Biofilm 7 (2024) 100171

Contents lists available at ScienceDirect

Biofilm

journal homepage: www.sciencedirect.com/journal/biofilm

# Alteration of oral microbial biofilms by sweeteners

Geum-Jae Jeong [a], Fazlurrahman Khan [b,c,d,*], Nazia Tabassum [c,d], Young-Mog Kim [a,c,d]

[a] Department of Food Science and Technology, Pukyong National University, Busan, 48513, Republic of Korea
[b] Institute of Fisheries Sciences, Pukyong National University, Busan, 48513, Republic of Korea
[c] Marine Integrated Biomedical Technology Center, The National Key Research Institutes in Universities, Pukyong National University, Busan, 48513, Republic of Korea
[d] Research Center for Marine Integrated Bionics Technology, Pukyong National University, Busan, 48513, Republic of Korea

ARTICLE INFO

*Keywords:*
Biofilm
Pathogen
Diet
Sweetener
Antibiofilm
Alteration

ABSTRACT

There is a growing interest in using sweeteners for taste improvement in the food and drink industry. Sweeteners were found to regulate the formation or dispersal of structural components of microbial biofilms. Dietary sugars may enhance biofilm formation and facilitate the development of antimicrobial resistance, which has become a major health issue worldwide. In contrast, bulk and non-nutritive sweeteners are also beneficial for managing microbial infections. This review discusses the clinical significance of oral biofilms formed upon the administration of nutritive and non-nutritive sweeteners. The underlying mechanism of action of sweeteners in the regulation of mono- or poly-microbial biofilm formation and destruction is comprehensively discussed. Bulk and non-nutritive sweeteners have also been used in conjunction with antimicrobial substances to reduce microbial biofilm formation. Formulations with bulk and non-nutritive sweeteners have been demonstrated to be particularly efficient in this regard. Finally, future perspectives with respect to advancing our understanding of mechanisms underlying biofilm regulation activities of sweeteners are presented as well. Several alternative strategies for the application of bulk sweeteners and non-nutritive sweeteners have been employed to control the biofilm-forming microbial pathogens. Gaining insight into the underlying mechanisms responsible for enhancing or inhibiting biofilm formation and virulence properties by both mono- and poly-microbial species in the presence of the sweetener is crucial for developing a therapeutic agent to manage microbial infections.

## 1. Introduction

A carbohydrate-rich diet facilitates the colonization of microbial species in the gut and thereby protects gut microbiota from the host immune system and antimicrobials [1]. Nutritive and non-nutritive sweeteners were shown to affect the gut microbial community positively or negatively [2–4]. The ingestion of dietary sugars, such as glucose, sucrose, lactose, fructose, and maltose, which are metabolized by microbes into organic acids, may drive microbial communities to shift toward more aciduric and acidogenic profiles [5]. Such microbial communities predominantly inhabit the oral cavity, where they use sugars to form biofilms on tooth surfaces for survival [6]. The shift toward aciduric and acidogenic bacterial species lowers the pH level within the biofilm and ultimately causes demineralization of the hard tissue of the tooth and the development of dental caries [7].

Microbes have the ability to self-secrete exopolysaccharides with the assistance of intracellular enzymes that are self-produced by the *eps* gene cluster [8,9]. Oral bacteria such as *Streptococcus mutans* are capable of using sugars as substrates for metabolic activities that create extracellular polymeric substances (EPS) in biofilms that have a high level of physical stability [10]. In this case, the synthesis of EPS takes place as a result of the activity of secreted enzymes, such as glucosyltransferase (Gtf), which act on dietary sugars (e.g., sucrose) to generate extracellular polysaccharides (e.g., α-glucan), which in turn aid the attachment of bacterial cells to the surface of the tooth [11]. Gtf also strongly binds *N*- and *O*-linked mannans on *Candida albicans* cell surface [12]. Thus, the Gtf-assisted synthesis of bacterial EPS as well as the high affinity of Gtf for the mannan residues on *C. albicans* leads to a synergistic interaction as well as the formation of a mixed bacterial-fungal biofilm in the oral cavity [13]. The synergistic interaction between bacterial and fungal pathogens has resulted in enhanced poly-microbial biofilm formation and a tolerance mechanism against antibiotics or antimicrobials [16–18]. As a result, new treatment approaches are required to combat poly-microbial biofilms of bacterial and fungal pathogens [14,15].

The biofilm matrix also includes polysaccharides that facilitate biofilm formation and increase the expression of related genes [19]. EPS

* Corresponding author. Institute of Fisheries Sciences, Pukyong National University, Busan, 48513, Republic of Korea.
*E-mail address:* fkhan055@pknu.ac.kr (F. Khan).

https://doi.org/10.1016/j.bioflm.2023.100171
Received 14 September 2023; Received in revised form 14 November 2023; Accepted 11 December 2023
Available online 13 December 2023

formation in the biofilm matrix in the presence of sweeteners further accelerates microbial colonization, and thus functions as a critical virulence factor and facilitates protection from the host immune system and host-derived antimicrobial peptides [11]. Biofilms also reduce the effectiveness of infectious disease treatment since they act as physical barriers between microbes and antimicrobial drugs [20]. To this purpose, it has been common practice to use bulk (sugar alcohols/polyols) and non-nutritive sweeteners with minimal calorie content to prevent the development of oral biofilms [21]. Bulk sweeteners, also known as nutritive sweeteners, are extensively employed in the food, medical, and pharmaceutical sectors because they have fewer calories and lower glycemic responses than sugar [22]. Aside from inhibiting biofilm formation, non-nutritive sweeteners have been frequently employed for a variety of reasons, including increased sweetening power relative to sucrose, inexpensive, and accessibility [23].

Despite these advantages, the intake of non-nutritive sweeteners was also shown to have significant adverse effects on health [24]. Although non-nutritive sweeteners cannot be digested to generate energy, they can be potentially toxic to cells following cellular absorption [25]. Nevertheless, non-nutritive sweeteners have also been shown recently to be beneficial in managing microbial infections [26–30]. Bulk sweeteners also exhibit antibacterial efficacy against a variety of drug-resistant pathogens [31–33]. These sweeteners have also been used in conjunction with antimicrobial substances to inhibit biofilm formation [34–36]. Formulations with bulk and non-nutritive sweeteners have been described to efficiently reduce biofilm-forming microbial pathogens [37–39].

The clinical significance of oral biofilms that are developed upon the use of nutritive and non-nutritive sweeteners is thoroughly examined here. The regulatory effects of sweeteners on microbial biofilms and the underlying mechanisms are discussed in detail. In addition, strategies involving the application of mixed formulations of nutritive and non-nutritive sweeteners are presented. Understanding the mechanisms involved in sweetener-regulated promotion or inhibition of mono- and

