

# Grape Products and Oral Health<sup>1-3</sup>

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## Abstract

Oral diseases, including dental caries, periodontal disease, and tooth loss, affect the majority of the population and can affect a person's overall health. Raisins contain polyphenols, flavonoids, and high levels of iron that may benefit human health. However, their oral health benefits are less well understood. We hypothesized that raisins contain antimicrobial phytochemicals capable of suppressing oral pathogens associated with caries or periodontal diseases and thus benefit oral health. Through antimicrobial assay-guided fractionation and purification, compounds identified with growth inhibition against oral pathogens were oleanolic acid, oleanolic aldehyde, linoleic acid, linolenic acid, betulin, betulinic acid, 5-(hydroxymethyl)-2-furfural, rutin,  $\beta$ -sitosterol, and  $\beta$ -sitosterol glucoside. Oleanolic acid suppressed in vitro adherence of cariogenic *Streptococcus mutans* biofilm. When the effect of raisins and raisin-containing bran cereal on in vivo plaque acidogenicity was examined in 7- to 11-y-old children, it was found that raisins did not reduce the plaque pH decline below pH 6 over the 30-min test period. Compared with commercial bran flakes or raisin bran cereal, a lower plaque pH drop was noted in children who consumed a raisin and bran flake mixture when no sugar was added ( $P < 0.05$ ). Grape seed extract, high in proanthocyanidins, positively affected the in vitro demineralization and/or remineralization processes of artificial root caries lesions, suggesting its potential as a promising natural agent for noninvasive root caries therapy. Raisins represent a healthy alternative to the commonly consumed sugary snack foods. J. Nutr. 139: 1818S-1823S, 2009.

## Oral health and disease

Oral diseases and conditions, including dental caries, periodontal disease, orofacial disorders, and tooth loss, affect more persons than any other disease in the United States. Millions of Americans suffer from these diseases and conditions of the oral cavity that result in pain and suffering; difficulty in speaking, chewing, and/or swallowing; and in extreme cases, death (1). Next to the common cold, dental diseases are the major cause of lost work or school days and have had a negative impact on

economic productivity and the learning ability of American children (2). Oral diseases and/or disorders can affect a person's overall health (3). Recent research has shown that oral bacteria may contribute to increased risk of heart attacks, strokes, and lung disease and may be associated with premature childbirth in some women (4,5).

Dental caries is a multi-factorial infectious disease that depends on diet and nutrition, microbial infection, and host response. Although the introduction of fluoride has resulted in the reduction of dental caries, the latter is still the most common infectious disease in humans and is especially prevalent in children and people with xerostomia (dry mouth) (6). In adults, the incidence of root caries was found to increase dramatically with age. Thus, control of caries is of major importance in dentistry and will continue to be for the foreseeable future. The mutans group of streptococci (MS),<sup>4</sup> found prominently in dental plaque, have been strongly implicated as one of the etiologic agents of dental caries in both humans and experimental animals (7). Epidemiological data indicate that *Streptococcus mutans* accounts for at least 90% of the isolates associated with human caries, with *Streptococcus sobrinus* being the second most common MS detected. The most prominent virulence factors of MS include their acidogenicity, aciduricity, and their ability to synthesize adherent glucans from

<sup>1</sup> Published in a supplement to *The Journal of Nutrition*. Presented at the conference "Grape Health Workshop," held in San Francisco, CA, December 2-3, 2008. The supplement coordinator for this supplement is John M. Pezzuto, University of Hawaii at Hilo. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact. The conference was organized by the National Grape and Wine Initiative (NGWI) (its contents are solely the responsibility of the authors and do not necessarily represent the official views of NGWI). *Supplement Coordinator disclosure*: John M. Pezzuto serves as Chair of the Grant Review Committee of the California Table Grape Commission. John M. Pezzuto received an honorarium to serve as moderator at the Grapes and Health Workshop. *Supplement Guest Editor disclosure*: Maria-Luz Fernandes has no relationships to disclose. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of *The Journal of Nutrition*.

<sup>2</sup> Supported by grants from the California Raisin Marketing Board; the Department of Pediatric Dentistry, College of Dentistry, University of Illinois at Chicago; and NIH-National Institute for Dental and Craniofacial Research no. DE017740.

<sup>3</sup> Author disclosure: C. D. Wu, no conflicts of interest.

