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Food components with anticaries activity

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Caries is the most common oral infectious disease in the world. Its development is influenced also by diet components that interfere with pathogen mutans group Streptococci (MGS) activity. A very active research to identify functional foods and their components that are generally recognised as safe has been ongoing, with the aim of developing alternative approaches, to the use of synthetic chlorhexidine, and at the reduction or prevention of caries. Until now convincing evidence exists only for green tea as a functional food for oral health, partly owing to its high content of catechins, especially epigallocatechin-gallate. A number of other foods showed potential anticaries activity. Some other foods able to act against MGS growth and/or their virulence factors in *in vitro* tests are: apple, red grape seeds, red wine (proanthocyanidins), nutmeg (macelignan), ajowan caraway (nafthalen-derivative), coffee (trigonelline, nicotinic and chlorogenic acids, melanoidins), barley coffee (melanoidins), chicory and mushroom (quinic acid). *In vivo* anticaries activity has been shown by cranberry (procyanidins), glycyrrhiza root (glycyrrhizol-A), myrtus ethanolic extract, garlic aqueous extract, cocoa extracts (procyanidins), and propolis (apigenin, *tt*-farnesol).

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Introduction

Among infectious chronic diseases whose development can be inhibited by the consumption of specific foods, oral diseases such as caries and gingivitis should be counted. Caries is the most common and diffuse oral infectious disease, widespread over the world in every segment of the population, but especially in young people including children. Caries spread is in decline in developed countries while it still represents an expanding problem in developing countries where the common nutritional deficiencies are worsened by the inability to chew normally [1]. The negative effects go beyond pain and those problems caused by a bad capability in biting because of an increasing evidence of close relation between oral diseases and serious chronic systemic pathologies such as cardiovascular diseases [2–4] and maybe pathological outcomes of pregnancy and infant oral health [5,6]. Today, the synthetic antiseptic chlorhexidine is generally accepted as the most efficient agent for the prevention or treatment of oral diseases. However, routine use of chlorhexidine is not advisable owing to local side effects [7,8].

So far, research has been very active to detect new natural, generally recognised as safe (GRAS) compounds for the successful development of alternative approaches with one goal: to reduce or prevent caries.

Caries development

Caries is an endogenous infection that causes tooth lesions by the action of bacteria able to produce, by glycosyltransferase (GTF) enzyme, insoluble bioadhesive polysaccharides, above all glucans, forming plaque that mediates the accumulation of mutans group Streptococci (MGS), allowing them to firmly adhere to the dental surface. These microbes produce organic acids (mainly lactic acid), that induce enamel demineralisation. This leads to an easier invasion of a tooth's deeper tissues by some secondary invaders that produce caries lesion.

The most common caries aetiological agents are considered *Streptococcus mutans* (*Sm*) and *Streptococcus sobrinus*; additional aetiological agents are *Lactobacillus* and *Actinomyces* [9]. Other factors, such as teeth susceptibilities to demineralisation, oral hygiene, diet habits (such as eating frequency) [10,11••], sugar intake, and the availability of some mineral elements, contribute to caries development. Fluoride, in particular, is important because it forms fluoro-apatite, a compound that resists acid attack better than hydroxyapatite (HA), and when the tooth is formed it promotes enamel re-mineralisation [12]. Fluoride also inhibits oral pathogen proliferation. Sucrose function is twofold because it is the substrate both for lactic acid and biofilm production.

Actually, caries prevention is based on the elimination of at least one of the causing factors. Fluoridated drinking water or fluoride administration have been very effective, as have the use of sweeteners different from sucrose, especially xylitol, a natural compound present in many fruits and vegetables [13,14].

Owing to the evidence that diet components have not only a negative influence, as in the case of sucrose, but

