치의과학박사 학위논문

The Effects of Xylitol and Sorbitol on the Enzymatic and Candidacidal Activities related with Lysozyme and Peroxidase

Xylitol과 Sorbitol이 Lysozyme과 Peroxidase에 관련된 효소활성 및 Candidacidal 활성에 미치는 영향

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치의과학과 구강내과·진단학 전공

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ABSTRACT

The Effects of Xylitol and Sorbitol on the Enzymatic and Candidacidal Activities related with Lysozyme and Peroxidase

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The purpose of this study was to investigate what xylitol and sorbitol could affect on the enzymatic and candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase system.

Xylitol and sorbitol were added to hen egg-white lysozyme, bovine lactoperoxidase, glucose oxidase-mediated peroxidase, and whole saliva in solution and on the hydroxyapatite surface phases. The enzymatic activities of lysozyme, peroxidase, and glucose oxidase-mediated peroxidase were determined by measuring the turbidity of a *Micrococcus lysodeikticus* suspension, the rate of oxidation to 5,5'-dithiobis-2-nitrobenzoic acid by hypothiocyanite, and the production of oxidized o-dianisidine, respectively. Candidacidal activities were determined by comparing colony forming units

and calculating the percent loss of cell viability using *Candida albicans* ATCC 10231, 11006, and 18804.

Both xylitol and sorbitol did not affect the enzymatic activity of hen egg-white lysozyme both in solution and on the hydroxyapatite surface phases, while both sugar alcohols inhibited the enzymatic activity of salivary lysozyme significantly in solution phase, but not on the surface phase. Both xylitol and sorbitol enhanced the enzymatic activities of both bovine lactoperoxidase and salivary peroxidase significantly by dose-dependent manner in solution phase, but not on the surface phase. Sorbitol, not xylitol, inhibited the enzymatic activity of glucose oxidase-mediated peroxidase significantly. Both xylitol and sorbitol did not affect the candidacidal activities of hen egg-white lysozyme, the bovine lactoperoxidase system, and the glucose oxidase-mediated bovine lactoperoxidase system.

Conclusively, xylitol and sorbitol inhibited salivary lysozyme activity, but enhanced both bovine lactoperoxidase and salivary peroxidase activities significantly in solution phase. Xylitol and sorbitol were not additive to the candidacidal activities related with lysozyme and peroxidase.

Keywords: Xylitol, Sorbitol, Lysozyme, Peroxidase, Glucose oxidase, Candidacidal activity

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I. INTRODUCTION

Xylitol and sorbitol, the most widely used sugar alcohols, have common properties such as the absence of reducing carbonyl group, the ability to complex with metal ions, and the ability to compete with water molecules.[1)] Sugar alcohols have been used as food ingredients for sweetener instead of sucrose, and could help prevent diabetes and obesity because of lowering blood glucose level.[2)] Especially xylitol among sugar alcohols, has been included in many oral health care products because xylitol was proved to be beneficial for the prevention of dental caries by inhibiting glycolytic pathway for bacterial growth and acid production.[3,4)] Xylitol and sorbitol have also been known for alleviating dry mouth by stimulation of salivary secretion like other sweeteners.[5)]

The increase of geriatric population which accompanies the increase of population under medications and chronic illnesses, leads to higher prevalence of individuals with dry mouth.[6)] Individuals who suffer from dry mouth usually experience a variety of signs and symptoms such as ulcerations and pain in oral mucosa, difficulty in chewing and swallowing, rampant dental caries, and recurrent oral candidal infections, which results in deteriorating the quality of life.[7)] These patients have been recommended to use artificial saliva or mouth rinse containing antimicrobials for the recovery of reduced salivary flow and impaired antimicrobial activities.[8)]

The most widely used antimicrobial host proteins are lysozyme and peroxidase mainly originated from animals. Lysozyme distributed in a wide range of biological fluids such as saliva, tears, and respiratory secretions,[9)] provides antimicrobial activity through muramidase-, cation-, and structure-dependent mechanisms.[10,11)] Peroxidase is also ubiquitous and involved in antimicrobial activity and preventing oral tissue damage from oxygen toxicity by consuming hydrogen peroxide and producing hypothiocyanite.[12)] Peroxidase can be activated

with the peroxidase system rather than enzyme alone, and a commercially available form is the glucose oxidase-mediated peroxidase (GO-PO) system.[13)]

