Eur J Nutr (2012) 51 (Suppl 2):S15–S25
DOI 10.1007/s00394-012-0323-7

SUPPLEMENT

Functional foods/ingredients and dental caries

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Introduction

In dentistry, there is a well-established practice of fluoridating water, salt and milk for the prevention of dental caries. The use of other foods to promote oral health is another step in the same direction, and the development of research into adding therapeutic benefit to food is welcome. As the mouth is at the beginning of the gastrointestinal tract, the potential to capture oral health benefits from the emerging developments in functional foods is considerable. This chapter will consider whether the prevention of dental caries could benefit substantially from the development of functional foods. 'Functional Foods' are foods or foods with components that may provide a health benefit beyond basic nutrition. Examples can include fruits and vegetables, whole grains, fortified or enhanced foods and beverages, and some dietary supplements. Functional attributes of many traditional foods are being discovered, while new food products are being developed with beneficial components. This chapter will present an overview of the possible role of functional foods in caries prevention.

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Caries process

Tooth, plaque and substrate (diet) are the three prerequisites for a caries lesion to develop as first postulated by Keyes [37] (see the inner three circles in Fig. 1). Teeth may vary in susceptibility to caries according to composition, morphology, location and position, which may promote plaque retention. The greater the tooth's susceptibility to disease, the greater the chance that caries will occur, although it will not develop without the presence of both bacteria and substrate. The bacteria circle indicates the need for the presence of acidogenic species for caries to develop. Caries will not develop in the absence of bacteria. *Streptococcus mutans*, a Gram-positive acidophilic bacterium, is usually associated with the initiation of caries. In addition to producing lactic acid when it ferments dietary saccharides, *S. mutans* produces by glycosyl tranferase (GTF) activity extracellular glucans, which adhere to enamel, allowing the bacterium to colonise the smooth enamel surface. In addition to the extracellular sticky glucans, *S. mutans* produces also intracellular polysaccharides that can be splitted to acidic end products when sugar food is not present in the oral cavity. Other acid-producing microorganisms such as *Lactobacillus species* contribute to the caries process once the initial demineralisation has taken place and a niche for non-adhesive bacteria is created.

The key to the third circle is that microorganisms need substrate to produce acid. Monosaccharides, disaccharides and fermentable carbohydrates can be used for bacterial plaque metabolism. The fourth circle encompassing the other three signifies the importance of time, as caries takes time to develop and the importance of other modifying factors like quantity and quality of saliva, variation in the availability of the substrate and protective factors like the

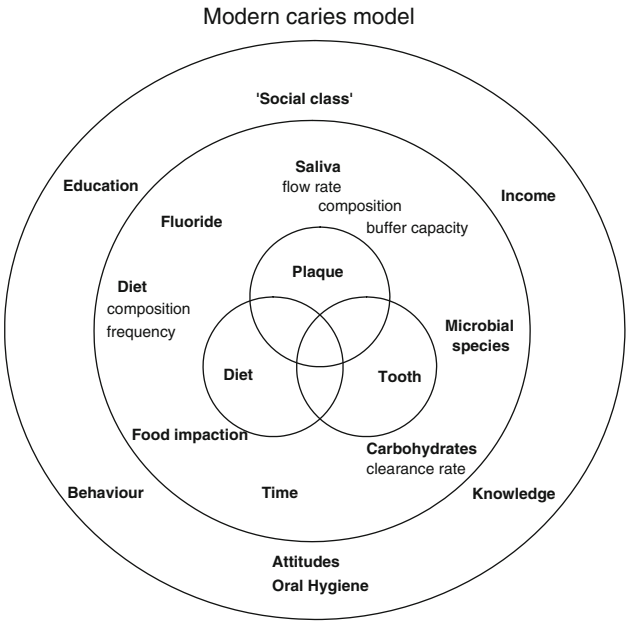

Fig. 1 Modern caries model. In 1960, Keyes designed the central triad in this *figure*. Plaque, tooth and diet were the three prerequisites for a caries lesion to develop. Subsequent researchers enlarged the model to include factors affecting the interplay between the three prerequisites. In the first ring, modifying factors that play a role in the oral cavity are shown. The *second*, *outer*, ring shows behavioural aspects that are associated with caries risk (modified from [22])

use of fluorides. The length of time the bacteria have access to substrate at the plaque enamel interface plays a major role in the progress of caries.

The tooth minerals, enamel, dentine and root cement consist of an inorganic component (approximately 86, 55 and 45 vol%, respectively), an organic component (approximately 4, 25 and 30 vol%, respectively) and water. The inorganic component is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. The oral and dental plaque fluids contain calcium and phosphate ions, and it depends on the pH whether the environment of the tooth is saturated, under- or supersaturated with respect to the mineral. When the environment is undersaturated, demineralisation will occur, and when the environment is supersaturated, remineralisation will take place. The critical pH for tooth mineral to dissolve varies between pH 5.0–5.7. The pH in dental plaque, physiologically around pH 7, will drop after the intake of fermentable carbohydrates to around or just below the critical value. The pH drop will be counteracted by the clearing and buffering effect of saliva. Furthermore, saliva will bring minerals to the dental plaque. Because caries develops not too far below the point of saturation, it can be assumed that relatively small changes in either factor involved in the caries process may shift the circumstances from being demineralising to remineralising or the other way around. So it may be assumed that the addition of minerals increasing the degree of saturation is beneficial, it may be assumed that factors reducing the acid formation in dental plaque (e.g. antimicrobial agents) are beneficial, and it may be assumed that gustatory, mechanical and psychological stimulation of salivary flow can be beneficial. All these incentives could be incorporated in a food making a functional food (Table 1). It should thereby be realised that any induced change in the oral conditions will be counteracted by the oral homoeostasis.

Methodology to assess the effect of functional food/active ingredients in dental caries control

Food claims towards reducing cariogenicity can be tested in vitro, in animal experiments, in situ and in vivo. In vitro studies mainly comprise the effect of food ingredients on growth, acid production and adherence of oral bacteria or mixed cultures of oral bacteria either in suspension or in biofilm. It is however difficult to validly recreate intra-oral conditions with in vitro systems, and therefore, the predictive value for cariogenicity is low. In animal experiments (usually with the laboratory rat), the animals can be orally infected with oral human bacteria and the feeding can be programmed to simulate diet patterns. In order to score caries, the animals have to be killed. In many countries, there is a move away from the use of animals in research for regulatory purposes and consumer attitudes.