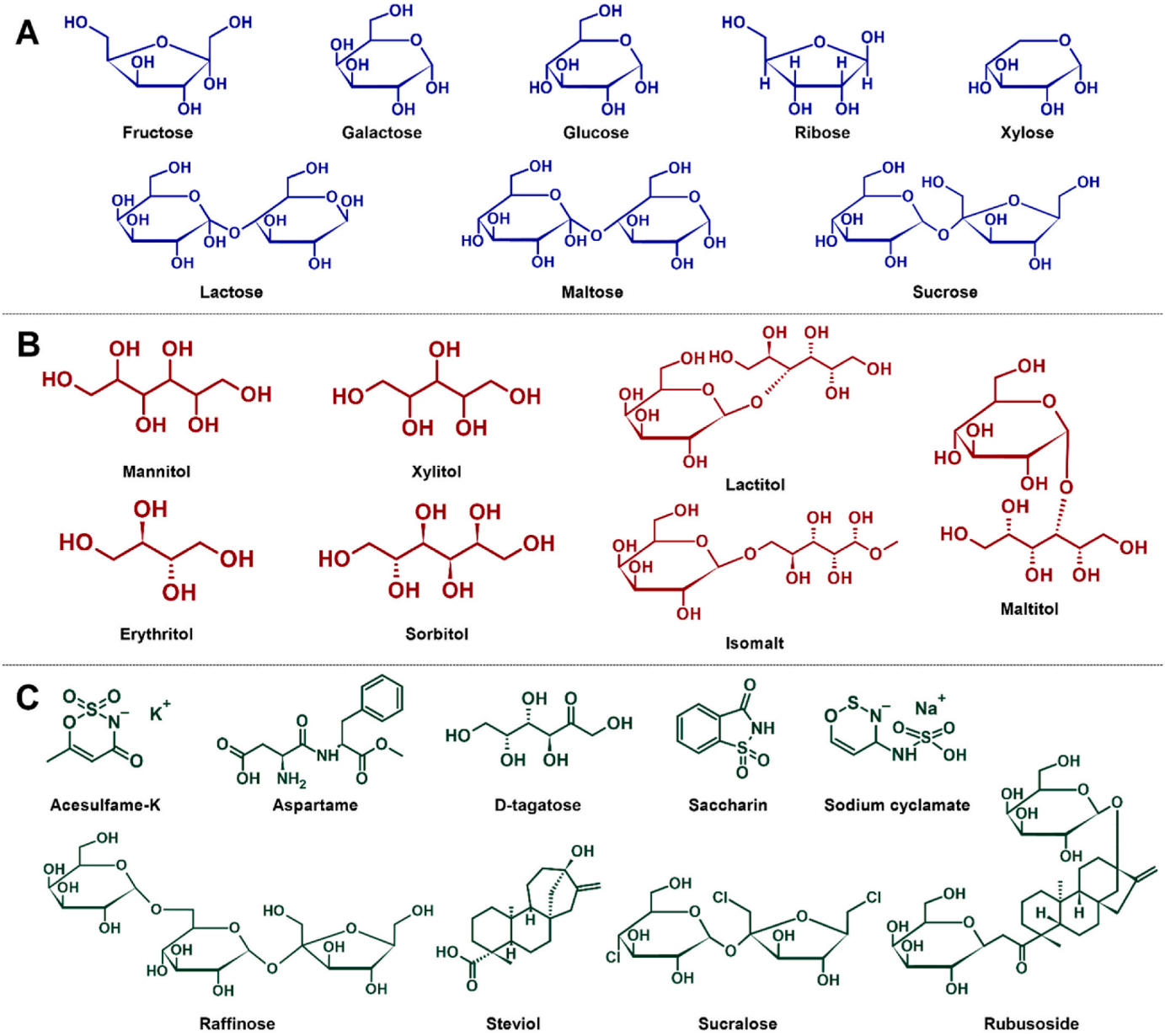


**Fig. 1.** Chemical structures of nutritive and non-nutritive sweeteners. **(A)** The blue color chemical structures are dietary sugars, **(B)** the red color chemical structures are bulk sweeteners, and **(C)** the green color chemical structures are non-nutritive sweeteners. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

poly-microbial biofilm formation will facilitate efforts in the discovery of therapeutics to control microbial infections.

## 2. Sweeteners

Sweeteners augment the taste of sweetness and are therefore used in a variety of foods and beverages. Sweeteners can be classified as nutritive or non-nutritive depending on whether they provide energy (Fig. 1) [40]. Nutritive sweeteners comprise dietary sugars such as mono- and di-saccharides as well as bulk sweeteners known as sugar alcohols/polyols [22]. Non-nutritive sweeteners are also known as 'artificial sweeteners', and are produced via chemical synthesis [21].

Glucose, fructose, and galactose are the major natural monosaccharide types found in plants [41]. These monosaccharides are combined to form disaccharides such as lactose (glucose and galactose), sucrose (fructose and glucose), and maltose (glucose and glucose) [42]. Sucrose is widely and readily available in various plant species such as sugarcane (*Saccharum* spp.) and sugar beets (*Beta vulgaris*) [43]. Sucrose is widely used in dishes; however, excessive intake of sucrose may cause numerous health issues such as obesity, diabetes, and hypertension [44]. In particular, dietary sucrose is directly related to dental caries since EPS synthesis by dental bacteria requires sucrose [45]. Lactose has a sweetness of approximately 20–40 % that of sucrose [46]. Lactose also causes dental caries; however, it is less carcinogenic than sucrose since it induces a lower level of reduction in pH level in the oral environment compared to sucrose [47,48]. Caries formation due to maltose is also lower than that due to sucrose [49]. Hence, despite being a major energy source for human metabolism, sugar can also cause various diseases and metabolic abnormalities, including dental caries [50].

Bulk sweeteners (sugar alcohols/polyols) include erythritol, mannitol, xylitol, lactitol, and maltitol [51]. These sweeteners are either produced naturally or synthesized from monosaccharides or disaccharides [50]. Owing to the negative effects of excessive sugar consumption on health, bulk sweeteners play an important role as sugar substitutes in the food industry [52]. Compared to sugars, bulk sweeteners are poorly absorbed by the body, resulting in lower levels of calorie intake and glycemic responses [53]. In addition, they are also non-cariogenic since oral bacteria are unable to utilize them [54]. Among these sweeteners, xylitol is a particularly good sugar substitute because it promotes salivation and reduces plaque formation [55].

Non-nutritive sweeteners include aspartame, acesulfame-K, sucralose, saccharin, and steviol glycosides [24]. They have a sweet taste when consumed in very small amounts with little or no calories [23]. Unlike fermentable carbohydrates, non-nutritive sweeteners cannot be fermented by oral microorganisms, and are therefore considered non-cariogenic [46]. Because of these properties, non-nutritive sweeteners are increasingly being employed in a variety of food and hygiene products, as well as drug formulations [24]. However, evidence also continues to emerge regarding the effects of non-nutritive sweeteners on carcinogenesis and metabolism, requiring critical evaluation of their use [25].

## 3. Clinical significance of oral biofilm

Oral microbes have a strong tendency to adhere onto tooth surfaces [56]. Oral microorganisms include bacteria that are beneficial to oral health as well as harmful species that cause various oral diseases [57]. Oral microbial communities adhere onto the surface of dental pellicles through adhesion-receptor interactions mediated by hydrophobic or electrostatic forces to initiate colonization and biofilm formation [58]. Dental caries is a biofilm-induced disease that causes damage to tooth enamel [59]. Uptake of sugars results in an acidic environment (pH 4.5–5.5) in the oral environment, and thus promotes the growth of cariogenic bacteria (*S. mutans*, *Actinomyces*, and *Lactobacillus*) that form oral biofilms, eventually leading to the development of oral diseases [5]. EPS in oral biofilms is mainly composed of *S. mutans*-derived glucans, in addition to soluble fructans and glucans produced by other oral microorganisms (*Actinomyces*, *S. gordonii*, and *S. salivarius*) [19]. *S. mutans* promotes carious biofilm formation by metabolizing various carbohydrates into organic acids on tooth surfaces [6]. Sucrose is a fermentable disaccharide that serves as a substrate for EPS-synthesizing bacterial enzymes [60]. *S. mutans* uses sugars, including sucrose, starch, glucose, and fructose as carbon sources to produce EPS and acids [10]. Thus, the pathogenicity of *S. mutans* in the oral microbiome is based on its ability to utilize extracellular insoluble glucans for EPS formation [61]. *S. mutans* secretes Gtf to degrade sucrose and produce extracellular glucan [62]. Gtf induces the production of glucan-rich exopolysaccharides to form a scaffold that provides protection against external stress and antimicrobial agents [11]. GtfB is responsible for the synthesis of insoluble glucans with primarily α-1,3 crosslinks, whereas GtfC is responsible for the synthesis of both insoluble and soluble glucans that are abundant in α-1,6 linkages [63,64]. GtfB and GtfC synthesize glucans that enhance the adhesion of bacterial cells onto the tooth surface, thereby improving the structural stability of the biofilm matrix [61]. GtfD produces soluble glucans that are easily metabolized and serve as primers for GtfB. In addition, *S. mutans* synthesizes fructans by secreting fructosyltransferase (Ftf), which uses sucrose as a substrate [65]. Fructans produced by Ftf are stored to be used as nutrients later on and also increase the virulence of oral biofilms by facilitating bacterial adhesion and colonization [66]. Glucan, produced in the oral environment, is bound by *S. mutans* cells via the glucan-binding protein (Gbp) [67]. Gbps, including GbpA, GbpB, GbpC, and GbpD, are considered caries-inducing factors due to their glucan-binding properties [68]. Moreover, *S. mutans* also regulates the expression of several genes that sense and adapt to external stressors through a two-component system [69]. In particular, the quorum sensing system encoded by *comCDE* is involved in biofilm formation and formation of the protective extracellular matrix in *S. mutans* [69]. Although different microorganisms are present in oral biofilms, most of them do not contribute to glucan synthesis until they are coated by Gtf produced by *S. mutans* [70]. Instead, GtfB binds to other oral microorganisms (*A. viscosus*, *L. casei*, and *C. albicans*) and non-Gtf-producing microorganisms, converting them into extracellular glucan producers [71,72]. Accordingly, *S. mutans* contributes to poly-microbial biofilm growth by building EPS together with other oral microorganisms [70]. In particular, *C. albicans* forms symbiotic relationships with oral bacteria in dental biofilms [73]. *S. gordonii* increases hyphae and biofilm formation of *C. albicans* by interacting with *C. albicans* [74]. In addition, *C. albicans* provides an adhesion site for *S. mutans*, which enhances the formation of poly-microbial biofilms [75].

The structural and genetic profile of *S. mutans* biofilms is affected by sugar availability as well [76]. Changes in the oral environment caused by sugar exposure can disturb the microbial balance within the biofilm, and promote the growth of pathogens such as *S. mutans*, *Actinomyces* spp., *S. salivarius*, and *S. gordonii* [77]. In contrast, bulk and non-nutritive sweeteners are mainly non-fermentable and, therefore, cannot be metabolized to acids by oral microorganisms and are therefore considered non-cariogenic [21,46,55].