Because these antimicrobial molecules are in the same environment, there must be evidently interactions between all components in oral health care products, and between these components and components in human saliva when applied to the oral cavity.[14)] These molecular interactions could occur not only in solution like saliva, but also on surface like tooth in the oral cavity. Immobilized proteins on the surface could cause conformational changes of them, which results in the alteration of their enzymatic activities.[15-17)] Therefore, the interactions on surface behave in a distinct way from those in solution. Interactions between antimicrobial proteins such as peroxidase and sIgA,[18)] peroxidase and lactoferrin,[19,20)] lysozyme and lactoferrin,[21)] lysozyme and histatins,[22)] lysozyme and the peroxidase system,[23,24)] and lysozyme and the GO-PO system,[25)] have been reported. There have also been reports on interaction between antimicrobial supplements and candidate substances of artificial saliva such as animal mucins,[26,27)] hyaluronic acid,[25,28,29)] and yam tuber mucilage.[30,31)] The results of interactions could be additive, synergistic, or inhibitory and the results of surface studies were not the same as those of solution ones. Sugar alcohols and antimicrobials could also be present in the same environment such as oral health care products and the oral cavity, therefore there might be interactions between them. The consumption of a xylitol diet has been reported to increase salivary peroxidase activity.[32)] However, there has been no report what sugar alcohols affect the enzymatic activities of important antimicrobials in solution and on the hydroxyapatite (HA) surface. There is also no report the effects of sugar alcohols on the candidacidal activities of antimicrobials commonly incorporated in artificial saliva products. The purpose of this study was to investigate the effects of xylitol and sorbitol on the enzymatic and candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system.

II. REVIEW OF LITERATURE

1. Sugar alcohols

(1) Chemical profiles

Sugar alcohols are white and sweet crystalline substances derived from aldose sugar. The general formula of them can be expressed as $HOCH_2(CHOH)_nCH_2OH$. Sugar alcohols are divided into different types according to the length of chain and further differentiated by the spatial orientation of hydroxyl groups.[33] The simple sugar alcohols have following common polyol properties.[34] Sugar alcohols do not have reducing carbonyl group, which makes them chemically less reactive than corresponding aldoses and ketoses. Thus, sugar alcohols can avoid acidogenic and cariogenic development in dental plaque by dietary hexose-based sugars. Sugar alcohols can change coenzymes into chemically reduced products because they have remnant hydrogen atoms. A large amount of hydroxyl groups makes most sugar alcohols readily soluble in saliva and can support the native conformation of salivary proteins. Sugar alcohols can form complex formation with polyvalent cation like Ca^{2+} via polyoxy structure, which facilitates remineralization of caries lesions.[35,36] Sugar alcohols are also potential source of free radical scavengers in many biological systems. However, sugar alcohols have differences in molecular masses and exert specific selective effects on biological reactions, although they are involved above common polyol properties.

(2) Functions

Ethylen glycol and methanol, the simplest sugar alcohol, are toxic and used as antifreeze, but most of the other sugar alcohols are nontoxic and used as food

additives. Sugar alcohols are not so sweet as sucrose and have less calories than sucrose. Especially xylitol and sorbitol are well known not to be involved in dental caries because they are not metabolized by oral microbes.[37] Most sugar alcohols are not absorbed completely in small intestines, which leads to lower blood glucose level compared with sucrose and be favorable to diabetic patients and low-caloric diet people.[38] Some sugar alcohols often cause gastrointestinal distress like diarrhea and flatulence when overconsumed. Sorbitol can be used as non-stimulant laxatives because of drawing water in to large intestine.[39] Some of sugar alcohols such as erythritol, xylitol, sorbitol and mannitol, extract chilling sense in oral cavity because endothermic reaction is required to dissolve them in saliva.[40]

