Archives of Oral Biology 80 (2017) 180–184

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Alternative sweeteners influence the biomass of oral biofilm

Fathilah Abdul Razak[a,*], Baizatul Amirah Baharuddin[a], Elisya Farha Mohd. Akbar[a], Amira Hanim Norizan[a], Nur Fazilah Ibrahim[a], Md. Yusoff Musa[b]

[a] Department of Oral and Craniofacial Sciences, Faculty of Dentistry University of Malaya, 50603, Kuala Lumpur, Malaysia
[b] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia

ARTICLE INFO

Keywords:
Oral biofilm
Adhesion
Plaque-mass
Sucrose
Xylitol
Cariogenic potential

ABSTRACT

Objective: Compact-structured oral biofilm accumulates acids that upon prolonged exposure to tooth surface, causes demineralisation of enamel. This study aimed to assess the effect of alternative sweeteners Equal Stevia®, Tropicana Slim®, Pal Sweet® and xylitol on the matrix-forming activity of plaque biofilm at both the early and established stages of formation.

Methods: Saliva-coated glass beads (sGB) were used as substratum for the adhesion of a mixed-bacterial suspension of *Streptococcus mutans, Streptococcus sanguinis* and *Streptococcus mitis*. Biofilms formed on sGB at 3 h and 24 h represented the early and established-plaque models. The biofilms were exposed to three doses of the sweeteners (10%), introduced at three intervals to simulate the exposure of dental plaque to sugar during three consecutive food intakes. The treated sGB were (i) examined under the SEM and (ii) collected for turbidity reading. The absorbance indicated the amount of plaque mass produced. Analysis was performed comparative to sucrose as control.

Results: Higher rate of bacterial adherence was determined during the early compared to established phases of formation. Comparative to the sweeteners, sucrose showed a 40% increase in bacterial adherence and produced 70% more plaque-mass. Bacterial counts and SEM micrographs exhibited absence of matrix in all the sweetener-treated biofilms at the early phase of formation. At the established phase, presence of matrix was detected but at significantly lower degree compared to sucrose ($p < 0.05$).

Conclusion: Alternatives sweeteners promoted the formation of oral biofilm with lighter mass and lower bacterial adherence. Hence, suggesting alternative sweeteners as potential antiplaque agents.

1. Introduction

Sugar is a common household ingredient that adds sweetness to enhance the taste of foods. The sugar consumed in our diet may come from fruits, vegetables, grains and dairy products. Simple sugars like glucose serves as an instant substrate for the generation of energy in the form of sweets and drinks, and often taken by athletes as instant energy booster prior to sport events. Sucrose is a disaccharide sugar that is easily available in the granulated form, and is the most commonly consumed sugar in human diet (Gupta et al., 2013). Today, a world population of seven billion people consumes roughly 165 million tonnes of sugar, that is 23 kg per capita on average and four billion of this consumers are concentrated in Asia (Arif, 2014). Despite its important stance in the food industry, excess consumption of sucrose has been implicated with several health conditions including diabetes and dental caries.

In relation to oral health, stability and health of the oral cavity is contributed by a number of biotic and abiotic components that co-exist to create dynamic interactions within the oral ecosystem. Many studies have highlighted the importance of sugar as one of the four etiological factors in caries formation (Colby & Russel, 1997). Metabolism of fermentable sugars such as sucrose, fructose and glucose by oral streptococci produces acidic by-products in oral biofilm which in thin plaque, are continuously cleansed by oral fluids to neutralize and maintain pH within the biofilm (Marsh, 1994; Samaranayake, 2002). The type of organic acids produced may vary from one strain of streptococci to another as it is determined by the availability and type of the sugar substrate. When the availability of sugar is high, its utilisation by *S. mutans* is more efficient comparative to other streptococci. Under such condition, lactate dehydrogenase is activated which then accelerates the production of lactic acid (Banas, 2004). When lactate becomes a major component of plaque acids, the enamel structure becomes vulnerable as demineralization of the enamel begins at a critical pH of 5.5 and less (Ferguson, 2006; Leme, Koo, Bellato,

* Corresponding author.
E-mail address: fathilah@um.edu.my (F. Abdul Razak).

http://dx.doi.org/10.1016/j.archoralbio.2017.04.014
Received 4 July 2016; Received in revised form 18 March 2017; Accepted 17 April 2017

Bedi, & Cury, 2006; Marsh & Martin, 1999). When the provision of substrate is low, pyruvate-formate lyase instead becomes activated to produce organic acids of lower acidity such as formate (Marsh & Martin, 1999; Washio & Takahashi, 2016).

Apart from efficiently carrying out acidogenic activities, streptococci possess extracellular glycosyltransferase (GTF) and/or fructosyltransferase (FTF) that catalyse the formation of extracellular polysaccharides (ECP) such as glucans and/or fructans when the supply of sucrose is in excess (Schonfeld, 1992; Weiger, Netuschil, von Ohle, Schlagenhauf, & Brecx, 1995). ECP forms the backbone of the biofilm matrix and contribute to 50–95% of its dry weight (Gupta et al., 2013). If a less soluble ECP such as the branched mutan that is synthesised by *S. mutans* is produced, a voluminous sticky biofilm matrix is formed (Bradshaw, Marsh, Watson, & Allison, 1997; Marsh & Bradshaw, 1999). The non-porous biofilm retains acid metabolites close to the tooth surface which upon long exposure, makes the enamel structure unstable and susceptible to demineralisation (Dawes, 2003; Ferguson, 2006). ECP also serves as a continuous source of substrate for saccharolytic resident bacteria. Due to these reasons, sucrose and other fermentable carbohydrates are categorised as cariogenic or caries-promoting sugars.