Relatively simple human studies are plaque pH measurements. There are three methods: the plaque sampling (post in vivo), the touch electrode and the interproximal telemetry method. Each of the three types of the methods can satisfactorily identify non-acidogenic foods when used properly with appropriate positive (10% sucrose) and negative (10% sorbitol) controls [45, 73]. It is important to realise that plaque pH methods can indicate acidogenic potential and only possibly the cariogenic potential of a product. If the plaque pH profile of a product is not

Table 1 Key targets of functional food in cariogenic attack and components of functional food

Cariogenic bacteria (as such)	Probiotics, polyphenols and plant extracts
Bacterial adherence, co-aggregation, glycosyltransferase (GTF) activity	Sugar polyols, bioactive peptides, polyphenols
Bacterial fermentation of carbohydrates	Artificial sweeteners, sugar polyols
Saliva secretion, composition and buffering capacity	Chewing gum as such sugar polyols
Availability of calcium and fluorides	Dairy products, other mineral sources

statistically significantly different from that of the 10% sorbitol rinse, then the food can effectively be considered as non-cariogenic. When the plaque pH profile falls below that of sorbitol but not to pH 5.7, an in situ cariogenicity test is required to further study the foodstuff. As the critical pH is higher for dentine than for enamel, the level of safety 'not below pH 5.7' may not be safe for exposed roots comprising dentine and cementum. The 'critical pH' of dentine and cementum has yet to be preconcerted.

In situ caries models involve the use of appliances or other devices that carries enamel or dentine samples and are worn intra-orally by participants [82]. The strength of these models is that all the multifactorial aspects of natural dental caries are generally included. Foods and beverages can be tested under clinically relevant conditions, and very sensitive laboratory methods can be applied to measure changes in mineral status of the tooth substrate.

Finally foods can be tested in clinical experiments including randomised controlled trials. Clinical studies with appropriate controls allow assessing local caries risk factors and clinical assessment of caries increment. Prospective clinical studies should continue at least 36 months in subjects of school age and at least 72 months in adult subjects.

Although there may be a substantial amount of research on a product and its health benefits, it may be difficult to compare in vitro and in vivo data across laboratories due to lack of standardised experimental procedures. Improvement in these aspects and the design of controlled clinical and multidisciplinary research studies is warranted.

Micronutrients and trace elements

During tooth formation, impurities may be incorporated in the tooth mineral making the mineral either more [e.g. incorporation of $(CO_3)^{2-}$ and $(HPO_4)^{2-}$] or less (e.g. incorporation of F^-) soluble. The impurities that promote enamel solubility will be washed out during de- and remineralisation cycles, while impurities that reduce enamel solubility continue to be incorporated further increasing the resistance against the caries process. A comparison of the (trace) element concentration in caries-affected and healthy teeth suggests that caries associates lower concentrations of fluoride, zinc, iron, copper, nickel, selenium and strontium and higher concentrations of chromium, cobalt, lead and cadmium [22]. The caries reducing effect of fluoride is indisputable. Zinc compounds are added to oral hygiene products to prevent the mineralisation of calculus. There is, however, no indication that this application of zinc would interfere with remineralisation or would adversely affect the anticaries effects of fluoride [30]. The association of copper (Cu^{2+}) with lower caries prevalence has been reported by various authors [18, 19]. Also, the ability of Cu^{2+} to inhibit cariogenesis in animals has been documented [47, 71, 72]. Cu^{2+} co-crystallised with sugar during manufacturing reduces the cariogenic potential of sugar [71, 72]. The mechanism involved has been attributed to a combination of its antimicrobial effects and to its ability to inhibit demineralisation directly [8]. In situ evidence showed a beneficial effect on dental enamel in response to a cariogenic challenge of a combination of Cu^{2+} and amine fluoride [1]. For the other trace elements, there is no strong evidence to reduce cariogenicity when incorporated in food or oral hygiene products.

Natural anticaries properties of (non-milk) food constituents and plant extracts

Some fruits and plants of human nutrition (e.g. tea, cocoa, coffee and raisins) contain polyphenols as phenolic acids or flavonoids that may suppress by antioxidant activity oral pathogens associated with caries and are thus hypothesised to benefit oral health (Table 2). Further substances identified with antimicrobial activity against oral pathogens are oleanolic acid, oleanolic aldehyde, linoleic acid, linolenic acid, betulin, betulinic acid, 5-(hydroxymethyl)-2-furfural, rutin, beta-sitosterol and beta-sitosterol glucoside.

Tea

Tea is an aqueous infusion of dried leaves of the plant *Camellia sinensis* L. (family Theaceae). The chemical composition of tea is complex: polyphenols, catechins, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compounds, fluoride, minerals and other undefined compounds [23]. Black tea has many more components than green tea, including bisflavanols, theaflavins, theaflagallins, epiflavic acids, and thearubigins. The most interesting components of tea leafs, in relation to oral health, are epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, gallocatechin and (+)-catechins [23, 53]. These oxidised polyphenols are often collectively called tannins, which are chemically very different from the commercial tannic acid or the plant tannins [92]. It is estimated that a cup of green tea (2.5 g of green tea leaves/200 mL of water) may contain 90 mg of epigallocatechin gallate.

Specific pathogen-free rats infected with *S. mutans* and fed a cariogenic diet containing green tea polyphenols had significantly lower caries scores than control animals [63]. Drinking tea (without added sugar) has been associated with lower caries levels in humans [60, 61]. Although tea is also a source of fluoride, studies have demonstrated that tea

Table 2 Natural anticaries properties of (non-milk) foods, food constituents and plant extracts

Foods and plant extracts	Anticaries activity		
	In vitro	Animal studies	In vivo
Tea	Antimicrobial	Reduced caries development	Antiplaque
	Anti-adhesion		Reduced caries development in epidemiological studies and clinical trials
	Inhibition of acid production		
Cranberries	Antimicrobial		Reduction in *S. mutans* counts in saliva
	Anti-adhesion		
	Antibiofilm		
	Inhibition of glycosyltransferase		
Cocoa	Antimicrobial	Cariostatic in hamsters	Reduction in *S. mutans* counts in plaque and saliva
	Antibiofilm		
	Inhibition of glycosyltransferase		
Edible fungi	Antimicrobial	Reduces cariogenicity	
	Antibiofilm		
	Inhibition of glycosyltransferase		
Roasted coffee	Anti-adhesion		
Variety of vegetables and fruits including apple and raisins	Anti-adhesion		
	Antibiofilm		
	Inhibition of glycosyltransferase		
Wine and grapes	Antimicrobial		
	Anti-adhesion		
	Antibiofilm inhibition of glycosyltransferase		
Propolis	Antimicrobial	Reduces cariogenicity	
	Anti-adhesion		
	Antibiofilm		

Adapted from Signoretto and Canepari [85]

polyphenols rather than fluoride contributed to the anticariogenic potential [35, 96]. Several mechanisms have been proposed for the observed anticariogenic properties of teas. These include tea's inhibitory effect on bacterial growth, bacterial viability, glycosyltransferase activity, adherence and salivary amylase activities [34, 50, 53, 94].