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<sup>4</sup> Abbreviations used: cRB, commercial raisin bran cereal; eRB, an experimental raisin bran cereal; GSE, grape seed extract; MS, mutans group of streptococci; PA, proanthocyanidin.

dietary sucrose via glucosyltransferases, facilitating dental plaque formation and its adherence to tooth surfaces (8).

Besides dental caries, gingivitis and periodontal disease affect most of the adult population, with the prevalence of severe disease increasing with age (3). Periodontal disease is a group of chronic inflammatory diseases caused by specific anaerobic Gram-negative bacteria that activate immunoinflammatory mechanisms within the local periodontal tissues, leading to the destruction of collagen and bone supporting the teeth (9). Periodontitis occurs at greatly different rates in different participants. The chronic forms of the disease are widespread among the population, whereas the aggressive, destructive form of the disease affects ~10% of the population, resulting in serious tooth loss before old age (10).

Dental plaque has been implicated as the prime etiologic factor in dental caries, gingivitis, and periodontal disease (11–13). It is a complex bacterial biofilm community for which the composition is governed by factors such as cell adherence, coaggregation, and growth and survival in the environment (14). Plaque bacteria utilize the readily fermentable carbohydrates on tooth surfaces to produce acids that promote and prolong the cariogenic challenge to teeth, leading to enamel demineralization and tooth decay. The development and progression of dental caries depends on the amount of food particles that become trapped on the surfaces of teeth that may serve as ready sources of fermentable carbohydrates, thereby promoting acid production by plaque bacteria. This prolongs the cariogenic challenge to the teeth, leading to enamel demineralization and tooth decay. To date, mechanical plaque elimination with assorted devices remains the primary and most widely accepted means of maintaining good oral hygiene and controlling plaque-mediated diseases (15,16).

In recent years, much attention has been focused on research and education related to the identification of food components and development of food products with disease-preventing and health-promoting benefits of the “functional foods.” Numerous naturally occurring components in foods and vegetables have been shown to promote health and reduce risks for many common diseases. Despite these advances, the general public seems less aware of foods that promote oral health. It is thought that plant-derived antimicrobial compounds may serve as alternatives to the commonly used chemicals for dental plaque and oral disease control. The hypothesis is that higher plants and selected foods possess antimicrobial phytochemicals capable of suppressing growth and virulence factors of oral pathogens, thereby benefiting oral health. The author has developed methodologies in her laboratory for the screening, fractionation, and identification of oral antimicrobials from these sources using an interdisciplinary research approach involving dentistry, oral microbiology, and natural products chemistry (17–23). The foods studied included honey, tea, cranberries, raisins, dried plums, grape seeds, and others. Many of the active compounds inhibited the growth, metabolism, virulence factors, acidogenicity, and accumulation of dental plaque bacteria. Recently, the effects of these phytochemicals on the *in vitro* remineralization of dental caries lesions have also been investigated. The following summarizes some of the studies related to grape products and their potential oral health benefits.

### Raisins and oral health

Raisins are dried grapes, fruits of *Vitis vinifera* L. (Vitaceae) (24). Today, most raisins are produced from Thompson seedless grapes, which were introduced to California in 1862 by William Thompson (23). This variety is classified as a raisin-type grape

that produces a green, seedless fruit. While dominating raisin production, it is also widely used for fresh consumption and for making juice concentrate and wine as well (24,25). Several other raisin grape varieties are used for raisins production, including Muscat, Black Corinth, and Sultana. The US per capita annual consumption of raisins is ~3.26 kg (25). Three types of raisins are economically important in the US. Natural raisins are sun-dried and account for the majority of the raisins produced and consumed. Dipped raisins are dried artificially and have higher moisture content than natural raisins. Golden raisins are treated with sulfur dioxide to preserve the golden color (24).

As a popular snack food, raisins contain polyphenols, flavonoids, iron, minerals, potassium, calcium, and certain B vitamins that may benefit overall human health. Raisins are cholesterol and fat free, rich in antioxidants, and a good source of fiber (26). Raisins consist of ~60% sugars by weight and their sweetness is contributed by mainly glucose and fructose, while no sucrose is detected (27). As described previously, sucrose, the main dietary sugar, serves as a substrate for the synthesis of adherent glucans in human dental plaque, the etiologic agent of tooth decay and gum disease (28). The various phytochemicals reported in raisins include triterpenes (29), fatty acids (30,31), flavonoids (32), amino acids (33), hydroxycinnamic acids (32), and 5-hydroxy-2-furaldehyde (34). 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.