## 4. Role of sweeteners in oral biofilm modulation

### 4.1. Enhancement of oral biofilm formation by dietary sugars

The cariogenic properties of *S. mutans* biofilms are mainly regulated by genes related to the extracellular polysaccharide, acid production, microbial adhesion, acid tolerance, and other biofilm-related genes (Fig. 2) [10,76,78]. Therefore, identification of the mechanisms used by *S. mutans* to adhere to the tooth surface may facilitate the development of new approaches for the treatment of dental caries [79]. Nutritive sweeteners, including sucrose, improve oral microbial biofilm properties through mechanisms shown in Table 1.

In an early study examining the correlation between the use of

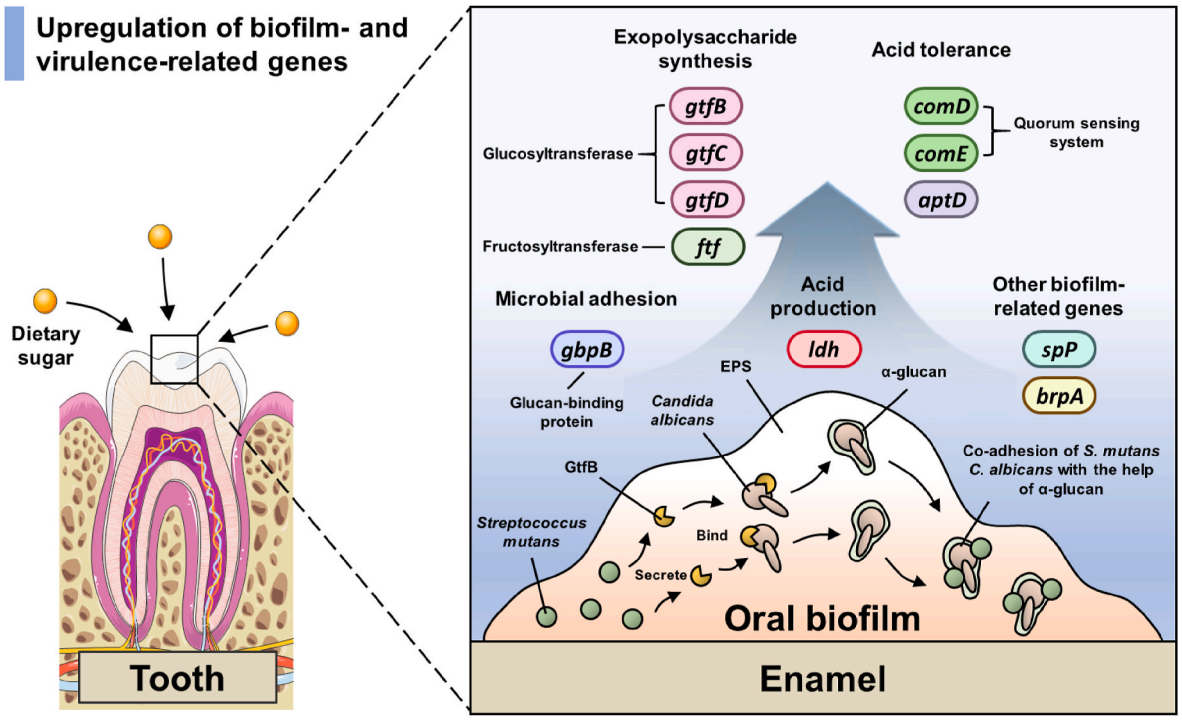


**Fig. 2.** Enhancement of oral biofilm properties by sucrose. In representative oral bacteria *Streptococcus mutans*, dietary sugars upregulate the expression of extracellular polysaccharide synthesis (*gtfB*, *gtfC*, *gtfD*, and *ftf*), acid production (*ldh*), microbial adhesion (*gbpB*), acid tolerance (*comD*, *comE*, and *aptD*), and other biofilm-related (*spP* and *brpA*) genes. Additionally, *S. mutans* GtfB binds to other oral microorganisms, transforming them into extracellular glucan producers. In an oral environment rich in dietary sugar, *S. mutans* GtfB binds to *N*- and *O*-linked mannans on the surface of *Candida albicans*, inducing the conversion of sucrose to α-glucan in the host. Then, α-glucan provides a binding site for *S. mutans*, leading to the formation of *C. albicans-S. mutans* mixed biofilms, which causes severe dental caries. Information obtained from the literature [11–13,70,71]. EPS, extracellular polymeric substances; Ftf, fructosyltransferase; Gbp, glucan-binding protein; Gtf, glucosyltransferase.

sweeteners and biofilm formation, sucrose (10 and 40 g/L) was found to enhance the properties of *S. mutans* biofilms formed in TY growth medium containing 1.4 % tryptone and 0.8 % yeast extract [78]. The expression levels of *gtfB*, *gtfC*, *comDE*, *ftf*, *gpbB*, and *spaP*, which promote biofilm formation, were found to increase in the presence of sucrose. In contrast, the expressions of these genes were suppressed when sucrose was introduced into the BHI medium. These results were attributed to the fact that the glucose in the BHI medium neutralized the effect of sucrose since microorganisms prefer simple sugars as their main carbon source. In a study investigating the effect of a combination of starch and sucrose on biofilms, a mixture including 1 % starch and 1 % sucrose was found to affect the exopolysaccharide composition and the expression of genes involved in exopolysaccharide formation in *S. mutans* biofilms [80]. *S. mutans* formed a tightly attached biofilm composed mostly of water-insoluble polysaccharides derived from a combination of starch and sucrose. Moreover, biofilms formed from the combination of starch and sucrose included higher levels of *gtfB* than those grown with sucrose alone or a combination of sucrose and glucose. In another study, the presence of 5 % sucrose in the growth medium was found to result in a higher percentage of vital cells in *S. mutans* biofilms compared to xylitol [76]. In addition, sucrose addition led to increased expression of genes associated with biofilm formation (*gtfB*, *gtfD*, and *ftf*). In another study that assessed the impact of sucrose on *S. mutans* biofilms, the effects of sucrose on bacterial adhesion, biofilm composition, and acidogenicity of *S. mutans* followed a second-order polynomial curve with sucrose concentration dependency [81]. The adhesion and biofilm development of *S. mutans* increased and subsequently decreased as sucrose concentration increased, with a turning concentration range of 0.45–2.40 %. Raffinose, together with sucrose, induced biofilm formation at concentrations lower than that required to induce *S. mutans* biofilm formation [82]. Sucrose increased bacterial cell-surface hydrophobicity and raffinose-induced fructan synthesis via Ftf, which enhances extracellular DNA-dependent cell aggregation. In mono- and co-culture studies of *C. albicans* and *C. tropicalis*, the application of 5 % sucrose enhanced the growth, adhesion, and biofilm formation of *Candida* spp. [83]. These findings were attributed to sucrose promoting the formation of aggregates and fibrillar layers and the subsequent biofilm formation by *Candida* spp.. In a study imitating human meal patterns, the application of 1–5 % sucrose increased acid production and accumulation in *S. mutans* biofilms [10]. However, biofilm development and acid production were found to decrease with increasing sucrose concentration at concentrations beyond 5 %, suggesting that the effect of sucrose on *S. mutans* biofilm formation follows a second-order polynomial curve. In contrast, EPS formation, acid production, and acid tolerance-related gene expression were upregulated with increasing sucrose concentrations. Hence, high sucrose concentrations could stimulate the expression of related genes to compensate for the EPS reduction. In an *in situ* study of humans wearing palatal devices containing titanium specimens, daily sucrose exposure was found to increase biofilm biomass and negatively affect the biochemical and microbiological composition of the biofilm formed [84]. In addition, the adhesion of *S. mutans* biofilms on titanium substrates was found to be induced by sucrose levels ranging from 0–750 mM [85]. In particular, *S. mutans* biofilms formed in the presence of 75 mM sucrose were found to show the maximum adhesion and mounds. In addition to these studies, 2–5 % lactose was also found to significantly enhance *S. mutans* biofilm formation [86]. Lactose was found to lead to increased expression of biofilm formation-related genes, such as *gtfB*, *gtfC*, *gtfD*, *ftf*, *brpA*, and *SMU.1039*. Furthermore, the biomass of *S. mutans* biofilms was also increased to a level similar to that obtained upon application of sucrose, yet with a different polysaccharide composition.