Table 1

Foods and food components studied for anticaries activity

Food	Extract	Compound/s	*In vitro* tests	*In vivo* studies	Reference/s
Ajowan caraway		Naphthalene derivative	MIC 156.25 μg/ml		[10]
Apple		Procyanidins	Virulence factors		[15]
Barley	Aqueous	LMM fraction, melanoidins	Virulence factors		[28,29]
Chicory	Aqueous	Quinic acid	Virulence factors		[unpublished]
Coffee	Aqueous	α-dicarbonyl compounds	MIC 11.8–34.3 μg/ml		[22]
		Trigonelline, Nicotinic and Chlorogenic acids, Melanoidins	Virulence factors		[20,21]
Mushroom	Aqueous	Quinic acid, Nucleosides	Virulence factors		[unpublished]
Nutmeg		Macelignan	MIC 3.9 μg/ml – Virulence factors		[18,19]
Red grape seeds	Hydro-propanone	Procyanidins?	MIC 0.5mg/ml		[16,17]
Wine	Dealcoholated	Organic acids Proanthocyanidins	MIC 97.8–220.2 μg/ml Virulence factors		[26,27]
Cocoa	Hydro-propanone	Procyanidins	MIC 4.0–1.0 mg/ml		[16]
	Hydro-propanone		Virulence factors	Rats	[45]
	Aqueous			Humans	[41,42]
Cranberry	Juice	HMM fraction	Virulence factors		[30]
	Hydro-organic	Proanthocyanidins	Virulence factors	Rats	[34•]
Garlic	Aqueous		MIC 0.5–32.0 mg/ml	Humans	[38,39••]
Glycyrrhiza root		Glycyrrhizol A		Humans	[35]
Green tea	Aqueous	Catechin (ECGg)	MIC 31.2 μg/ml – Virulence factors	Rats – Humans	[63••]
Myrtus	Ethanolic	Flavonoid?	MIC 106.6 μg/ml	Humans	[37]
Propolis		Apigenin, tt-farnesol	MIC 14.0–28.0 μg/ml	Rats	[53]
	Ethanolic	C16 and C18 fatty acids		Rats	[56]

also a positive one on oral health, as in the case of fluoride, xylitol, and fiber-rich foods, recent research has been directed towards foods that could be protective against oral pathologies and to food components that are able to inhibit oral Streptococci growth and/or their virulence traits.

Today a number of foods and food components are known to act potentially as anti-caries agents, (Table 1). Characterization of their effectiveness has been obtained through *in vitro* or *in vivo* tests, using both animal models and humans.

In vitro studies

Apple (*Malus pomila* cv. Fiji)

Inhibitory effects of apple polyphenols (APP) on the synthesis of water–insoluble glucans by GTF of MGS and on the adherence of the bacterial cells were found through *in vitro* studies [15]. APP showed no effect on MGS growth. The strongest GTF-inhibitors were apple condensed tannins (ACT), a mixture of proanthocyanidins (PACs). The 50% GFT inhibitory dose was 5 μg/ml for *Sm*. The ACT efficacy depended upon the degree of polymerization (DP), with the fraction higher than pentamers showing the strongest activity. Epigallocathechin-gallate (EGCg) showed weak inhibitory GTF activity, whereas tannic acid showed strong inhibitory activity. These findings should indicate that plant polyphenol-GTF-inhibitors have common structural feature shared with either PACs (condensed tannins) and/or gallate-ester compounds (hydrolysable tannins). Inhibiting compounds in oolong and black tea are classified as complex tannin compounds.

Red grape seeds (*Vitis vinifera*)

Among the minimum inhibitory concentrations (MICs) determined for the aqueous propanone extracts (P70) from edible plants, the lowest MICs were found for the extracts of red grape seeds (0.5 mg/ml), green tea and sloe berry skin (2 mg/ml) [16]. P70 from both red and green grapes seeds (0.5 and 4.0 mg/ml) were more active than P70 grape-skins (16 and 8 mg/ml) which were richer in oligomeric PACs [17].

Nutmeg (*Myristica fragrans*)

The extract of nutmeg, widely used as a spice, possesses strong inhibitory activity against *Sm* [18]. The anticariogenic compound was identified as macelignan ((8R, 8′S)-7-(3,4-methylenedioxyphenyl)-7′-(4-hydroxy-3-methoxyphenyl)-8,8′-dimethybutane) with a MIC of 3.9 μg/ml, which is much lower than that of thymol (500 μg/ml). Twenty μg/ml macelignan concentration completely inactivated *Sm* in 1 min. Macelignan is active also against lactobacilli at the 2–31.3 μg/ml MIC range. Macelignan in

particular conditions reduces biofilm formation by more than 50% [19].

The specific activity and fast-effectiveness of macelignan ascribes it as a potent natural anti-biofilm agent.

Ajowan caraway (*Trachyspermum ammi*)

It is an aromatic spice closely resembling thyme in flavour. A naphthalene derivative, (4aS, 5R, 8aS) 5,8a-di-1-propyl-octahydronaphthalen-1-(2H)-one, with MIC of 156.25 μg/ml was identified, possessing powerful anticariogenic power. Almost 50% reduction was observed in adherence (at 39.06 μg/ml), in biofilm formation (at 78–13 μg/ml), in glucan synthesis (at 19.53 μg/ml), and in hydrophobicity (at 9.76 μg/ml). This compound might bind to cell surface proteins reducing cell hydrophobicity. The maximum reduction in acid production was at 78.13 μg/ml. Confocal microscopy revealed distorted biofilm architecture [10].