Cranberry

Cranberry components are potential anticaries agents since they inhibit acid production, attachment and biofilm formation by *S. mutans*. Glucan-binding proteins, extracellular enzymes, carbohydrate production and bacterial hydrophobicity are all affected by cranberry components.

It is often suggested that cranberry components, especially those with high molecular weight, could serve as bioactive molecules for the prevention and/or treatment of oral diseases [5]. After 42 days of using a mouthwash enriched with a high molecular weight polyphenols cranberry fraction, total bacterial and *S. mutans* counts in saliva were reduced [90].

Cocoa

Cocoa polyphenol pentamers significantly reduce biofilm formation and acid production by *S. mutans* and *Streptococcus sanguinis* [77]. Extracts from cocoa mass have been shown to have anticariogenic potential but not strong enough to suppress the cariogenicity of sucrose [62]. Cacao bean husk extract (CBH) has been shown to possess antibacterial and antiglycosyltransferase activities. The number of mutans streptococci in dental plaque was significantly reduced when human dental plaque was exposed to CBH extract for 1 h. A mouth rinse with CBH extract in ethanol, before and after each intake of food and before sleeping at night for 4 days without using other oral hygiene procedures, reduced plaque depositions and the numbers of mutans streptococci, compared with rinsing with 1% ethanol alone [50].

Coffee

Trigonelline, caffeine and chlorogenic acid occurring in green and roasted coffee interfere with *S. mutans* adsorption to saliva-coated hydroxyapatite beads. The anti-acidogenic effects against alpha-haemolytic streptococci showed by polyphenols from coffee suggest further studies to a possible application of this beverage in the prevention of pathogenesis of dental caries [21].

Apple

The inhibitory effects of apple polyphenols on the synthesis of water-insoluble glucans by glycosyltransferases (GTF) of the mutans streptococci and on the sucrose-dependent adherence of the bacterial cells were examined in vitro. Apple-derived polyphenols markedly inhibited the activity of GTF purified from the cariogenic bacterial cells; however, they showed no significant effect on the growth of the cariogenic bacteria [95].

Raisins

When the effect of raisins and raisin-containing bran cereal on in vivo plaque acidogenicity was examined in 7–11-year-old children, it was found that raisins did not lower the plaque pH below pH 6 over the 30-min test period [41]. Compared with commercial bran flakes or raisin bran cereal, a smaller plaque pH drop was noted in children who consumed a raisin and bran flake mixture when no sugar was added [93].

Grapes and wine

When the minimally inhibitory concentration of plant polyphenols was tested in *S. mutans* cultures and in the cultures of other microorganisms, the lowest MICs were found for the extracts of red grape skin 0.5 mg/mL. Grape seed extract, high in proanthocyanidins, positively affected the in vitro demineralisation and remineralisation processes of artificial root caries lesions [93]. The compounds responsible for the antimicrobial activity of wine, however, have been shown not to be the polyphenols but the various organic acids in wine.

Propolis

Propolis is a resinous mixture that honeybees collect from tree buds, sap flows or other botanical sources and contains numerous flavonoids. It is used as a sealant for unwanted open spaces in the hive. Propolis has been shown to exhibit good antimicrobial activity against a range of oral bacteria and inhibit the adherence of *S. mutans* and *S. sobrinus* to glass [39]. It was also shown to be a potent inhibitor of water-soluble glucan synthesis (GTF-activity inhibitor). The activity of propolis against a number of microorganisms, including *S. mutans* and *Streptococcus sobrinus*, was demonstrated [85]. Ethanol extracts of four samples of propolis collected from different geographical regions in Anatolia exhibited MIC values of 2–64 μg/mL. Propolis showed antimicrobial activity similar to chlorhexidine and greater than clove or sage extracts in a study investigating the ability of these chemicals to inhibit the growth of microbes obtained from the saliva of periodontally healthy subjects and those with chronic periodontitis [20].

Nidus vespae, the honeycomb of *Polistes olivaceous* (De Geer), *P. japonicus de Saussure* and *P. Parapolybiavaria fabricius*, is a traditional Chinese medicine that has a number of pharmacological properties. While *N. vespae* is similar to propolis, it contains additional materiál including waxes and aromatic oils. Like propolis, extracts and fractions of *N. vespae* have been shown to exert antimicrobial activity towards a number of oral microorganisms, in particular *S. mutans* [94]. In addition, the extracts showed significant anti-acidogenic activity.

Food preservatives

There has been a large increase in the use of food preservatives over the last few decades. For instance, in the last decades of the previous century, the use of preservatives in the USA increased over 20-fold and it is estimated that the average ingestion of benzoate in the USA is currently 2.3 mg/kg bw per person per day. Manufactured foods and soft drinks are the primary sources of preservatives. Many toothpastes and mouthwashes also contain benzoate.

Sodium benzoate, potassium sorbate and sodium nitrite are commonly used food preservatives. Food preservatives behave like weak acids and can dissipate the ΔpH across the bacterial cell membrane causing acidification of the cytoplasm. In an in vitro study, food preservatives inhibited *S. mutans* biofilm formation [3]. In another study, the maximum permitted use levels of sorbic and benzoic acid was shown to inhibit the growth of oral streptococci but not the in vitro glycolysis at tested concentrations [42]. The combination of benzoate and fluoride reduced caries activity more effectively in rodents fed a cariogenic diet ad libitum than fluoride alone [17]. In a double-blind, controlled crossover study, Arweiler et al. [4] evaluated the influence of food preservatives (0.1% benzoate, BA, and 0.1% sorbate, SA) on in situ dental biofilm growth in comparison with 0.2% chlorhexidine (CHX) and saline. After 5 days, the developed biofilms were scanned after staining by confocal laser scanning microscopy for biofilm thickness and bacterial vitality. The use of SA, BA and

CHX resulted in a significantly reduced biofilm thickness and bacterial vitality compared to saline ($p < 0.001$). Differences between SA and BA were not statistically significant, while CHX showed significantly lower values. Thus, both preservatives showed antibacterial and plaque-inhibiting properties, but not to the extent of 0.2% CHX.