**Table 1**
Enhancement of oral biofilm properties by dietary sugars.

| Product | Studied microorganism | Testing method | Active concentration | Surface material | Mechanism | References |
|---|---|---|---|---|---|---|
| Sucrose | *Streptococcus mutans* | *In vitro* | 10, 40 sucrose g/L in TY media (1.4 % tryptone, 0.8 % yeast extract) | Polystyrene multi dishes | Increased expression of genes that positively regulate biofilm formation (*gtfB*, *gtfC*, *comDE*, *ftf*, *gpbB*, and *spP*) | [78] |
| Sucrose | *S. mutans* | *In vitro* | 1 % sucrose in tryptone yeast-extract broth containing 1 % starch | Saliva-coated hydroxyapatite (sHA) discs | Increased expression of genes involved in extracellular polysaccharide matrix formation (*gtfB*) | [80] |
| Sucrose | *S. mutans* | *In situ* | 5 % sucrose in Schaedler broth | Methacrylate-based mounting material embedded with intact human third molars | Increased expression of *gtfB*, *gtfD*, and *ftf* genes | [76] |
| Sucrose | *S. mutans* | *In vitro* | 0.45–2.40 % (w/v) | sHA discs | Increased the accumulation and virulence of biofilms | [81] |
| Sucrose + raffinose | *S. mutans* | *In vitro* | 0.002 % (w/v) sucrose + 0.25 % raffinose (w/v) | sHA discs | Increased cell surface hydrophobicity by the reaction of Gtf-I (sucrose)<br>Contributed to the aggregation of extracellular DNA in the biofilm (raffinose) | [82] |
| Sucrose | *Candida albicnas* + *C. tropicalis* | *In vitro* | 5 % | Microtiter plates | Promoted the production of a floccular and fibrillar layer, which mediated adhesion and subsequent biofilm formation | [83] |
| Sucrose | *S. mutans* | *In vitro* | 1–5 % | sHA discs | Upregulated expressions of genes related to exopolysaccharide formation (*gtfB*, *gtfC*, and *gtfD*), glycolysis (*ldh*), and acid tolerance (*atpD*) | [10] |
| Sucrose | *Actinomyces israelii*<br>*S. sanguinis*<br>*S. mitis*<br>*Fusobacterium periodonticum*<br>*Tannerella forsythia*<br>*Prevotella melaninogenica*<br>*Eubacterium saburreum*<br>*S. mutans* | *In situ* | – | Titanium surfaces | – | [84] |
| Sucrose | *S. mutans* | *In vitro* | 37.5, 75.0, 375, 750 mM | Titanium surfaces | – | [85] |
| Lactose | *S. mutans* | *In vitro* | 2–5 % (w/v) | Polystyrene plates | Increased expression of biofilm-related genes (*gtfB*, *gtfC*, *gtfD*, *ftf*, *brpA*, and *SMU.1039*) | [86] |

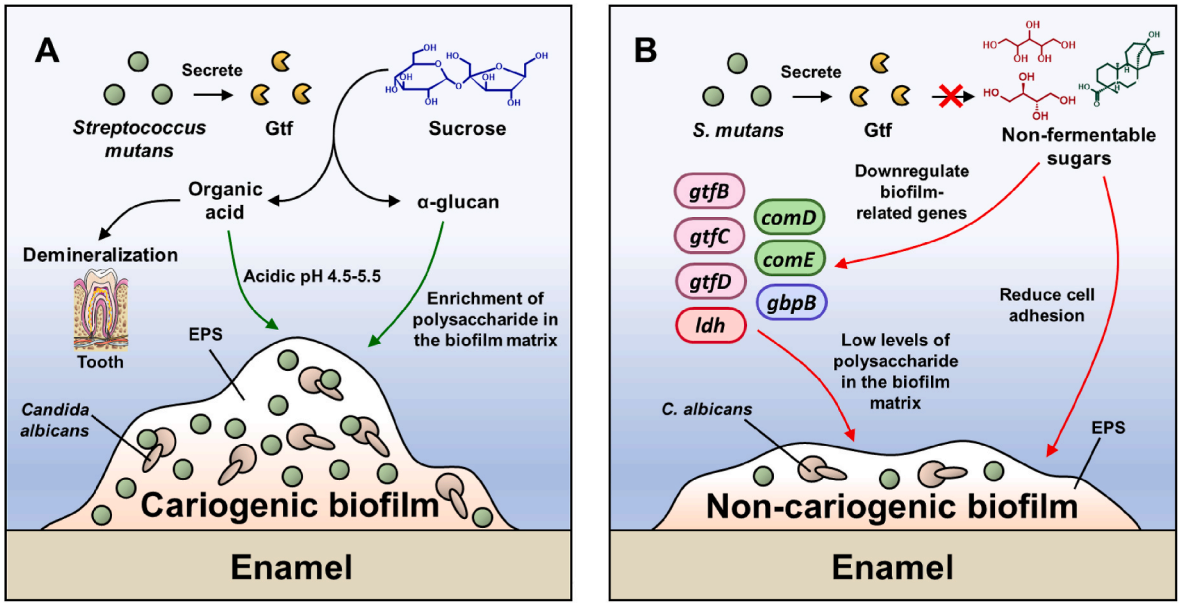


**Fig. 3.** Regulation of oral biofilms by sweeteners. **(A)** Enhancement of oral biofilm formation by sucrose. The Gtf of *Streptococcus mutans* degrades sucrose to produce glucan, which contributes to the accumulation of polysaccharides. Additionally, organic acids produced during fermentation metabolism contribute to reducing the pH of the oral environment, which leads to demineralization and the dental caries process. **(B)** Inhibition of oral biofilm formation by bulk and non-nutritive sweeteners. Non-fermentable sugars, including bulk sweeteners and non-nutritive sweeteners, cannot be utilized by *S. mutans* as a substrate for biofilm matrix synthesis. In addition, bulk sweeteners and non-nutritive sweeteners inhibit biofilm- and virulence-related gene expression, leading to a non-cariogenic biofilm with low levels of polysaccharide. Information obtained from the literature [6,26–28,32,87]. EPS, extracellular polymeric substances; Gtf, glucosyltransferase.

### 4.2. Inhibition of oral biofilm formation by bulk and non-nutritive sweeteners

Sugar substitutes are often used to prevent caries. However, the mechanisms underlying the inhibition of oral biofilm formation and plaque reduction due to the use of sugar substitutes have not been fully elucidated. Bulk and non-nutritive sweeteners have been shown to reduce oral biofilm formation and activity by suppressing the expression of biofilm- and virulence-related genes (Fig. 3) [26–28,87]. In addition, unlike sucrose, bulk and non-nutritive sweeteners cannot be utilized by *S. mutans* as a substrate for biofilm matrix synthesis, resulting in reduced biofilm formation [32]. The details of mechanisms involved in biofilm inhibition by sweeteners, especially bulk and non-nutritive sweeteners, are listed in Table 2.

Xylitol at 5 % was found to inhibit early-stage (8 h) biofilms (formed in a medium containing 0.3 % sucrose) of *S. mutans*, *S. sanguinis*, and *A. naeslundii* [31]. This result was attributed to reduced polysaccharide-mediated cell adhesion upon xylitol usage without a reduction in exopolysaccharide production. Both 1 and 4 % D-tagatose were found to inhibit biofilm formation in *S. mutans* exposed to sucrose at a level much higher than that of xylitol [26]. D-tagatose inhibited Gtf, and thus reduced the production of water-insoluble glucans from sucrose. Hence, the formation of *S. mutans* biofilms was inhibited by restricting the access to released free D-fructose. In another study, xylitol and sorbitol were found to inhibit mono- and poly-microbial biofilm formation by *S. mutans* and *C. albicans* under sucrose-free conditions [32]. In contrast, the inclusion of 1 % sucrose was found to attenuate the inhibitory effects of xylitol and sorbitol on biofilm development. This was attributed to the fact that oral bacteria prefer hexose sugars over sugar alcohols such as xylitol and sorbitol. In a real-time monitoring study of biofilm formation, xylitol and erythritol were found to inhibit *S. mutans* biofilm formation in media containing 1 % sucrose [33]. Xylitol and erythrol strongly inhibited the initial biofilm formation, but not the biofilm at 10 h. However, the quantity of RNA in the biofilm after 10 h was much lower than that in the control group. This was due to the sugar alcohol starving bacterial cells, resulting in lower RNA levels in the biofilm. Rubusoside is another non-nutritive sweetener that was found to affect cariogenic characteristics and expression of virulence-related genes in *S. mutans* biofilms [27]. When *S. mutans* was exposed to each medium supplemented with 1 % rubusoside, 1 % xylitol, and 1 % sucrose, the presence of rubusoside was found to result in a lower level of acid production compared to the presence of sucrose and xylitol and reduce the level of biofilm accumulation and viability. Rubusoside also inhibited the expression of virulence-related genes such as *atpF, spaP, gbpB, gtfB*, *gtfC*, *gtfD*, *ftf*, *ldh*, *comD,* and *vicR*. Raffinose inhibited the biofilm formation of non-oral pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Rhodococcus qingshengi*, *Clostridium tropical*, and *Bacillus amyloliquefaciens* [87]. In particular, raffinose at 1 μM or higher was also shown to inhibit the formation of mixed biofilms of *S. aureus* and *P. aeruginosa*. In particular, raffinose was found to inhibit *S. mutans* biofilm (formed in a medium supplemented with 10 μM sucrose) formation and Gtf-related gene expression [28]. In addition, 10 % acesulfame-K and 7.5 % sucralose inhibited the biofilm formation of *Porphyromonas gingivalis*, an anaerobic periodontal pathogen, and showed bactericidal activity against bacteria within the biofilm [88]. Stevioside also inhibited the formation of mixed biofilms of *C. albicans* and *S. mutans* and acid production [29]. Stevioside facilitated the metabolic utilization of galactose and intracellular polysaccharides while reducing that of sucrose. In addition, the presence of stevioside was also found to inhibit the transformation of *C. albicans*, which reduces pathogenicity. The artificial sweetener acesulfame-K reduced the expression of genes encoding Bap (biofilm-associated protein) as well as genes encoding Csu pili (related to twitching motility) in *Acinetobacter baumannii* [30]. In particular, acesulfame-K decreased the twitching motility of *A. baumannii* in a dose-dependent manner. These results showed that acesulfame-K could be employed as a therapeutic agent by reducing *A. baumannii* biofilm development and twitching motility.