Coffee (*Coffea arabica* and *robusta*)

Roasted coffee, but not green coffee, showed antibacterial activity against *Sm* owing to its α-dicarbonyl compounds content. Green and roasted coffee extracts interfere with *Sm* adsorption to HA. Both the roasted coffee dialyzable and non-dialyzable (melanoidin) fractions had anti-adhesive properties [20,21]. Low molecular mass (MM) components, that is, trigonelline, and nicotinic and chlorogenic acids, proved active as anti-adhesives [20,22,23]. Coffee also promoted anti-adhesive properties of dental surfaces [24]. A recent cross sectional data analysis indicated that people who drank more cups of coffee a day had lower number of teeth; however the authors note that coffee is usually consumed with the addition of sugar [25•].

Wine (from *Vitis vinifera*)

In addition to exerting antibacterial activity against MGS [26], wine was found to interfere strongly with *Sm* adhesion to and detachment from HA and to strongly inhibit biofilm formation. The main responsible substances for these activities were found to be the PAC fraction. It was also demonstrated that red wine could inhibit *ex vivo Sm* biofilm formation on the occlusal surface of extracted human teeth [27].

Barley coffee (*Hordeum vulgare*)

Barley coffee (BC), made from roasted barley, in different experimental conditions at sub-MICs promoted variable, but significant inhibition of MGS adherence to HA and inhibited *Sm* biofilm formation.

Both the dialyzable (MM < 1000 Da) fraction containing polyphenols, zinc, and fluoride, and the non-dialyzable fraction (MM > 1000 kDa) consisting of melanoidins, were devoid of antimicrobial activity, but displayed anti-adhesive and anti-biofilm properties [28,29].

Chicory (*Cichorium intybus* L. var. *Silvestre*) and Mushroom (*Lentinus edodes*)

Both the low and high MM fractions (cut-off 5000 Da) of both foods inhibited MGS adherence to, and detachment from HA, inhibited biofilm formation (although not chicory), and induced biofilm disruption. The more active low MM fractions contained organic acids, with those active in biofilm disruption being quinic acid. Adenosine from mushrooms was able to inhibit biofilm formation.

Both extracts were found to inhibit important virulence factors and *Sm* comDe gene expression [data unpublished].

In vivo studies

Cranberry (*Vaccinium macrocarpum*)

In a clinical study the non-dialyzable cranberry fraction (NDCf) used as mouthwash reduced significantly the total oral microflora, especially *Sm*. NDCf inhibited *S. sobrinus* adhesion to HA when studied with an *in vitro* model system [30].

Significant inhibitory effects of cranberry juice on MGS attachment and inhibition, even to 95% of biofilm formation by NDCf, were verified [31]. These findings were confirmed by Duarte *et al.* who also found cranberry polyphenols reducing *Sm* acidogenicity [32]. Once combined, specific flavonols and PACs inhibited *Sm* GTFs-activity, acid production, and tolerance [33]. Koo *et al.* found that cranberry PACs do not display antibacterial activity but PACs with DP of 4–12 were potent inhibitors of surface GTF and procyanidin-A2 reduced biofilm formation and *Sm* acidogenicity [34•]. *In vivo* studies on caries development, carried out in rats, showed that topical application of PACs (1.5 mg/ml, 60 s exposure, twice daily) resulted in less biofilm mass and polysaccharides, and reduced the incidence of smooth surface caries. These effects were attributed to epicatechin-A-type dimers and oligomers.

Glycyrrhiza uralensis

He *et al.* found that glycyrrhizol-A in the extract of glycyrrhiza roots possesses strong bactericidal activity against *Sm* [35]. The extract was used to develop a sugar-free lollipop that when applied twice a day for ten days promoted a marked reduction of cariogenic bacteria in human studies [36••].

Myrtus communis

Myrtus ethanolic extract is used in Mediterranean regions to prepare an alcoholic drink. Al-Anbori *et al.* found the leaf ethanolic extract to have a MIC value of 106.6 μg/ml against MGS in adults with dental caries. A single mouth rinse with ethanolic extract showed a marked reduction in salivary MGS. The effect was attributed to flavonoids that were identified in the extract by UV–Vis spectra analysis [37]. Ethanol could influence the activity.