### Identification of antimicrobial compounds in raisins against oral pathogens

The antimicrobial compounds present in raisins capable of suppressing growth and/or virulence properties of oral pathogens have been fractionated and identified (23). Thompson seedless raisins were chosen in the study because the hexane-soluble fraction of the crude methanol extract demonstrated growth inhibitory activity against 2 oral pathogens, the cariogenic *S. mutans* and the periodontopathic *Porphyromonas gingivalis*. Through bioassay-guided fractionation of hexane- and ethyl acetate-soluble partitions of *V. vinifera*, antimicrobial compounds were isolated and identified. All of the compounds were previously reported from species in the family Vitaceae. The substances, oleanolic acid (1) (35), oleanolic aldehyde (2) (36), linoleic acid (3) (37), linolenic acid (4) (37), betulin (5) (38), betulinic acid (6) (39), 5-(hydroxymethyl)-2-furfural (7) (40), rutin (8) (41),  $\beta$ -sitosterol (42), and  $\beta$ -sitosterol glucoside (43), were identified by comparing their physical and spectroscopic data with those of published values. The results in this study were in general in agreement with expected chemotaxonomic pattern for a member of Vitaceae. After their purification, the triterpenoids (1-2 and 5-6), linoleic acid (3), linolenic acid (4), betulin (5), betulinic acid (6), 5-(hydroxymethyl)-2-furfural (7), rutin (8), and the derivatives (1a-f) were tested for antimicrobial activity against *S. mutans* and *P. gingivalis*. Compounds 1, 2, 7, 1d, 1e, and 1f were inhibitory to the growth of *P. gingivalis*, with minimum inhibitory concentration values ranging from 0.0035 to 0.488 mg/mL. Compounds 1, 2, 7, 8, 1a, 1e, and 1f were active against *S. mutans* (0.0078–0.0625 mg/mL). Among these, compounds 1, 2, 7, and 8 were either equally or more potent than their respective crude extract of origin. The hexane and ethyl acetate extracts were more potent than the chloroform, methanol, and 1-butanol extracts. The differential antimicrobial activity observed against *P. gingivalis* suggests that compounds 2, 7, 1d, 1e, and 1f may benefit periodontal health.

Earlier *in vitro* studies have shown that oleanolic acid (1) inhibited insoluble glucan synthesis of mutans streptococci in the oral cavity (44–46). Several pharmacological properties of oleanolic acid have been demonstrated: antiinflammatory, antitumor, hepatoprotective, cytotoxic, antidiabetogenic, antibacterial, and anti-HIV activities (45). It was observed that oleanolic acid inhibited the *in vitro* biofilm formation of *S. mutans* (data not shown). Studies to elucidate the mechanism of actions of the bioactive compounds from raisins are currently underway.

### Raisins and their effect on *in vivo* dental plaque acidogenicity in children

The development and progression of dental caries depends on both their frequency of consumption of cariogenic carbohydrates and on the amount of food particles that become trapped on the surfaces of teeth. Both of these serve as ready sources of fermentable carbohydrates that promote acid production by plaque bacteria. When plaque is not removed, the prolonged cariogenic challenge (pH below the threshold of 5.5) leads to enamel demineralization and tooth decay. However, the frequency of consumption of cariogenic carbohydrates plays a much larger role in caries progression than the amount of food particles trapped on the surfaces of teeth (47).

Raisins have been shown to possess a moderate to high cariogenic potential in laboratory rats (48). Dental plaque pH studies in humans categorized raisins as acidogenic (49–51). Some health professionals believe that sweet and sticky foods such as raisins are more cariogenic because they are difficult to clear off the tooth surfaces (52). Studies have shown that sticky foods are not necessarily all retentive and may be cleared relatively fast from the oral cavity. Oral clearance properties vary markedly among individuals and depend on factors such as salivary flow, metabolism by microorganisms, and degradation by plaque and salivary enzymes (53). Kashket et al. (54) found no correlation between stickiness and retention of foods on teeth. They also reported a poor correlation between the consumer ratings of stickiness of foods and their actual clearance rates from tooth surfaces. Among the foods evaluated, raisins were almost completely cleared from tooth surfaces 5 min after chewing and swallowing.