### 4.3. Combined use of sweeteners to target microbial biofilm formation

Due to the biofilm-forming capabilities of some bacteria, such as *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp., it may be challenging to treat infections caused by these ESKAPE bacterial strains [89]. These pathogenic biofilms are enclosed by EPS, which protects cells from antimicrobial agents [20]. Limited penetration of drugs due to the

**Table 2**
Inhibition of oral biofilm formation by bulk and non-nutritive sweeteners.

| Product | Studied microorganism | Testing method | Active concentration | Surface material | Mechanism | References |
|---|---|---|---|---|---|---|
| Xylitol | *Streptococcus mutans*<br>*S. sanguinis*<br>*Actinomyces naeslundii* | *In situ* | 5 % | Hydroxyapatite discs | – | [31] |
| D-tagatose | *S. mutans* | *In vitro* | 1, 4 % | Plastic discs | Interfered with glucosyltransferase (Gtf) activity | [26] |
| Sorbitol<br>Xylitol | *Candida albicans* + *S. mutans* | *In vitro* | 10 % | 96-well plates | – | [32] |
| Erythritol<br>Xylitol | *S. mutans* | *In vitro* | 1, 2, 5 % | 16-well electronic microtiter plates | Modified the expression levels of *gbpB*, *gtfB*, *gtfC,* and *gtfD* genes that were important in polysaccharide-mediated adherence of *S. mutans* | [33] |
| Rubusoside | *S. mutans* | *In vitro* | 1 % | 24-well microtiter plates | Downregulated virulence gene expression (*gtfB*, *gtfC*, *gbpB*, *ldh*, and *comD*) | [27] |
| Raffinose | *Staphylococcus aureus* + *Pseudomonas aeruginosa* | *In vitro* | 1–1000 μM | Polystyrene microtiter plates | – | [87] |
| Raffinose | *S. mutans* | *In vitro* | 1000 μM | Saliva-coated hydroxyapatite discs | Inhibited Gtf-related gene expression | [28] |
| Acesulfame-K<br>Sucralose | *Porphyromonas gingivalis* | *In vitro* | 10 % acesulfame-K<br>7.5 % sucralose | 96-well plates | – | [88] |
| Stevioside | *C. albicans* + *S. mutans* | *In vitro* | 1 % | 24-well microplate plates | Decreased sucrose metabolism and increased galactose and intracellular polysaccharide metabolism in *S. mutans*<br>Decreased genes related to glycosylphosphatidylinositol-modified proteins and secreted aspartyl proteinase family in *C. albicans* | [29] |
| Acesulfame-K | *Acinetobacter baumannii* | *In vitro*<br>*Ex vivo* | 8.85 % | Glass microscope slides<br>Porcine skins | Disabled virulence behaviors such as biofilm formation, motility, and the ability to acquire exogenous antibiotic-resistant genes | [30] |

presence of EPS within the biofilm contributes to high levels of tolerance to conventional antibiotics [90]. To address this issue, a treatment strategy has been developed in which biofilm-dispersing and antimicrobial agents are co-administered [91]. The biofilm-dispersing agent effectively dispersed the biofilm and thus facilitated the penetration of antimicrobial agents into bacterial cells [92]. Moreover, this combination therapy not only produces a greater antibacterial effect at a lower dose but also reduces the possibility of the emergence of multidrug-resistant bacteria [7]. The combination of sweeteners and antimicrobial agents this way was also effective in controlling several other pathogenic biofilms as well, including oral biofilms (Table 3).

The combination of xylitol and lactoferrin exhibited a cooperative inhibitory effect on *P. aeruginosa* biofilms [34]. Here, lactoferrin treatment destabilized *P. aeruginosa* cell membrane through iron chelation. The combination with xylitol produced a synergistic effect, as membrane destabilization by lactoferrin enhanced the penetration of xylitol into bacterial cells. The combination of xylitol and ursolic acid was also found to significantly inhibit biofilm formation by *S. mutans* and *S. sobrinus* via synergistic interactions [7]. This combination also prevented the pH from falling below 5.5, effectively preventing tooth demineralization. The combination treatment with ribose and xylitol inhibited *S. mutans* and *S. sobrinus* biofilms more effectively than treatment with ribose or xylitol treatment alone [35]. Moreover, ribose-xylitol combination treatment also significantly downregulated biofilm formation and expression of dextran-dependent aggregation-related genes (*gpbC* and *dblB*) compared to treatment with ribose or xylitol alone. The combination of xylitol and bacteriophages inhibited biofilm formation as well through the release of DNA and proteins from a mixed biofilm of *P. aeruginosa* and *Klebsiella pneumoniae* [93]. This finding was attributed to the bacteriophages facilitating the penetration of xylitol by destroying the cell layers. In combination with the zwitterionic molecule betaine and sugar alcohol erythritol, the betaine-erythritol complex was found to induce spontaneous detachment of *S. mutans* biofilm from the surface [36]. The anionic site of betaine binding erythritol, and the remaining cationic site allowed the complex to be transferred to the negatively charged exopolysaccharide of *S. mutans* biofilm. In addition, the hydroxyl group of the sugar alcohol interfered with hydrogen bonding between the hydroxyl groups of the exopolysaccharide, which was promoted by the formation of a complex with the zwitterion. Combined treatment with xylitol and isothiazolones showed a synergistic inhibitory effect on early biofilm formation by *S. aureus* and *P. aeruginosa* [94]. Furthermore, a combination of xylitol and erythritol showed cooperative inhibitory effects against cariogenic biofilms of *S. mutans*, *S. sobrinus*, and *Scardovia wiggsiae* [95]. The combination with a high xylitol ratio effectively inhibited the growth of *S. sobrinus* and *S. wiggsiae*, whereas the combination with a high erythritol ratio effectively inhibited the growth of *S. mutans*.

### 4.4. Applications of sweeteners as formulation forms to target microbial biofilms

New types of antibacterial substances in forms such as conjugates and nanoparticles have been developed to combat biofilm infections. Nanoparticles can easily interact with microorganisms owing to their small sizes and high surface-area-to-volume ratios [96,97]. In addition, they can also serve as carriers of antibacterial agents for drug administration [39,98]. Nanoparticles loaded with sweeteners have been developed to improve biofilm penetration of xylitol [37]. These sweetener formulations were found to effectively control mono- or poly-microbial biofilms, the mechanisms of which are described in Table 4.

A coordination compound composed of zinc chloride and erythritol effectively eliminated mature *S. mutans* biofilms [38]. The antibiofilm activity of the zinc-erythritol complex was attributed to the facilitation of zinc penetration into mature biofilms by erythritol. Poly-lactic-*co*-glycolic acid (PLGA) nanoparticles incorporating xylitol have also been shown to exert antibiofilm activities against the poly-microbial biofilms of *S. aureus* and *P. aeruginosa* [37]. PLGA nanoparticles containing xylitol showed high levels of biofilm activity by enhancing the penetration of *S. aureus* and *P. aeruginosa* biofilms into EPS. In a study synthesizing non-nutritive decorated gold nanoparticles, aspartame-decorated gold nanoparticles showed stronger antibacterial and antibiofilm effects against Carbapenem-resistant *Enterobacteriaceae* than gold nanoparticles decorated with saccharin, sucralose, and acesulfame [39]. Gold nanoparticles decorated with aspartame showed an antibacterial effect by accumulating reactive oxygen species in bacteria and improving internal membrane permeability. In addition, the inhibition of biofilm formation by aspartame was also observed, indicating that the antibiofilm activity of aspartame-gold nanoparticles may be due to the decoration of aspartame.