Garlic (*Allium sativum*)

Groppo *et al.* determined garlic water extract antimicrobial activity *in vitro* against MGS and *in vivo* when used as a mouthwash in a five week study [38]. The MIC depends on the *Allium* clone. For the most active white clone, MIC ranged from 0.5 mg/ml for *S. sobrinus* to 32 for MGS isolated from volunteers. However, only weak *in vivo* activity was found from a 2.5% garlic solution in 30 volunteers (who showed halitosis and nausea). A subsequent study [39••] showed a 3% garlic extract solution able to induce an inhibition zone against *Sm*. A daily mouthwash with a solution of garlic extract–water–sorbitol–spearmint oil, after seven days, promoted a highly significant reduction in salivary *Sm* counts in 15 volunteers.

Cocoa (*Theobroma cacao*)

No significant increase in caries in individuals whose diet contained chocolate-milk products, was found [40,41]. Ooshima *et al.* [42,43] reported that the inclusion of cocoa bean husk extract, rich in polyphenols, in cocoa mass extract, significantly reduced caries incidence. Whole cocoa and water extract significantly suppressed the decline in plaque pH induced by dietary glucose in human volunteers [44]. The addition of a water soluble extract from cocoa extracted powder to a cariogenic model food significantly reduced caries scores and glucan synthesis in rats infected with *S. sobrinus* 6715. Its high MM fraction (>10 kDa) containing sugar, protein, and polyphenols effectively inhibited GTFs-activity [45]. Percival *et al.* found that pre-treatment with cocoa pentamer reduced biofilm formation and acid production at pH 7 in a caries model [46].

Smullen *et al.* reported that a 70% aqueous-propanone extract of unfermented cocoa, or fermented cocoa, yielded MIC values of 4 mg/ml and 8 mg/ml, respectively [16]. Fractionated extract activity increased with the epicatechin DP. The procyanidin polymer had a MIC of 1 mg/ml and MBC of 64 mg/ml. The unfermented cocoa extract was bacteriostatic, prevented *Sm* acid production when added at MIC, and reduced adherence to glass.

Cocoa bean husk extract was found highly effective in reducing MGS counts and plaque deposition when used as a mouth rinse by children [47].

Propolis

Propolis is a resinous substance produced by *Apis mellifera* (honey bees) when they mix their own secretions with resin collected from different parts of different plants. This leads to a strong variability in propolis chemical composition and bioactivity [48]. Many studies reported potential application of propolis against caries [49–52].

Koo *et al.* in *in vitro* studies found reductions in *Sm* counts and interference with their adhesion capacity and GTF-activity; *in vivo* studies demonstrated reductions in *Sm* counts in saliva, plaque index, and insoluble polysaccharide formation, mostly attributed to flavonoids [53]. Apart from the flavanoid apigenin, also sesquiterpene *tt*-farnesol was found strongly active. *tt*-Farnesol presented a higher anti-*Sm* effect (MIC of 14–28 μg/ml and MBC of 56–112 μg/ml). Both compounds affected biofilm synthesis. Their combination with sodium fluoride, significantly reduced glucans in biofilm. *In vivo* topical application of apigenin-*tt*-farnesol reduced the incidence of caries in rats. The combination of 1 mM apigenin with 5 mM *tt*-farnesol and 250 ppm fluoride was as effective in preventing caries as chlorhexidine plus fluoride alone [54]. Recently, antimicrobial activity on MGS has reported for *Melipona fasciculata* bee propolis. Decreased MGS counts of 62–81% were observed in saliva samples after 1–7 days of treatment, respectively [55].

A flavonoid-free brasilian propolis hexane extract that lacked killing activity against *Sm* nonetheless reduced caries development in rats under cariogenic challenge [56]. As the extract contained fatty acids (oleic, palmitic, linoleic and stearic acids), these were indicated as the putative active compounds that inhibited *Sm* acid production and F-ATPase and GTF-activity.

Tea (*Camellia sinensis*)

In Japan, it is believed that tea drinkers have a healthy mouth. Probably also owing to this belief, tea is one of the first and best scientifically studied beverages for its effects on oral health.

Since the end of the 1980s, Sakanaka *et al.* found green tea extracts are active against *Sm* and Kawamura *et al.* attributed such activity to tea catechin (TC) [57]. Epigallocatechin-gallate (EGCg) and epigallocatechin (EGC) were found to strongly inhibit GTF-activity and to reduce caries scores in pathogen free rats infected with *Sm*, when fed with cariogenic diet/drinking water containing tea polyphenols (TPs).