The sweetness of raisins makes them a popular additive to snack foods and cereals, among which raisin bran cereal is a good example. Studies have shown that bran flakes were acidogenic and contributed to high levels of total carbohydrate in saliva (55,56). Utreja et al. (57) investigated the effects of raisins and raisin bran cereal on *in vivo* plaque acidogenicity in young children. The underlying hypothesis was that raisins or raisin-containing cereals without added sugar were not more acidogenic than cereal without raisins in lowering the plaque pH of young children. When raisins were mixed with bran flakes without additional sugar, the combination is no more acidogenic than bran flakes alone.

Twenty healthy children between the ages of 7–11 y participated in this randomized, controlled, cross-over study. The Institutional Review Board, Office for the Protection of Research Subjects of the University of Illinois at Chicago approved this study. Informed consent and assent to participate were obtained for each participant prior to the start of the study. The author enrolled all participants and assigned to them identifier codes in accordance with the Institutional Review Board guidelines. The order in which participants consumed the test foods was determined by a computer-generated randomized sequence.

The foods used in this study were: 1) raisins; 2) commercial bran flakes; 3) a commercial raisin bran cereal (cRB); and 4) an experimental raisin bran cereal (eRB). All commercial products were purchased from the Jewel-Osco department store. A 10-g serving of cRB contained 7 g of bran flakes and 3 g of raisins from the prepackaged commercial cereal. eRB was prepared in the laboratory by mixing 7 g of commercial bran flakes and 3 g of raisins. No sugar was added. Sucrose and sorbitol (10% solution) were used as positive and negative controls, respectively. Sugar profiles of bran flakes, raisins, cRB, and eRB were determined by GC (Covance Laboratories) as described by Mason and Slover (58).

All children participated in testing once per week when they either consumed a test food (without milk) or rinsed with a sugar solution. All participants thus consumed 4 test foods and rinsed with 2 solutions during the study period. At each visit, they were asked to chew and ingest 10 g of a test food in 2 min or rinse with 10 mL of a solution for 1 min. The *in vivo* plaque pH of the participant was measured at baseline to determine the resting pH and at time intervals of 2, 5, 10, 15, 20, and 30 min after either eating 1 of the 4 test foods or rinsing with a control solution. *In vivo* plaque pH was measured with a touch microelectrode (NMPH3 Dental Beetrode, World Precision Instruments) and a glass reference electrode (DRIFEF-5, World Precision Instruments) (59). The measuring electrode was inserted in interproximal plaque just below the contact area in the upper left and right maxillary premolar regions. The individual mean of pH from the 2 sites was tabulated, following which the mean of the all the participants for each food group was calculated for each time point. Comparisons were performed between the various foods at individual time points using ANOVA and the Scheffé post hoc test (SPSS 15.0 for Windows) (57).

Results obtained from this study showed that the mean resting plaque pH of the 20 participants at 0 min ranged from 6.34 to 6.42. Consumption of bran flakes resulted in a steady drop in plaque pH, reaching a maximum between 5 and 10 min (pH 5.89 and 5.99), and remained at  $6.23 \pm 0.28$  at the end of 30 min, which lower than the  $6.46 \pm 0.31$  ( $P < 0.001$ ) observed 30 min after rinsing with sucrose.

When participants consumed raisins, the mean plaque pH dropped from  $6.40 \pm 0.30$  to  $6.01 \pm 0.32$  at 10 min, followed by a gradual recovery toward baseline pH at 30 min. Consuming raisins and bran flakes without added sugar (eRB) resulted in a plaque pH decline to  $6.19 \pm 0.28$  in 10 min, followed by a steady increase to  $6.35 \pm 0.29$  at 30 min. Therefore, consumption of raisins, or mixing raisins and bran flakes without sugar (eRB), did not reduce plaque pH below 6 during the 30-min testing period. Moreover, consumption of eRB promoted a lower plaque pH decrease beyond 10 min compared with bran flakes alone ( $P < 0.001$ ).

Although the carbohydrate profile of raisins revealed 68% sugars (6.84 g in 10 g), the highest among all test foods, they were less acidogenic than cRB, bran flakes, and 10% sucrose solution, i.e. the mean plaque pH did not fall below 6 after consumption by participants. We concluded that that raisins, albeit sweet, were less retentive on tooth surfaces and were rapidly cleared after chewing. This concurs with the findings of Kashket et al. (54) that raisins were poorly retained, similar to apples, bananas, and white bread and were rapidly cleared 5 min after chewing and swallowing. Raisins, when added to bran flakes without additional sugar, may enhance the clearance rate of the chewed bran particles from the tooth surfaces, thus rendering eRB less acidogenic than bran flakes alone.