Alternative sweeteners

Sugar-free sweets, confectionery, chewing gum and drinks are formulated with sugar alcohols, intense sweeteners, non-cariogenic disaccharides and non-cariogenic bulking agents. The relevant alternative sweeteners, sugar replacers and bulking agents currently used are described in Table 3.

The most widely used sugar alcohols are xylitol (pentitol), sorbitol, mannitol (both hexitols), maltitol, lactitol (both glucosyl-hexitols) and hydrogenated starch hydrolysates (6–8% sorbitol, 50–55% maltitol (1,4-glucosyl-sorbitol), 20–25% maltotriitol (di-glucosyl-sorbitol) and 10–20% poly glucosyl alcohols) and isomalt (1:1 mixture of 1,6-glucosyl-sorbitol and 1,1-glucosyl-mannitol, two glucosyl-polyols). Other sugar alcohols such as erythritol are emerging with promising results [7]. Erythritol may reduce the numbers of mutans streptococci and the amount of dental plaque to the same extent as xylitol [36, 46]. The relative sweetness of the sugar alcohols compared to sucrose varies from 0.5 to 1. All sugar alcohols have been tested in vitro for fermentation by oral microorganisms and can be classified as hypo- or non-acidogenic. There is a reduced or virtually no extracellular polysaccharide production from sugar alcohols. Hypo- and non-acidogenicity of the sugar alcohols is confirmed by plaque pH measurements. From animal experiments and intra-oral cariogenicity tests (ICT), it is concluded that sugar alcohols are (extremely) low or non-cariogenic. In vitro, adaptation of mutans streptococci by frequent subculturing in sorbitol, maltitol, lactitol and hydrogenated starch hydrolysates occur, but this is not likely to be important in vivo when the sugar alcohols are given in combination with a diet rich in sucrose. In all these experiments, xylitol stands out. With rare exceptions, xylitol is not fermented by oral microorganisms. Xylitol inhibits the growth of mutans streptococci [86] even selectively in mixed chemostat cultures [6]. It interferes with the glycolysis when glucose is used as energy source [89] although this may not be a stable phenomenon in vivo [74]. In vivo, there was also no reduction of the acidogenic response of dental plaque to sucrose after periods of using xylitol chewing gums [91] or xylitol mouth rinses [44]. It has been proposed that xylitol weakens the caries-inductive properties of dental plaque colonising newly erupting tooth surfaces [31] and that such a caries protective effect might persist several years after the cessation of use of xylitol products [28]. From animal experiments, it has been concluded that xylitol is anticariogenic [26]. Recently, it has been demonstrated that children of mothers who frequently use xylitol-sweetened chewing gum were less colonised with mutans streptococci. For all this, it is widely believed that xylitol is superior to the other sugar alcohols for potential caries control (for reviews: [81, 83]), although the clinical evidence for this superiority has been challenged and classified as weak [29, 74, 84, 88]. For all sugar alcohols, there is a limited use due to laxative effects in particular in beverages.

Meanwhile, novel disaccharides of very low acidogenicity and with good gastrointestinal tolerance for manufacturing tooth-friendly sweets have been developed and are on the market (isomaltulose and leucrose).

Intense sweeteners are not substrates for oral microorganisms and, as such, can be classified as non-cariogenic. As a result of their intense sweetness, only very small amounts are needed. Therefore, these sweeteners are mainly used to sweeten beverages such as soft drinks,

Table 3 Commonly used sugar substitutes (sugar alcohols, bulking agent, novel disaccharides and intense sweeteners) for the manufacture of tooth-friendly confectionery and for use in oral care products

Category	Structure	Examples
Sugar alcohols (hydrogenated carbohydrates)	Hydrogenated monosaccharides	Sorbitol, mannitol, xylitol, erythritol
	Hydrogenated disaccharides	Isomalt, lactitol, maltitol
	Hydrogenated oligosaccharides	Maltitol syrups, hydrogenated starch hydrolysates
Bulking agent	Polymer	Polydextrose
Novel disaccharides	Disaccharide (isomers to sucrose)	Leucrose, isomaltulose (Palatinose™)
Non-caloric, intense sweeteners	Acesulfame K	
	Aspartame	
	Cyclamate	
	Saccharin	
	Sucralose	

All substances are non-cariogenic, and some of the intense sweeteners can interfere with microbial fermentation

coffee and tea. Although the light or diet soft drinks may not cause caries, they may contain high amounts of acids that could cause tooth erosion.

Bioactive peptides in dairy products

Dairy products were recognised in the late 1950s as a food group that is effective in preventing dental caries. Shaw et al. [76] observed that milk, ice cream and cheese lowered incidence of dental caries in rats. Desalivated rats given 2% milk or lactose-reduced milk remained essentially caries-free [6]. Epidemiological studies in recent years indicate that children [65, 66] and adolescents [67] with low incidence of dental caries drank more milk than those with high caries incidence. Elderly people who eat cheese several times per week had a lower incidence of root surface caries development [64]. Several reviews describe the role of milk and dairy products in dental caries prevention [33, 35, 54].

The dairy components that have anticariogenic properties are calcium, phosphate, casein and lipids. Casein added to food (e.g. chocolate) reduced cariogenicity, but casein's adverse organoleptic properties and the large amount required for efficacy precluded its use in a food. Digestion of casein did not destroy the proteins' ability to prevent enamel demineralisation in a human oral caries model. Two casein digestives, caseinophosphopeptides (CPP) and glycomacropeptide (GMP), have been patented for use in common personal hygiene products to prevent dental caries. Research has shown CPP and GMP to be growth inhibitory to the cariogenic bacteria *S. mutans* and other species [32, 56, 57]. Additionally, CPP forms nanoclusters with amorphous calcium phosphate at the tooth surface to provide a reservoir of calcium and phosphate ions to maintain a state of super saturation with respect to tooth enamel.