Sugar substitutes or alternative sweeteners have been shown to be effective in reducing the prevalence of dental caries as many of them are not metabolised to acid by plaque bacteria (ElSalhy, Sayed Zahid, & Honkala, 2012; Gupta et al., 2013; Matsukubo & Takazoe, 2006). Sweeteners can be categorised as carbohydrate or non-carbohydrate origin. Carbohydrate sweeteners include sugar alcohols such as sorbitol and xylitol while non-carbohydrate sweeteners are chemically synthesised such as saccharin, aspartame and sucralose (Gupta et al., 2013; Maguire & Rugg-Gunn, 2003; Matsukubo & Takazoe, 2006). Sorbitol is the most frequently used non-sugar sweetener. Although sorbitol could be adapted by the oral microbiota as a substrate, studies showed no increase in caries upon its frequent use (Hogg & Rugg-Gun, 1991; Marsh & Bradshaw, 1999). Non-carbohydrate sweeteners are usually calorie-free high-intensity sweeteners which accounts for their popular usage in slimming and health care products. Plant derived sweeteners such as stevioside and thaumatins are also calorie free (Matsukubo & Takazoe, 2006). A lot of previous works had shown the inability of alternative sweeteners to produce acids but not many had focus on their effects on the ecology and structure of dental plaque.

This study aimed to assess the effect of several alternative sweeteners which include Equal Stevia®, Tropicana Slim®, Pal Sweet® and xylitol on the matrix-forming activity of plaque bacteria, at both early and established stages of biofilm formation. The microbial component of the plaque biofilm includes *S. mutans, S. mitis* and *S. sanguinis* which constitute the dominant species in early dental biofilm. The cariogenic potential of the sweeteners was comparatively evaluated and analysed with reference to sucrose.

2. Materials and methods

2.1. Preparation of bacterial suspension

Stock cultures of *S. mutans, S. sanguinis* and *S. mitis* were separately revived in brain heart infusion (BHI) broth and incubated at 37 °C for 18 h. The bacteria cells were then washed in PBS and harvested by centrifugation at 3000 rpm, 4 °C, for 15 min. Bacterial suspension of each species was prepared in nutrient broth and the turbidity was standardised at an optical density (OD) of 0.144 at 550 nm. At this absorbance, the concentration of cells is standardised to about 10^6 cells/ml, an equivalent of McFarland Standard # 0.5 (BioMerieux, France) (Fathilah & Rahim, 2003).

2.2. Collection of saliva and preparation of saliva-coated glass beads

3 ml of whole stimulated saliva was collected from a healthy subject. The saliva was first clarified by low speed centrifugation to remove debris and then passed through a filter of 0.2 μm pore size to sterilize before it was poured into a Petri dish. Filtration is the most common method used for saliva sterilization although the process may reduce the amount of total salivary protein (Ruhl et al., 2011). Sterile glass beads of 3 mm diameter were introduced and the dish was gently swirled on a rocker for 2 min to allow coating of the beads by the saliva. The saliva-coated glass beads (sGB) represent the pellicle-coated substratum for plaque formation.

Table 1
Composition list and manufacturer's information of each of the test sweeteners used in the study.

Test sweeteners	Presentation	Constituents
Sucrose	Powder	Pure sucrose
Xylitol	Powder	Pure xylitol
Tropicana Slim®	Powder	Aspartame Sorbitol Corn powder
Pal Sweet®	Powder	Aspartame Acesulfame Lactose
Equal Stevia®	Powder	Stevia Erythritol Cellulose powder

Table 2
Adherent streptococci to saliva-coated glass beads indicated the colonization of bacteria at the early 3 h and established 24 h phases of biofilm formation.

Plaque age (h)	Adherent cells ($\times 10^6$ cells/mL)	Colonization rate ($\times 10^3$ adhering cells/h)
0	0	–
3	1.4 ± 0.6	467
24	3.5 ± 0.4	146

2.3. Preparation of sucrose and alternative sweeteners

Equal Stevia®, Pal Sweet®, Tropicana Slim® and xylitol were used as test sweeteners and sucrose was included as the positive control. Treatment with distilled water was also performed to represent a negative control. Details on the composition and manufacturer's information of the respective sweeteners are presented in Table 1. All test sweeteners were prepared to a standardised concentration of 10%. The test sweeteners were filter-sterilised and stored at 4 °C in sterile centrifuge tubes prior to use.

2.4. Preparation of dental plaque models

6 ml of mixed-bacterial suspension consisting of equal ratio (1:1:1) of *S. mutans, S. sanguinis* and *S. mitis* were pipetted into sterile Petri dishes. sGBs were aseptically introduced and immersed into the suspension. The Petri dishes were then incubated in a shaking incubator at 37 °C. After 3 h the Petri dishes were removed and the beads with biofilm formed on the surfaces were used to represent a 3 h-plaque model. Similar procedure was repeated with an extension of the incubation period to 24 h for the 24 h-plaque model. The former represented biofilm at the early stage, while the later at the established stage of formation. The population of adherent bacteria in the 3 h-plaque and 24 h-plaque models was determined and compared.

2.5. Determination of effect of test sweeteners on 3 h- and 24 h-plaque models

The 3 h- and 24 h-plaque models were separately placed in Petri dishes containing basic Nutrient broth as growth media. Three doses of test sweeteners (10%) were introduced at three intervals to simulate the