**Table 4**
Applications of sweeteners as formulation forms to target pathogenic biofilm.

| Formulation type | Studied microorganism | Mechanism | References |
|---|---|---|---|
| Zinc-erythritol complex | *Streptococcus mutans* | Removed mature biofilms due to metal ions and the coordination properties of sugar alcohols | [38] |
| PLGA/xylitol nanoparticles | *Pseudomonas aeruginosa* + *Staphylococcus aureus* | Penetrated the biofilm matrix as compared to the xylitol solution and hence facilitated the release of the drug inside the biofilm matrix | [37] |
| Aspartame-gold nanoparticles | Carbapenem-resistant *Enterobacteriaceae* | Inhibited biofilm formation from the decoration of aspartame | [39] |

**Table 3**
Combinatorial applications of sweeteners with antimicrobials to target pathogenic biofilm.

| Sweeteners | Other product | Studied microorganism | Mechanism | References |
|---|---|---|---|---|
| Xylitol | Lactoferrin | *Pseudomonas aeruginosa* | Inhibited the ability of biofilms to respond to environmental iron restriction | [34] |
| Xylitol | Ursolic acid | *Streptococcus mutans* *S. sobrinus* | Exhibited antibiofilm activity while preventing tooth demineralization by raising the pH above the threshold of 5.5 | [7] |
| Ribose | Xylitol | *S. mutans* *S. sobrinus* | Inhibited the expression of dextran-dependent aggregation-responsible genes | [35] |
| Xylitol | Bacteriophages | *Klebsiella pneumoniae* + *P. aeruginosa* | Promoted bacteriophage host penetration by xylitol | [93] |
| Erythritol | Betaine | *S. mutans* | Reduced adhesive forces of the biofilms due to an increase in solubility of exopolysaccharides | [36] |
| Xylitol | Isothiazolone | *P. aeruginosa* *S. aureus* | – | [94] |
| Erythritol | Xylitol | *S. mutans* *S. sobrinus* | – | [95] |

## 5. Conclusion and future perspectives

The use of nutritive as well as non-nutritive sweeteners has increased in food, beverage, and pharmaceutical industries in recent years to enhance palatability. On the other hand, with technological advances, evidence suggests that excessive dietary sugar consumption, in conjunction with unhealthy nutrition and physical activity habits, can have detrimental health consequences [99,100]. In particular, dietary sugars promote the growth of aciduric and acidogenic bacteria, which form acids from sugars and thus lead to tooth demineralization and dental cavities when used regularly. Additionally, dietary sugars facilitate the synthesis of EPS by bacteria, thereby contributing to biofilm antimicrobial tolerance. Therefore, bulk and non-nutritive sweeteners have gained popularity as alternatives to dietary sugars in this regard. Furthermore, numerous studies have also indicated that bulk and non-nutritive sweeteners show antibacterial effects that reduce virulence and inhibit biofilm formation. With a growing number of studies on antibiofilm activities of bulk and non-nutritive sweeteners, a variety of alternative formulations for use in industry have also been developed. Solutions include combining bulk sweeteners or non-nutritive sweeteners with antimicrobials as well as formulations, all of which were found to boost antibiofilm activities against microbial pathogens. Future perspectives that will assist in improving our understanding of the mechanism of action of sweeteners on microbial biofilms are summarized as follows.

- To determine the mechanism of actions involved, it will be necessary to establish structure-activity relationships for sweetener-mediated regulation of biofilms and virulence-regulating proteins.
- Encapsulating sweeteners in nanomaterials may allow for targeted applications for the elimination of microbial biofilms.
- Most of the studies on sweetener-enhanced biofilm development have been conducted *in vitro*. However, *in vivo* research is also required to imitate the host environment and elucidate the influence of host factors on biofilm activity.
- Because the majority of nutritive and non-nutritive sweeteners contain hydroxyl groups, which act as a good reducing and capping agent [99,101,102], nanoparticle production utilizing these sweeteners as a reducing and capping agent is required to broaden their antibiofilm action against oral and non-oral biofilm-forming microbial pathogens.
- Due to the presence of poly-microbial interactions in the host system, antibiofilm and antivirulence activities must be performed utilizing poly-microbial pathogens *in vitro* or *in vivo* for future applications in the host system.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

Not applicable.

## Authorship contribution statement

Geum-Jae Jeong: Literature Search, Writing-original Draft & Editing, Fazlurrahman Khan: Conceptualization, Funding, Supervising, Literature Search, Writing-review & Editing, Nazia Tabassum: Literature search, Writing & Editing, and Young-Mog Kim: Supervision, Funding, Writing-review & Editing.

## Funding

This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) of Korea, funded by the Ministry of Education (RS-2023-00241461 to F. Khan). This research was also supported by the Basic Science Research Program through the NRF of Korea grant funded by the Ministry of Education (2021R1A6A1A03039211 and 2022R1A2B5B01001998).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Acknowledgments

Parts of Figs. 2 and 3 were created using templates from Servier Medical Art, which is available under a Creative Commons Attribution 3.0 Unported License.

## Abbreviations

| | |
|---|---|
| EPS | extracellular polymeric substances |
| Ftf | fructosyltransferase |
| Gbp | glucan-binding protein |
| Gtf | glucosyltransferase |
| PGLA | polylactic-*co*-glycolic acid |

## References

[1] Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148:1258–70. https://doi.org/10.1016/j.cell.2012.01.035.
[2] Bokulich NA, Blaser MJ. A bitter aftertaste: unintended effects of artificial sweeteners on the gut microbiome. Cell Metabol 2014;20:701–3. https://doi.org/10.1016/j.cmet.2014.10.012.
[3] Wang Q-P, Browman D, Herzog H, Neely GG. Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. PLoS One 2018;13:e0199080. https://doi.org/10.1371/journal.pone.0199080.
[4] Di Rienzi SC, Britton RA. Adaptation of the gut microbiota to modern dietary sugars and sweeteners. Adv Nutr 2020;11:616–29. https://doi.org/10.1093/advances/nmz118.
[5] Leme AP, Koo H, Bellato C, Bedi G, Cury J. The role of sucrose in cariogenic dental biofilm formation—new insight. J Dent Res 2006;85:878–87. https://doi.org/10.1177/154405910608501002.
[6] Lemos J, et al. The biology of *Streptococcus mutans*. Microbiol Spectr 2019;7. https://doi.org/10.1128/microbiolspec.gpp3-0051-2018. 10.1128/microbiolspec. gpp.1123–0051-2018.
[7] Zou Y, Lee Y, Huh J, Park J-W. Synergistic effect of xylitol and ursolic acid combination on oral biofilms. Restor. Dent. Endod. 2014;39:288–95. https://doi.org/10.5395/rde.2014.39.4.288.
[8] Nguyen P-T, et al. Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications. AIMS Microbiol 2020;6:451. 10.3934/microbiol.2020027.
[9] Donot F, et al. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carbohydr Polym 2012;87:951–62. https://doi.org/10.1016/j.carbpol.2011.08.083.
[10] Cai J-N, Jung J-E, Lee M-H, Choi H-M, Jeon J-G. Sucrose challenges to *Streptococcus mutans* biofilms and the curve fitting for the biofilm changes. FEMS Microbiol Ecol 2018;94:fiy091. https://doi.org/10.1093/femsec/fiy091.
[11] Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol 2015;5:10. https://doi.org/10.3389/fcimb.2015.00010.
[12] Hwang G, et al. *Candida albicans* mannans mediate *Streptococcus mutans* exoenzyme GtfB binding to modulate cross-kingdom biofilm development *in vivo*. PLoS Pathog 2017;13:e1006407. https://doi.org/10.1371/journal.ppat.1006407.
[13] Khan F, et al. Mixed biofilms of pathogenic *Candida*-bacteria: regulation mechanisms and treatment strategies. Crit Rev Microbiol 2021;47:699–727. https://doi.org/10.1080/1040841X.2021.1921696.

[14] Khan F, et al. Surface adherence and vacuolar internalization of bacterial pathogens to the *Candida* spp. cells: mechanism of persistence and propagation. J Adv Res 2022. https://doi.org/10.1016/j.jare.2022.12.013.

[15] Ciofu O, et al. Tolerance and resistance of microbial biofilms. Nat Rev Microbiol 2022;20:621–35. https://doi.org/10.1038/s41579-022-00682-4.

[16] Alam F, et al. *Candida albicans* enhances meropenem tolerance of *Pseudomonas aeruginosa* in a dual-species biofilm. J Antimicrob Chemother 2020;75:925–35. https://doi.org/10.1093/jac/dkz514.

[17] Harriott MM, Noverr MC. Ability of *Candida albicans* mutants to induce *Staphylococcus aureus* vancomycin resistance during polymicrobial biofilm formation. Antimicrob Agents Chemother 2010;54:3746–55. https://doi.org/10.1128/aac.00573-10.

[18] Harriott MM, Noverr MC. *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrob Agents Chemother 2009;53:3914–22. https://doi.org/10.1128/aac.00657-09.

[19] Bowen WH, Burne RA, Wu H, Koo H. Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. Trends Microbiol 2018;26:229–42. https://doi.org/10.1016/j.tim.2017.09.008.

[20] Costa OY, Raaijmakers JM, Kuramae EE. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol 2018;9:1636. https://doi.org/10.3389/fmicb.2018.01636.

[21] Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners–a review. J Food Sci Technol 2014;51:611–21. https://doi.org/10.1007/s13197-011-0571-1.

[22] Edwards CH, Rossi M, Corpe CP, Butterworth PJ, Ellis PR. The role of sugars and sweeteners in food, diet and health: alternatives for the future. Trends Food Sci Technol 2016;56:158–66. https://doi.org/10.1016/j.tifs.2016.07.008.