Caseinophosphopeptides (CPP) can be produced forming colloidal complexes with calcium and phosphate in solution (CPP–ACP). The use of CPP–ACP would increase the level of amorphous calcium phosphate in plaque increasing the degree of saturation. Specific pathogen-free rats orally infected with *S. sobrinus* had a reduced incidence of smooth surface caries after CPP–ACP solutions were applied to the animal's teeth twice daily. The in situ caries model has shown the ability of CPP–ACP to prevent enamel demineralisation and promote remineralisation. Clinical experiments have been conducted with CCP–ACP in chewing gum (for overview see: [70]).

Schupbach et al. [75] demonstrated that GMP could prevent cariogenic bacterial adhesion in an in vitro model. The researchers speculate that GMP reduces dental caries by changing the microbial population of dental plaque from being predominated by *S. mutans* and *S. sangius* to a less cariogenic population predominated by *Actinomyces viscous*. In vitro experiments, Reynolds et al. [69] showed GMP to have an inhibitory activity to enamel demineralisation.

Other bioactive components of milk that might play a role in prevention of dental caries include lactoferrin, lysozyme, lactoperoxidase, folate-binding protein, immunoglobulin proteins, growth factors and others (Table 4). For example, lactoferrin inhibits adherence of *S. mutans* to saliva-coated hydroxyapatite beads. Lactoperoxidase and lysozyme synergistically inhibit glucose metabolism by *S. mutans* [43]. Proteose peptone fractions 3 and 5 were shown to inhibit demineralisation of hydroxyapatite in vitro [24].

Probiotics

The idea to use bacteria to modify plaque virulence has been a topic of dental research for many years. There have been several approaches. It has been investigated how the resident oral flora associated with health could be favoured over the species associated with disease. Many early studies concentrated on utilising bacteria that were known to compete with or that expressed bacteriocins or bacteriocin-like inhibitory substances against cariogenic bacteria [27, 80, 87]. Another approach was to replace for instance

Table 4 Bioactive proteins secreted in bovine milk [3]

Protective proteins	Immunoglobulins
	Proteose peptones
	Lactoferrin
	Transferrin
Growth factors	Epidermal growth factor (EGF)
	Tissue growth factor β (TGFβ)
	Insulin-like growth factor (IGF-1)
Enzymes	Lactoperoxidase
	Lysozyme
	Plasmin
	Xanthine oxidase
	Glucose oxidase
Hormones	Thyrotropin-releasing hormone (TRH)
	Somatostatin (SIH)
	Calcitonin
	Insulin
	Relaxin
	Thyroid-stimulating hormone (TSH)
	Luteinising-releasing hormone (LRH)
	Gastrin-releasing peptide (GRP)
	Adrenocorticotropic hormone (ACTH)
	Prolactin

S. mutans strains with strains of attenuated virulence (lactate dehydrogenase deficiency) and increased competitiveness [27]. A recent study utilised a recombinant strain of *S. mutans* expressing urease, which was shown to reduce the cariogenicity of plaque in an animal model [15]. Recently, there has been a focus to maintain a healthy oral flora by modification by non-resident bacteria, the probiotic approach. The rationale for their use in oral healthcare stems from the increase in evidence that supports their claims for benefit for a range of diseases, especially in the gastrointestinal tract [48, 59, 68]. The use of probiotica fits with the paradigm shift in oral microbiology away from treating dental diseases by targeting specific oral pathogens towards an ecological and microbial community-based approach [49, 78]. These approaches recognise the importance of maintaining the natural balance of the resident oral microbiota and the need to carefully modulate host immune responses to the microflora at a site.

The probiotic approach needed to identify food grade and probiotic bacteria that may have potential in caries prevention. These have been selected because of their likely ability to colonise teeth and influence the supragingival plaque; in vitro models for this selection have included adhesion to hydroxyapatite and mixed species biofilm models [16, 25]. Also, strains have been screened for suitable antagonistic activity against relevant oral bacteria [66]. In vitro studies of the antibacterial activity of live yoghurts showed inhibition of *S. mutans* but not of some other oral streptococci, including *S. sobrinus*; this activity was heat-sensitive implying that the effect was not simply due to acid [66].

Recently, oral lactobacilli have also been screened for their utility as potential probiotic strains [79], and strains of oral lactobacilli have been isolated that are inhibitory against *S. mutans, Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*, as well as being tolerant of relevant environmental stresses [38].

Recently, a probiotic mouthwash product containing three selected species of naturally occurring oral streptococci strains, *S. oralis KJ3sm, S. uberis KJ2sm and S. rattus JH145*, successfully affected the levels of dental pathogens (*S. mutans*) in saliva and periodontal pathogens in subgingival plaque (*P. gingivalis* and *Campylobacter rectus*) [97].

Genetically modified probiotics with enhanced properties can be developed ('designer probiotics'). For example, a recombinant strain of *Lactobacillus* that expressed antibodies targeting one of the major adhesins of *S. mutans* (antigen I/II) was able to reduce both the viable counts of *S. mutans* and the caries score in a rat model [40].

Clinical studies have indicated that bacteria with established probiotic effects (lactobacilli and bifidobacteria) have some promise for the prevention of caries. LGG ingested in dairy products (milk and cheese) reduced salivary mutans streptococcal counts in adults and protected against caries in children [2, 55]. Other lactobacilli have also been shown to reduce mutans streptococcal counts in saliva. *Lactobacillus reuteri*, when delivered by yoghurt [58], straw or tablet [11], by chewing gum [12] or as a lozenge [13], significantly reduced the counts of mutans streptococci in saliva ($p < 0.05$). The short-term consumption of yoghurt [9, 10] or ice cream [14] containing *Bifidobacterium* spp. resulted in a significant reduction in salivary mutans streptococci ($p < 0.05$) but not in lactobacilli. Other studies have reported reductions in mutans streptococci levels in saliva following use of probiotic-containing yoghurts [65].

Despite the fact that some products have reached the market, there remains a paucity of clinical evidence to support the effectiveness of probiotics to prevent or treat caries [51, 52].

