tea consumption represents a beneficial dietary habit and if sequenced properly between meals and normal oral hygiene, a reduction in plaque and perhaps, dental caries may be possible<sup>(38)</sup>.

## II. Raisins (*Vitis vinifera* L.)

Raisins, a popular snack food, are known to contain polyphenols, antioxidants, flavonoids and iron that benefit overall human health. The sweetness of raisins is contributed by mainly glucose and fructose, but not sucrose. Although various *in vitro* studies have been performed to investigate the mode of actions of these phytochemicals and their effects on bodily functions, much less attention has been paid to their effects on oral health and disease prevention. Through bioassay-guided fractionation, we have identified compounds that inhibited growth of *S. mutans* and *P. gingivalis*. These were oleanolic acid, oleanolic aldehyde, linoleic acid, linolenic acid, betulin, betulinic acid, 5-(hydroxymethyl)-2-furfural, rutin,  $\beta$ -sitosterol and  $\beta$ -sitosterol glucoside<sup>(39)</sup>. A clinical study was conducted in twenty 7 - 11 years old children to investigate the effect of raisins and raisin-containing cereals on *in vivo* plaque acidogenicity. The plaque pH was measured with a touch microelectrode (Beetrode<sup>®</sup>) prior to (baseline) and 2, 5, 10, 15, 20 and 30 min after consumption of a test food. It was found that addition of raisins to bran flakes, without any extrinsic sugars, promoted less plaque pH drop beyond 10 min when compared to bran flakes alone ( $p < 0.05$ ). Consumption of raisins did not reduce plaque pH below pH 6 over the 30 min test period<sup>(40)</sup>. Data obtained from these studies support the fact that raisins represent a healthy alternative to the commonly consumed sugary snack foods. Further studies to evaluate the long-term effect of raisins consumption on plaque microflora and acidogenicity are underway.

## III Grape Seed Extract

Proanthocyanidin (PA) is a naturally occurring plant metabolite widely available in fruits, vegetables, nuts, seeds, flowers and bark. Commonly used as natural antioxidants and free-radical scavengers, PAs have been proven to be safe in various clinical applications and as dietary supplements<sup>(41,42)</sup>. Grape seed extract (GSE) is a rich source of PAs, which has been reported to strengthen collagen-based tissues by increasing collagen cross-links<sup>(43)</sup>. PA from cranberries inhibited the surface-adsorbed glucosyltransferases and acid production by *Streptococcus mutans*<sup>(44)</sup>. Studies have also shown that PAs increased collagen synthesis and accelerated the conversion of soluble collagen to insoluble collagen during development. PA-treated collagen matrices were demonstrated to be nontoxic and resisted enzyme digestion *in vitro* and *in vivo*<sup>(45)</sup>.

*in vivo*<sup>(45)</sup>.

To evaluate the effect of GSE on the remineralization of artificial root caries, an *in vitro* pH-cycling model was used<sup>(46)</sup>. We have found that fluoride treatment inhibited further demineralization of existed artificial root lesions and increased the microhardness value of lesions. Treatment with GSE was also found to increase the microhardness of the lesions significantly when compared to the control group ( $p < 0.05$ ). Polarized light microscopy data revealed a significantly thicker mineral precipitation band on the surface layer of the GSE treated lesions, which was further confirmed by CLSM. The data supported the fact that GSE positively affected the demineralization and/or remineralization processes of artificial root caries lesions, most likely through a different mechanism than that of fluoride<sup>(46)</sup>. GSE may contribute to mineral deposition on the superficial layer of the lesion, and may also interact with the organic portion of the root dentin through PA-collagen interaction, thereby stabilizing the exposed collagen matrix. Grape seed extract may be a potential adjunct or alternative to fluoride in the treatment of root caries during minimally invasive therapy.

Diet has been considered an identifiable risk factor in preventing dental caries. The American Dietetic Association position paper on the role of diet and nutrition in oral health states: "Diet and nutrition have a direct implication on the progression of tooth decay, a preventable oral infectious disease. The primary risk factors to consider in determining the cariogenic, cariostatic, and anticariogenic properties of the diet are food (liquid, solid and sticky, long-lasting), frequency of consumption of sugar and other fermentable carbohydrates, nutrient composition, sequence of food intake, and combination of foods." By understanding the role of various food components play in oral health will provide the general public the opportunity to modify their dietary habits and to prevent or reverse the cariogenic effects of many foods. More studies investigating the "functional" aspects of foods and how they impact the oral cavity, thus overall wellbeing of a person are warranted.

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