[23] Liauchonak I, Qorri B, Dawoud F, Riat Y, Szewczuk MR. Non-nutritive sweeteners and their implications on the development of metabolic syndrome. Nutrients 2019;11:644. https://doi.org/10.3390/nu11030644.

[24] Rother KI, Conway EM, Sylvetsky AC. How non-nutritive sweeteners influence hormones and health. Trends Endocrinol Metabol 2018;29:455–67. https://doi.org/10.1016/j.tem.2018.04.010.

[25] Praveena SM, Cheema MS, Guo H-R. Non-nutritive artificial sweeteners as an emerging contaminant in environment: a global review and risks perspectives. Ecotoxicol Environ Saf 2019;170:699–707. https://doi.org/10.1016/j.ecoenv.2018.12.048.

[26] Hasibul K, et al. D-Tagatose inhibits the growth and biofilm formation of *Streptococcus mutans*. Mol Med Rep 2018;17:843–51. https://doi.org/10.3892/mmr.2017.8017.

[27] Guan C, et al. Effect of rubusoside, a natural sucrose substitute, on *Streptococcus mutans* biofilm cariogenic potential and virulence gene expression *in vitro*. Appl Environ Microbiol 2020;86:e01012-01020. https://doi.org/10.1128/AEM.01012-20.

[28] Ham S-Y, et al. Raffinose inhibits *Streptococcus mutans* biofilm formation by targeting glucosyltransferase. Microbiol Spectr 2022;10:e02076-02021. https://doi.org/10.1128/spectrum.02076-21.

[29] Guo M, et al. Inhibitory effects of Stevioside on *Streptococcus mutans* and *Candida albicans* dual-species biofilm. Front Microbiol 2023;14:1128668. https://doi.org/10.3389/fmicb.2023.1128668.

[30] de Dios R, et al. Artificial sweeteners inhibit multidrug-resistant pathogen growth and potentiate antibiotic activity. EMBO Mol Med 2023;15:e16397. https://doi.org/10.15252/emmm.202216397.

[31] Marttinen AM, et al. Effects of xylitol on xylitol-sensitive versus xylitol-resistant *Streptococcus mutans* strains in a three-species *in vitro* biofilm. Curr Microbiol 2012;65:237–43. https://doi.org/10.1007/s00284-012-0151-2.

[32] Chan A, et al. Inhibitory effects of xylitol and sorbitol on *Streptococcus mutans* and *Candida albicans* biofilms are repressed by the presence of sucrose. Arch Oral Biol 2020;119:104886. https://doi.org/10.1016/j.archoralbio.2020.104886.

[33] Loimaranta V, Mazurel D, Deng D, Söderling E. Xylitol and erythritol inhibit real-time biofilm formation of *Streptococcus mutans*. BMC Microbiol 2020;20:1–9. https://doi.org/10.1186/s12866-020-01867-8.

[34] Ammons MCB, Ward LS, Dowd S, James GA. Combined treatment of *Pseudomonas aeruginosa* biofilm with lactoferrin and xylitol inhibits the ability of bacteria to respond to damage resulting from lactoferrin iron chelation. Int J Antimicrob Agents 2011;37:316–23. https://doi.org/10.1016/j.ijantimicag.2010.12.019.

[35] Lee H-J, et al. Synergistic inhibition of Streptococcal biofilm by ribose and xylitol. Arch Oral Biol 2015;60:304–12. https://doi.org/10.1016/j.archoralbio.2014.11.004.

[36] Lim JH, Song S-H, Park H-S, Lee JR, Lee S-M. Spontaneous detachment of *Streptococcus mutans* biofilm by synergistic effect between zwitterion and sugar alcohol. Sci Rep 2017;7:8107. https://doi.org/10.1038/s41598-017-08558-x.

[37] Anjum A, Chung P-Y, Ng S-F. PLGA/xylitol nanoparticles enhance antibiofilm activity via penetration into biofilm extracellular polymeric substances. RSC Adv 2019;9:14198–208. https://doi.org/10.1039/C9RA00125E.

[38] Lim JH, et al. Penetration of an antimicrobial zinc-sugar alcohol complex into *Streptococcus mutans* biofilms. Sci Rep 2018;8:16154. https://doi.org/10.1038/s41598-018-34366-y.

[39] Liu H, et al. A potential strategy against clinical carbapenem-resistant *Enterobacteriaceae*: antimicrobial activity study of sweetener-decorated gold nanoparticles *in vitro* and *in vivo*. J Nanobiotechnol 2023;21:1–23. https://doi.org/10.1186/s12951-023-02149-x.

[40] Whitehouse CR, Boullata J, McCauley LA. The potential toxicity of artificial sweeteners. AAOHN J 2008;56:251–61. https://doi.org/10.1177/216507990805600604.

[41] Lee CC, Shallenberger R, Vittum M. Free sugars in fruits and vegetables. 1970.

[42] Voragen AG. Technological aspects of functional food-related carbohydrates. Trends Food Sci Technol 1998;9:328–35. https://doi.org/10.1016/S0924-2244(98)00059-4.

[43] Ni D, et al. Comprehensive utilization of sucrose resources via chemical and biotechnological processes: a review. Biotechnol Adv 2022;60:107990. https://doi.org/10.1016/j.biotechadv.2022.107990.

[44] Rippe J, Angelopoulos T. Added sugars and risk factors for obesity, diabetes and heart disease. Int J Obes 2016;40:S22–7. https://doi.org/10.1038/ijo.2016.10.

[45] Sheiham A. Sucrose and dental caries. Nutr Health 1987;5:25–9. https://doi.org/10.1177/026010608700500202.

[46] Al Humaid J. Sweetener content and cariogenic potential of pediatric oral medications: a literature. Int J Health Sci 2018;12:75.

[47] Koulourides T, et al. Cariogenicity of nine sugars tested with an intraoral device in man. Caries Res 1976;10:427–41. https://doi.org/10.1159/000260235.

[48] Edgar W, Dodds M. The effect of sweeteners on acid production in plaque. Int Dent J 1985;35:18–22.

[49] Shaw J, Krumins I, Gibbons R. Comparison of sucrose, lactose, maltose and glucose in the causation of experimental oral diseases. Arch Oral Biol 1967;12:755–68. https://doi.org/10.1016/0003-9969(67)90211-7.

[50] Castro-Muñoz R, et al. Natural sweeteners: sources, extraction and current uses in foods and food industries. Food Chem 2022;370:130991. https://doi.org/10.1016/j.foodchem.2021.130991.

[51] Ding S, Yang J. The effects of sugar alcohols on rheological properties, functionalities, and texture in baked products–a review. Trends Food Sci Technol 2021;111:670–9. https://doi.org/10.1016/j.tifs.2021.03.009.

[52] Rice T, Zannini E, Arendt E K, Coffey A. A review of polyols–biotechnological production, food applications, regulation, labeling and health effects. Crit Rev Food Sci Nutr 2020;60:2034–51. https://doi.org/10.1080/10408398.2019.1625859.

[53] Gwak M-J, Chung S-J, Kim YJ, Lim CS. Relative sweetness and sensory characteristics of bulk and intense sweeteners. Food Sci Biotechnol 2012;21:889–94. https://doi.org/10.1007/s10068-012-0115-0.

[54] Edgar W. Sugar substitutes, chewing gum and dental caries-a review. Br Dent J 1998;184:29–32. https://doi.org/10.1038/sj.bdj.4809535.

[55] Nayak PA, Nayak UA, Khandelwal V. The effect of xylitol on dental caries and oral flora. Clin Cosmet Invest Dent 2014:89–94. https://doi.org/10.2147/CCIDE.S55761.

[56] Gibbons RJ, Houte J. Bacterial adherence in oral microbial ecology. Annu Rev Microbiol 1975;29:19–42. https://doi.org/10.1146/annurev.mi.29.100175.000315.

[57] Meurman JH. Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci 2005;113:188–96. https://doi.org/10.1111/j.1600-0722.2005.00191.x.

[58] Kuboniwa M, Lamont RJ. Subgingival biofilm formation. Periodontol 2000;52:38. https://doi.org/10.1111/j.1600-0757.2009.00311.x. 2010.

[59] Pitts NB, et al. Dental caries. Nat Rev Dis Prim 2017;3:1–16. https://doi.org/10.1038/nrdp.2017.30.

[60] Kreth J, Zhu L, Merritt J, Shi W, Qi F. Role of sucrose in the fitness of *Streptococcus mutans*. Oral Microbiol Immunol 2008;23:213–9. https://doi.org/10.1111/j.1399-302X.2007.00413.x.

[61] Bowen W, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res 2011;45:69–86. https://doi.org/10.1159/000324598.

[62] Russell R, Donald AC, Douglas C. Fructosyltransferase activity of a glucan-binding protein from *Streptococcus mutans*. Microbiology 1983;129:3243–50. https://doi.org/10.1099/00221287-129-10-3243.

[63] Aoki H, Shiroza T, Hayakawa M, Sato S, Kuramitsu H. Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. Infect Immun 1986;53:587–94. https://doi.org/10.1128/iai.53.3.587-594.1986.