Conclusions

Caries is the result of an interaction of microbes (plaque), dietary factors and their fermentation on teeth. The effect of dietary components on microbial fermentation can be studied in situ and in vitro. Many dietary factors have been identified that enhance or reduce the formation of cariogenic lesions. Together with improved oral hygiene and fluoridated toothpaste, functional food ingredients/constituents that inhibit oral microflora and/or their fermentation thus contribute to a reduction of dental caries. Some products such as sugar alcohols are already on the market for a long time. Research has also identified the beneficial effects of certain phytochemicals. For other concepts such as probiotics, evidence is just starting to emerge.

Acknowledgments This publication was commissioned by the Functional Foods Task Force of the European branch of the International Life Sciences Institute (ILSI Europe). Industry members of the task force are Abbott Nutrition, Barilla G. & R. Fratelli, BASF, Bionov, Biosearch Life, Cargill, Chiquita Brands International, Coca-Cola Europe, Danone, Dow Europe, DSM, DuPont Nutrition & Health, Institut Mérieux, International Nutrition Company, Kellogg Europe, Kraft Foods Europe, Mars, Martek Biosciences Corporation, McNeil Nutritionals, Naturex, Nestlé, PepsiCo International, Pfizer Consumer Healthcare, Red Bull, Rudolf Wild, Schwabegroup, Royal Friesland-Campina, Soremartec Italia—Ferrero Group, Südzucker/BENEO Group, Tate & Lyle Ingredients, Tereos-Syral, Unilever and Yakult Europe. This publication was coordinated by Dr. Alessandro Chiodini, Scientific Project Manager at ILSI Europe. For further information about ILSI Europe, please email info@ilsieurope.be or call +32 2 771 00 14. The opinions expressed herein and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies.

Declaration of interest C. van Loveren and Z. Broukal received an honorarium from ILSI Europe for their participation in this publication and reimbursement of their travel and accommodation costs for attending the related meetings.

References

1. Abdullah AZ, Strafford SM, Brookes SJ, Duggal MS (2006) The effect of copper on demineralization of dental enamel. J Dent Res 85:1011–1015
2. Ahola AJ, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlstrom A, Meurman JH et al (2002) Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol 47:799–804
3. Al-Ahmad A, Wiedmann-Al-Ahmad M, Auschill T, Follo M, Braun G, Hellwig E, Arweiler N (2008) Effects of commonly used food preservatives on biofilm formation of *Streptococcus mutans* in vitro. Arch Oral Biol 53:765–772
4. Arweiler NB, Lenz R, Sculean A, Al-Ahmad A, Hellwig E, Auschill TM (2008) Effect of food preservatives on in situ biofilm formation. J Clin Oral Investig 12:203–208
5. Bodet C, Grenier D, Chandad F, Ofek I, Steinberg D, Weiss EI (2008) Potential oral health benefits of cranberry. Crit Rev Food Sci Nutr 48:672–680
6. Bowen WH, Pearson SK, Van Wuyckhuyse BC, Tabak LA (1991) Influence of milk, lactose-reduced milk, and lactose on caries in desalivated rats. Caries Res 25:283–286
7. Bradshaw DJ, Marsh PD (1994) Effect of sugar alcohols on the composition and metabolism of a mixed culture of oral bacteria grown in a chemostat. Caries Res 28:251–256
8. Brookes SJ, Shore RC, Robinson C, Wood SR, Kirkham J (2003) Copper ions inhibit the demineralisation of human enamel. Arch Oral Biol 48:25–30
9. Caglar E, Kargul B, Tanboga I (2005) Bacteriotherapy and probiotics' role on oral health. Oral Dis 11:131–137
10. Caglar E, Sandallii N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S (2005) Effect of yogurt with *Bifidobacterium* DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scand 63:317–320
11. Caglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S (2006) Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets. Acta Odontol Scand 64:314–318
12. Caglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S (2007) Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. Clin Oral Investig 11:425–429
13. Caglar E, Kuscu OO, Cildir SK, Kuvvetli SS, Sandalli N (2008) A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli. Int J Paediatr Dent 18:35–39
14. Caglar E, Kuscu OO, Kuvvetli SS, Cildir SK, Sandalli N, Twetman S (2008) Short-term effect of ice-cream containing *Bifidobacterium lactis* Bb-12 on the number of salivary mutans streptococci and lactobacilli. Acta Odontol Scand 66:154–158
15. Clancy KA, Pearson S, Bowen WH, Burne RA (2000) Characterization of recombinant, ureolytic *Streptococcus mutans* demonstrates an inverse relationship between dental plaque ureolytic capacity and cariogenicity. Infect Immun 68:2621–2629
16. Comelli EM, Guggenheim B, Stingele F, Nesser JR (2002) Selection of dairy bacterial strains as probiotics for oral health. Eur J Oral Sci 110:218–224
17. Davis BA, Raubertas RF, Pearson SK, Bowen WH (2001) The effects of benzoate and fluoride on dental caries in intact and desalivated rats. Caries Res 35:331–337
18. Derise NL, Ritchey SJ (1974) Mineral composition of normal human enamel and dentin and the relation of composition to dental caries. II. Microminerals. J Dent Res 53:853–858
19. Duggal MS, Chawla HS, Curzon ME (1991) A study of the relationship between trace elements in saliva and dental caries in children. Arch Oral Biol 36:881–884
20. Feres M, Figueiredo LC, Barreto IM, Coelho MH, Araujo MW, Cortelli SC (2005) In vitro antimicrobial activity of plant extracts and propolis in saliva samples of healthy and periodontally-involved subjects. J Int Acad Periodontol 7:90–96
21. Ferrazzano GF, Amato I, Ingenito A, De Natale A, Pollio A (2009) Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). Fitoterapia 80:255–262
22. Gierat-Kucharzewska B, Karasiński A (2006) Influence of chosen elements on the dynamics of the cariogenic process. Biol Trace Elem Res 111:53–62
23. Graham HN (1992) Green tea composition, consumption, and polyphenol chemistry. Prev Med 21:334–350
24. Grenby TH, Andrews AT, Mistry M, Williams RJH (2001) Dental caries-protective agents in milk and milk products: investigations in vitro. J Dent 29:3–92
25. Haukioja A, Yli-Knuuttila H, Loimaranta V, Kari K, Ouwehand AC, Meurman JH et al (2006) Oral adhesion and survival of probiotic and other lactobacilli and bifidobacteria in vitro. Oral Microbiol Immunol 21:326–332
26. Havenaar R (1984) The anti-cariogenic potential of xylitol in comparison with sodium fluoride in rat caries experiments. J Dent Res 63:120–123
27. Hillman JD (2002) Genetically modified *Streptococcus mutans* for the prevention of dental caries. Antonie Van Leeuwenhoek 82:361–366
28. Hujoel PP, Mäkinen KK, Bennett CA, Isotupa KP, Isokangas PJ, Allen P, Mäkinen PL (1999) The optimum time to initiate habitual xylitol gum-chewing for obtaining long-term caries prevention. J Dent Res 78:797–803
29. Imfeld T (1994) Cariogenicity tests. Adv Dent Res 8:225–228
30. Ingram GS, Horay CP, Stead WJ (1992) Interaction of zinc with dental mineral. Caries Res 26:248–253
31. Isokangas P, Alanen P, Tiekso J (1993) The clinician's ability to identify caries risk subjects without saliva tests—a pilot study. Community Dent Oral Epidemiol 21:8–10
32. Janer C, Rohr LM, Peláez C, Laloi M, Cleusix V, Requena T, Meile L (2004) Hydrolysis of oligofructoses by the recombinant beta-fructofuranosidase from *Bifidobacterium lactis*. Syst Appl Microbiol 27:279–285
33. Johansson I (2002) Milk and dairy products: possible effects on dental health. Scan J Nutr 46:119–122
34. Kashket S, Paolino VJ, Lewis DA et al (1985) In vitro inhibition of glucosyltransferase from the dental plaque bacterium *Streptococcus mutans* by common beverages and food extracts. Arch Oral Biol 30:821–826
35. Kashket S, DePaola DP (2002) Cheese consumption and the development and progression of dental caries. Nutr Rev 60:97–103
36. Kawanabe J, Hirasawa M, Takeuchi T, Oda T, Ikeda T (1992) Noncariogenicity of erythritol as a substrate. Caries Res 26:358–362
37. Keyes PH (1960) The infectious and transmissible nature of experimental dental caries. Arch Oral Biol 1:304–320
38. Koll P, Mandar R, Marcotte H, Leibur E, Mikelsaar M, Hammarstro L (2008) Characterization of oral lactobacilli as potential probiotics for oral health. Oral Microbiol Immunol 23:139–147