These studies were followed by many other investigations carried out *in vitro* and *in vivo* in animals and humans, about the effects of the different types of tea, that is, dried, unfermented green leaves, partially fermented oolong tea, and fully fermented black tea. Fermentation reduces green tea monomeric polyphenols and produces their higher MM polymerization products. So, black tea contains less monomeric polyphenols than green tea, in addition to containing theaflavins, thearubigins, and having a fluoride content five times higher. Green-TP mainly consists of catechin and PACs. Most studies reported the main anticaries compounds in green tea as EGC and especially EGCg, with oxidized complex polyphenols (theaflavins and thearubigins) as the main anticariogenic compounds in black tea [58,59].

Since 2002, a large body of papers attributed anticariogenic green tea properties to catechins owing to their ability to inhibit bacterial growth, bacterial viability, GTF-activity and salivary α-amylase activities, and black tea activity to its polymeric polyphenols by their action in inhibiting dental plaque, acidity, and cariogenic microflora [60]. The authors concluded that teas ''can be considered a functional food for oral health by controlling, through prevention, the most prevalent infectious disease of mankind: caries''. However, owing to discrepancies among the different studies, research continued. Taylor *et al.* [61] confirmed tea anti-microbial properties and Hirasawa *et al.* [62] found EGCg able to reduce MGS and acid production in dental plaque bacteria inhibiting lactate dehydrogenase-activity.

Most recently, Xu *et al.* found that EGCg provided efficacy against *Sm* UA159 (31.25 μg/ml MIC and 62.50 μg/ml MBC); however, the authors found that the antimicrobial activity depended on the culture medium composition, because medium proteins may bind to or even precipitate TC, compromising their efficacy [63••]. This can explain the discrepancies among previous studies. Furthermore, many authors found that bacteria living in dental plaque are generally more resistant than culture bacteria normally used for the *in vitro* studies [38,64]. EGCg was demonstrated to inhibit biofilm formation by 90% at 15.6 μg/ml and to reduce preformed biofilm viability at 625 μg/ml. At sub-MIC levels, EGCg worked to also inhibit acidogenicity and acidurity attributable to the suppression of F1-F2-ATPase, agmatine deiminase, enolase, and lactate-dehydrogenase of *Sm* UA159 at the transcriptional and enzymatic levels.

The results of a cross-sectional data analysis showed green tea consumption to be significantly associated with decreased odds for tooth decay when at least a cup of tea/day was consumed [25]. The association was not found for oolong tea. As TC shows antibacterial activity at the concentration of 1 mg/ml and a typical green tea preparation contains 50–150 mg/100 ml (oolong tea presents 13 mg/100 ml catechin concentration), the authors report that the association seems to fit the threshold model so that people consuming at least a cup of green tea/day might receive some benefit.

In a pilot intervention study, significant differences were found in the *Sm* counts in saliva and plaque, in salivary and plaque pH values, and Gengival Bleeding Index (values determined pre and post-rinsing with 2% green tea for 5 min) [11••]. Meaningful reductions in MGS and lactobacilli colony counts were found also by another *in vivo* study [65].

Conclusions

This literature survey shows that the research about food functionality to oral health is very active. Currently, their exists convincing evidence only for tea, especially green tea, to be a functional food for oral health. This is because for the other food/beverages, clinical and epidemiological investigations are still lacking. Most studies are performed *in vitro* or *in vivo* in animals and the results so obtained are difficult to transpose to humans, especially because 1) the human oral environment could influence antibacterial activity and the effects of the active compounds on the virulence factors of oral pathogens; 2) most investigations are carried out on food extracts where the active compounds are in solution allowing them an easier contact with bacteria. Such situation could be comparable with beverages but not with solid foods; and 3) in most cases food/beverage consumption probably allows insufficient contact time between the oral tissues and the active components. Other than tea catechin, a number of other food components with potential or actual anticaries activity have been pointed out. Most of them belong to the flavonoids chemical class and in a number of food/beverages, procyanidins are indicated as the main active compounds. The possibility of using the isolated compounds in bioadhesive time-specific and site-specific controlled release systems to enrich other foods or to prepare chewing-gum or sugar-free candies could offer some other functional foods with high anticaries efficacy, overcoming the increasing problem of antibiotic resistance without altering oral microflora.

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