[64] Hanada N, Kuramitsu HK. Isolation and characterization of the *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucans. Infect Immun 1988;56:1999–2005. https://doi.org/10.1128/iai.56.8.1999-2005.1988.

[65] Baird J, Longyear V, Ellwood D. Water insoluble and soluble glucans produced by extracellular glycosyltransferases from *Streptococcus mutans*. Microbios 1973;8:143–50.

[66] Rozen R, Bachrach G, Bronshteyn M, Gedalia I, Steinberg D. The role of fructans on dental biofilm formation by *Streptococcus sobrinus, Streptococcus mutans, Streptococcus gordonii* and *Actinomyces viscosus*. FEMS Microbiol Lett 2001;195:205–10. https://doi.org/10.1111/j.1574-6968.2001.tb10522.x.

[67] Banas J, Vickerman M. Glucan-binding proteins of the oral streptococci. Crit Rev Oral Biol Med 2003;14:89–99. https://doi.org/10.1177/154411130301400203.

[68] Matsumoto-Nakano M. Role of *Streptococcus mutans* surface proteins for biofilm formation. Jpn. Dent. Sci. Rev. 2018;54:22–9. https://doi.org/10.1016/j.jdsr.2017.08.002.

[69] Kawada-Matsuo M, Komatsuzawa H. Role of *Streptococcus mutans* two-component systems in antimicrobial peptide resistance in the oral cavity. Jpn. Dent. Sci. Rev. 2017;53:86–94. https://doi.org/10.1016/j.jdsr.2016.12.002.

[70] Koo H, Falsetta M, Klein M. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. J Dent Res 2013;92:1065–73. https://doi.org/10.1177/0022034513504218.

[71] Klein MI, et al. The role of extracellular polysaccharides matrix in virulent oral biofilms. Norfolk, UK: Caister Academic Press; 2013. p. 69. 67.

[72] Vacca-Smith A, Bowen W. Binding properties of streptococcal glucosyltransferases for hydroxyapatite, saliva-coated hydroxyapatite, and bacterial surfaces. Arch Oral Biol 1998;43:103–10. https://doi.org/10.1016/S0003-9969(97)00111-8.

[73] Du Q, Ren B, Zhou X, Zhang L, Xu X. Cross-kingdom interaction between *Candida albicans* and oral bacteria. Front Microbiol 2022;13:911623. https://doi.org/10.3389/fmicb.2022.911623.
[74] Bamford CV, et al. *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. Infect Immun 2009;77:3696–704. https://doi.org/10.1128/iai.00438-09.
[75] Falsetta ML, et al. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms *in vivo*. Infect Immun 2014;82:1968–81. https://doi.org/10.1128/iai.00087-14.
[76] Decker E-M, Klein C, Schwindt D, Von Ohle C. Metabolic activity of *Streptococcus mutans* biofilms and gene expression during exposure to xylitol and sucrose. Int J Oral Sci 2014;6:195–204. https://doi.org/10.1038/ijos.2014.38.
[77] Marsh PD. Ecological events in oral health and disease: new opportunities for prevention and disease control? J Calif Dent Assoc 2017;45:525–37. https://doi.org/10.1080/19424396.2017.12222502.
[78] Shemesh M, Tam A, Steinberg D. Expression of biofilm-associated genes of *Streptococcus mutans* in response to glucose and sucrose. J Med Microbiol 2007;56:1528–35. https://doi.org/10.1099/jmm.0.47146-0.
[79] Forssten SD, Björklund M, Ouwehand AC. *Streptococcus mutans*, caries and simulation models. Nutrients 2010;2:290–8. https://doi.org/10.3390/nu2030290.
[80] Duarte S, et al. Influences of starch and sucrose on *Streptococcus mutans* biofilms. Oral Microbiol Immunol 2008;23:206–12. https://doi.org/10.1111/j.1399-302X.2007.00412.x.
[81] Cai J-N, et al. Functional relationship between sucrose and a cariogenic biofilm formation. PLoS One 2016;11:e0157184. https://doi.org/10.1371/journal.pone.0157184.
[82] Nagasawa R, Sato T, Senpuku H. Raffinose induces biofilm formation by *Streptococcus mutans* in low concentrations of sucrose by increasing production of extracellular DNA and fructan. Appl Environ Microbiol 2017;83:e00869-00817. https://doi.org/10.1128/AEM.00869-17.
[83] Weerasekera MM, et al. The effect of nutritive and non-nutritive sweeteners on the growth, adhesion, and biofilm formation of *Candida albicans* and *Candida tropicalis*. Med Princ Pract 2018;26:554–60. https://doi.org/10.1159/000484718.
[84] Souza JG, et al. Effect of sucrose on biofilm formed *in situ* on titanium material. J Periodontol 2019;90:141–8. https://doi.org/10.1002/JPER.18-0219.
[85] Waldman LJ, Butera T, Boyd JD, Grady ME. Sucrose-mediated formation and adhesion strength of *Streptococcus mutans* biofilms on titanium. Biofilms 2023;100143. https://doi.org/10.1016/j.bioflm.2023.100143.
[86] Assaf D, Steinberg D, Shemesh M. Lactose triggers biofilm formation by *Streptococcus mutans*. Int Dairy J 2015;42:51–7. https://doi.org/10.1016/j.idairyj.2014.10.008.
[87] Kim H-S, et al. Effect of broad-spectrum biofilm inhibitor raffinose, a plant galactoside, on the inhibition of co-culture biofilm on the microfiltration membrane. J Hazard Mater 2021;402:123501. https://doi.org/10.1016/j.jhazmat.2020.123501.
[88] Shinohara M, et al. Analysis of the effects of food additives on *Porphyromonas gingivalis*. Pathogens 2022;11:65. https://doi.org/10.3390/pathogens11010065.
[89] Bennett AN, et al. Spectrum of activity of *Salmonella* anti-biofilm compounds: evaluation of activity against biofilm-forming ESKAPE pathogens. Biofilms 2023;6:100158. https://doi.org/10.1016/j.bioflm.2023.100158.
[90] McDougald D, Rice SA, Barraud N, Steinberg PD, Kjelleberg S. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nat Rev Microbiol 2012;10:39–50. https://doi.org/10.1038/nrmicro2695.
[91] Hawas S, Verderosa AD, Totsika M. Combination therapies for biofilm inhibition and eradication: a comparative review of laboratory and preclinical studies. Front Cell Infect Microbiol 2022;12:850030. https://doi.org/10.3389/fcimb.2022.850030.
[92] Kiedrowski MR, Horswill AR. New approaches for treating staphylococcal biofilm infections. Ann N Y Acad Sci 2011;1241:104–21. https://doi.org/10.1111/j.1749-6632.2011.06281.x.
[93] Chhibber S, Bansal S, Kaur S. Disrupting the mixed-species biofilm of *Klebsiella pneumoniae* B5055 and *Pseudomonas aeruginosa* PAO using bacteriophages alone or in combination with xylitol. Microbiology 2015;161:1369–77. https://doi.org/10.1099/mic.0.000104.
[94] Zhou G, et al. Enhanced synergistic effects of xylitol and isothiazolones for inhibition of initial biofilm formation by *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538. J Oral Sci 2019;61:255–63. https://doi.org/10.2334/josnusd.18-0102.
[95] Kõljalg S, Smidt I, Chakrabarti A, Bosscher D, Mändar R. Exploration of singular and synergistic effect of xylitol and erythritol on causative agents of dental caries. Sci Res 2020;10:6297. https://doi.org/10.1038/s41598-020-63153-x.
[96] Yin IX, et al. The antibacterial mechanism of silver nanoparticles and its application in dentistry. Int J Nanomed 2020;2555–2562. https://doi.org/10.2147/IJN.S246764.
[97] Khan F, et al. Retrospective analysis of the key molecules involved in the green synthesis of nanoparticles. Nanoscale 2022;14:14824–57. https://doi.org/10.1039/D2NR03632K.
[98] Jeong G-J, Khan F, Tabassum N, Cho K-J, Kim Y-M. Controlling biofilm and virulence properties of Gram-positive bacteria by targeting wall teichoic acid and lipoteichoic acid. Int J Antimicrob Agents 2023;106941. https://doi.org/10.1016/j.ijantimicag.2023.106941.
[99] Hemmati S, et al. Artificial sweeteners and sugar ingredients as reducing agent for green synthesis of silver nanoparticles. J Nanomater 2019:1–16. https://doi.org/10.1155/2019/9641860. 2019.
[100] Ma X, et al. Excessive intake of sugar: an accomplice of inflammation. Front Immunol 2022;13:988481. https://doi.org/10.3389/fimmu.2022.988481.
[101] Katti KK, et al. Facile and general method for synthesis of sugar-coated gold nanoparticles. Int J Green Nanotechnol Biomed 2009;1:B53–9. https://doi.org/10.1080/19430850902983848.
[102] Tavakoli F, et al. Application of glucose as a green capping agent and reductant to fabricate CuI micro/nanostructures. Mater Res Bull 2014;49:14–20. https://doi.org/10.1016/j.materresbull.2013.08.037.