Upon consuming a cRB, a decline in plaque pH began at 2 min and remained below 6 for the remainder of the 30-min testing period. The pH decline during this period was lower than in the sucrose, eRB, bran flakes, or raisin groups ( $P < 0.001$ ). Based on sugar profile analysis, the cRB was found to contain higher total sugar than the eRB. During preparations of the test food samples, it was observed that the raisins in the cRB were covered with visible sweet-tasting white granules whose identity was unknown. According to a manufacturer, coating of raisins is routinely carried out to prevent clumping and to improve the appearance of the product. The sweet granules on the surfaces of raisins may be a readily fermentable sugar source for acid production by plaque bacteria. In addition, the combination of starch from the bran flakes might also have added to the rapid drop in plaque pH over most of the testing period after consumption of cRB. Ribeiro et al. (60) have reported that starch in combination with sucrose, as in many processed foods today, can be highly acidogenic.

Based on data obtained from this study, the ranking of study test foods in promoting plaque acidogenicity was: cRB > bran flakes > raisins > eRB. Raisins represent a healthy alternative to the commonly consumed sugary snack foods. Further studies to evaluate the long-term effect of raisin consumption on plaque microflora and acidogenicity are warranted.

### Grape seed extract and dental health

Root caries is especially prevalent among the elderly population due to gingival recession and the exposure of susceptible root surface (61). Approximately 8% of the population are expected to acquire one or more new root caries lesions yearly in North America (62). During root caries development, the dentin mineral is dissolved by acid produced from oral bacterial biofilm and the demineralized dentin matrix is further degraded, allowing bacteria to infiltrate the intertubular area (63). Dentin is a complex mineralized tissue composed of ~70% mineral, 20% organic component, and 10% fluid (64). The organic matrix of dentin consists of ~90% fibrillar type I collagen, while the remaining 10% is composed of noncollagenous proteins such as phosphoproteins and proteoglycans (64,65). The preservation and stability of dentin collagen may be essential during the remineralization process, because it acts as a scaffold for mineral deposition. It has also been suggested that the presence of an organic matrix may reduce the progression of erosion in dentin (66,67). One of the important strategies regarding preventive therapies for root caries is to promote remineralization of demineralized dentin (68–71).

Proanthocyanidin (PA) is a naturally occurring plant metabolite widely available in fruits, vegetables, nuts, seeds, flowers, and bark (72). Commonly used as natural antioxidants and free-radical scavengers, PA has been proven to be safe in various clinical applications and as dietary supplements (27,73). Grape seed extract (GSE) is a rich source of PA, which has been reported to strengthen collagen-based tissues by increasing collagen cross-links (74). PA from cranberries inhibited the surface-adsorbed glucosyltransferases and acid production by *S. mutans* (75). Studies have also shown that PA increased collagen synthesis and accelerated the conversion of soluble collagen to insoluble collagen during development (76,77). PA-treated collagen matrices were demonstrated to be nontoxic and resisted enzyme digestion in vitro and in vivo (78). Xie et al. (79) performed a study to examine the effect of GSE on the remineralization and demineralization of the collagen-rich root tissue of human teeth.

To evaluate the effect of GSE on the remineralization of artificial root caries, an in vitro pH-cycling model was used (79). Teeth fragments obtained from the cervical portion of the root were stored in a demineralization solution for 96 h at 37°C to induce artificial root caries lesions. The fragments were then divided into 3 treatment groups: 6.5% GSE, 1000 mg/L fluoride (NaF), and an untreated control. The demineralized samples were pH-cycled through treatment solutions, acidic buffer, and neutral buffer for 8 d at 6 cycles/d. The samples were subsequently evaluated using a microhardness tester, polarized light microscopy, and confocal laser scanning microscopy. Data were analyzed using ANOVA and Fisher's tests ( $P < 0.05$ ).

Results obtained from this study showed 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 compared with the untreated 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 confocal laser scanning microscopy. 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 (79). 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. GSE may be a potential adjunct or alternative to fluoride in the treatment of root caries during minimally invasive therapy. Further investigation is warranted.

Other articles in this supplement include (80–86).

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