39. Koo H, Gomes BP, Rosalen PL, Ambrosano GM, Park YK, Cury JA (2000) In vitro antimicrobial activity of propolis and *Arnica montana* against oral pathogens. Arch Oral Biol 45:141–148
40. Kruger C, Hu YZ, Pan Q, Marcotte H, Hultberg A, Delwar D et al (2002) In situ delivery of passive immunity by lactobacilli producing single-chain antibodies. Nat Biotechnol 20:702–706
41. La Monte D, Fadavi A, Wu CD (2004) Effects of raisin-containing cereals on acidogenicity of dental plaque. Abstract no. 1443, 82nd IADR/AADR general session
42. Leikanger S, Bjertness E, Aamdal Scheie A (2007) Effects of food preservatives on growth and metabolism of plaque bacteria in vitro and in vivo. Eur J Oral Sci 100:371–376
43. Lenander-Lumikari M, Mansson-Rahemtulla B, Rahemtulla F (1992) Lysozyme enhances the inhibitory effects of the perioxidase system on glucose metabolism of *Streptococcus mutans*. J Dent Res 71:484–490
44. Lingström P, Lundgren F, Birkhed D, Takazoe I, Frostell G (1997) Effects of frequent mouth rinses with palatinose and xylitol on dental plaque. Eur J Oral Sci 105:162–169
45. Lingström P, Birkhed D, Granfeldt Y, Björck I (1993) pH measurements of human dental plaque after consumption of starchy foods using the microtouch and the sampling method. Caries Res 27:394–401
46. Mäkinen KK, Saag M, Isotupa KP, Olak J, Nõmmela R, Soderling E, Mäkinen PL (2005) Similarity of the effects of Erythritol and Xylitol on some risk factors of dental caries. Caries Res 39:207–215
47. Maltz M, Emilson CG (1988) Effect of copper fluoride and copper sulphate on dental plaque, *Streptococcus mutans* and caries in hamsters. Scand J Dent Res 96:390–392
48. Marco ML, Pavan S, Kleerebezem M (2006) Towards understanding molecular modes of probiotic action. Curr Opin Biotechnol 17:204–210
49. Marsh PD (2003) Are dental diseases examples of ecological catastrophes? Microbiol 149:279–294
50. Matsumoto M, Hamada S, Ooshima T (2003) Molecular analysis of the inhibitory effects of oolong tea polyphenols on glucan-binding domain of recombinant glucosyltransferases from *Streptococcus mutans* MT8148. FEMS Microbiol Lett 228:73–80
51. Meurman JH (2005) Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci 113:188–196
52. Meurman JH, Stamatova I (2007) Probiotics contributions to oral health. Oral Dis 13:443–451
53. Mitscher LA, Jung M, Shankel D et al (1997) Chemoprotection: a review of the potential therapeutic antioxidant properties of green tea (*Camellia sinensis*) and certain of its constituents. Med Res Rev 17:327–365
54. Moynihan P (2000) The British nutrition foundation oral task force report—issues relevant to dental health professionals. Br Dent J 188:308–312
55. Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka A, Poussa T et al (2001) Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res 35:412–420
56. Neeser JR, Chambaz A, Del Vedovo S, Prigent MJ, Guggenheim B (1988) Specific and nonspecific inhibition of adhesion of oral actinomyces and streptococci to erythrocytes and polystyrene by caseinoglycopeptide derivatives. Infect Immun 56:3201–3208
57. Neeser JR, Golliard M, Woltz A, Rouvet M, Dillmann ML, Guggenheim B (1994) In vitro modulation of oral bacterial adhesion to saliva-coated hydroxyapatite beads by milk casein derivatives. Oral Microbiol Immunol 9:193–201
58. Nikawa H, Makihira S, Fukushima H et al (2004) *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. Int J Food Microbiol 95:219–223
59. Nomoto K (2005) Prevention of infections by probiotics. J Biosci Bioeng 100:583–592
60. Ooshima T, Minami T, Aono W et al (1993) Oolong tea polyphenols inhibit experimental dental caries in SPF rats infected with mutans streptococci. Caries Res 27:124–129
61. Ooshima T, Minami T, Matsumoto M et al (1998) Comparison of the cariostatic effects between regimens to administer oolong tea polyphenols in SPF rats. Caries Res 32:75–80
62. Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumoto M (2000) Cariostatic activity of cacao mass extract. Arch Oral Biol 45:805–808
63. Otake S, Makimura M, Kuroki T et al (1991) Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Res 25:438–443
64. Papas AS, Joshi A, Belanger AJ, Kent RL Jr, Palmer CA, De Paola PF (1995) Dietary models for root caries. Am J Clin Nutr 61:417S–422S
65. Petti S, Tarsitani G, D'Arca AS (2001) A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Arch Oral Biol 46:705–712
66. Petti S, Tarsitani G, Simmonetti D'Arca A (2008) Antibacterial activity of yoghurt against viridans streptococci in vitro. Arch Oral Biol 53:985–990
67. Petridou E, Athanassouli T, Panagopoulos H, Revinthi K (1996) Sociodemographic and dietary factors in relation to dental health among Greek adolescents. Community Dent Oral Epidemiol 24:307–311
68. Reid G, Jass J, Sebulsky MT, McCormick JK (2003) Potential uses of probiotics in clinical practice. Clin Microbiol Rev 16:658–666
69. Reynolds EC, Riley PF, Storey E (1982) Phosphoprotein inhibition of hydroxyapatite dissolution. Calcif Tiss Int 34:552–556
70. Reynolds EC (2009) Casein phosphopeptide–amorphous calcium phosphate: the scientific evidence. Adv Dent Res 21:25–29
71. Rosalen PL, Pearson SK, Bowen WH (1996) Effects of copper, iron and fluoride co-crystallized with sugar on caries development and acid formation in deslivated rats. Arch Oral Biol 41:1003–1010
72. Rosalen PL, Bowen WH, Pearson SK (1996) Effect of copper co-crystallized with sugar on caries development in deslivated rats. Caries Res 30:367–372
73. Scheie AA (1992) Dentifrices in the control of dental caries. In: Embery G, Rolla G (eds) Clinical and biological aspects of dentifrices. Oxford University Press, New York, pp 29–60
74. Scheie AA, Fejerskov OB (1998) Xylitol in caries prevention: what is the evidence for clinical efficacy? Oral Dis 4:268–278
75. Schüpbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B (1996) Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. J Dent Res 75:1779–1788
76. Shaw JH, Ensfield BJ, Wollman DH (1959) Studies on the relation of dairy products to dental caries in caries-susceptible rats. J Nutr 10:253–273
77. Smullen J, Koutsou GA, Foster HA, Zumbé A, Storey DM (2007) The antibacterial activity of plant extracts containing polyphenols against *Streptococcus mutans*. Caries Res 41:342–349
78. Socransky SS, Haffajee AD (2005) Periodontal microbial ecology. Periodontology 2000(38):135–187
79. Strahinic I, Busarcevic M, Pavlica D, Milasin J, Golic N, Topisirovic L (2007) Molecular and biochemical characterizations of human oral lactobacilli as putative probiotic candidates. Oral Microbiol Immunol 22:111–117
80. Tagg JR, Dierksen KP (2003) Bacterial replacement therapy: adapting 'germ warfare' to infection prevention. Trends Biotechnol 21:217–223

81. Tanzer JM (1995) Xylitol chewing gum and dental caries. Int Dent J 45(Suppl 1):65–76
82. ten Cate JM (1994) In situ models, physico-chemical aspects. Adv Dent Res 8:125–133
83. Trahan L (1995) Xylitol: a review of its action on mutans streptococci and dental plaque—its clinical significance. Int Dent J 45(1 Suppl 1):77–92
84. Twetman S (2009) Consistent evidence to support the use of xylitol- and sorbitol-containing chewing gum to prevent dental caries. Evid Based Dent 10:10–11
85. Uzel A, Sorkun K, Oncag O, Cogulu D, Gencay O, Salih B (2005) Chemical compositions and antimicrobial activities of four different *Anatolian propolis* samples. Microbiol Res 160:189–195
86. Vadeboncoeur C, Trahan L, Mouton C, Mayrand D (1983) Effect of xylitol on the growth and glycolysis of acidogenic oral bacteria. J Dent Res 62:882–884
87. van der Hoeven JS, de Jong MH, Rogers AH, Camp PJ (1984) A conceptual model for the co-existence of *Streptococcus* spp. and *Actinomyces* spp. in dental plaque. J Dent Res 63:389–392
88. Van Loveren C, Scheper WA, Eijkman MA (2005) Sports diets and oral health. Ned Tijdschr Tandheelkd 112:136–140
89. Wåler SM, Rölla G (1983) Effect of xylitol on dental plaque in vivo during carbohydrate challenge. Scand J Dent Res 91:256–259
90. Weiss EI, Kozlovsky A, Steinberg D, Lev-Dor R, Bar Ness Greenstein R, Feldman M, Sharon N, Ofek I (2004) A high molecular mass cranberry constituent reduces mutans streptococci level in saliva and inhibits in vitro adhesion to hydroxyapatite. FEMS Microbiol Lett 12:89–92
91. Wennerholm K, Arends J, Birkhed D, Ruben J, Emilson CG, Dijkman AG (1994) Effect of xylitol and sorbitol in chewing-gums on mutans streptococci, plaque pH and mineral loss of enamel. Caries Res 28:48–54
92. Wu CD, Guo-Xian Wei MS (2002) Tea as a functional food for oral health. Nutrition 18:443–444
93. Wu CD (2009) Grape products and oral health. J Nutr 139:1818–1823
94. Xiao J, Liu Y, Zuo YL, Li JY, Ye L, Zhou XD (2006) Effects of *Nidus vespae* extract and chemical fractions on the growth and acidogenicity of oral microorganisms. Arch Oral Biol 51:804–813
95. Yanagida A, Kanda T, Tanabe M, Matsudaira F, Oliveira Cordeiro JG (2000) Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of mutans streptococci. J Agric Food Chem 48:5666–5671
96. Yu H, Oho T, Xu LX (1995) Effects of several tea components on acid resistance of human tooth enamel. J Dent 23:101–105
97. Zahradnik RT, Magnusson I, Walker C, McDonell E, Hillman CH, Hillman JD (2009) Preliminary assessment of safety and effectiveness in humans of ProBiora3, a probiotic mouthwash. J Appl Microbiol 107:682–690