(3) Xylitol

Xylitol is pentitol and all five carbon atoms of the molecule bind an hydroxyl group. This tridentate ligand $(H\text{-}C\text{-}OH)_3$ reacts with various polyvalent cations and oxyacids in reversible reactions. Xylitol is roughly as sweet as sucrose. One gram of ingested xylitol provides about two calories in human diets, while three calories in sucrose. Thus, xylitol has been widely used as sweetener in the diets of diabetic and hyperglycemic patients. Clinical research of xylitol called Truku sugar studies, were designed to be 2-year heavy loading with xylitol, fructose and sucrose, and 1-year trial with xylitol- or sucrose-sweetened chewing gum in Finland. These studies showed that the replacement of sugar by xylitol reduced caries progression.[42,43] Several other trials are followed such as Yliviesk studies in Finland, Belize studies in central America, mother-child studies in Finland, Sweden and Japan.[44-47] These long-term clinical trials and consensus all over the world suggest that there are sufficient evidences that positive dental benefits result from the use of xylitol as food component. Similar results were obtained from the examples of animal experiments.[48-50] The safety of xylitol

had been thoroughly studied in humans and the long-term use of xylitol was proved not to be harmful.[51] Studies in Hungary and French Polynesia showed that substitution of xylitol for sugar resulted in more effective prevention of dental caries than fluoride use only.[52,53] The recent mother-child studies showed that maternal use of xylitol gum prevented caries in infants more effectively than the topical use of fluoride.[46] Some studies reported that substitution of xylitol for sugar led to remineralization of caries lesions. Other studies suggested that xylitol could exert specific effects on dental caries not shown by hexitols. However, all xylitol studies did not reach positive clinical and biologic findings. A recommended practice is to use 6-7 g of xylitol daily, preferably in 3–5 separate chewing and sucking episodes after main meals.[34] Not only chewing gum but also other xylitol-containing products such as tablets, dentifrice, pacifier, mouth wash, spray, gels and artificial saliva have also been available.

(4) Sorbitol

Sorbitol is a hexitol-type bulk sweetener and its molecular structure is similar to that of D -glucose. Sorbitol is crystalline substance with lower sweet taste and calories compared with sucrose. Sorbitol also shares the common polyol properties, but it is different from xylitol in molecular mass and ligands. Some clinical caries trials were carried out on sorbitol. Five-minutes chewing has been found to provide more meaningful results because prolonged salivation may mask desired pharmacologic sugar alcohol effects. Sorbitol has significantly reduced the incidence of caries when compared with sucrose. However, the efficacy seems to be weaker, when compared with xylitol.[54] The reason may be explained by microbiologic and molecular parameters of sorbitol which normally stimulates the growth of some strains of *Streptococcus mutans*, retards the growth of dental plaque, and is readily convertible to glucose and

fructose serving as substrates for cariogenic, plaque-building organisms in dental plaque. Mixtures of xylitol and sorbitol do exert positive dental effects, more significant decrease of plaque mass and more distinct lowering of oral counts of cariogenic bacteria have been observed.[55)]

2. Salivary antimicrobials

(1) Developments of salivary antimicrobial molecules

Saliva is indispensable for good oral health, because it not only maintains oral functions but it also protects oral tissues from noxious agents derived from microorganisms, food and environmental stress. Saliva protects oral tissues in several ways. Saliva contains many innate or acquired defense systems. During early childhood innate salivary factors work already at almost full capacity. Lysozyme, salivary peroxidase, and hypothiocyanite are already at levels similar to those of adults, while lactoferrin, myeloperoxidase, and total protein are still insufficient in the saliva of infants.[56)] All non-immune factors reach adult levels by the early teens.[57)] These factors remain at high concentrations even among elderly people.[58)] Salivary antibodies against oral pathogens are induced by the gut- or duct-associated lymphoid tissues, which produces protective antibodies on mucosal surfaces.[59)] Salivary sIgA antibodies to mucosal bacteria begin to appear as early as the first weeks of life, and they approach adult levels by 1 to 2 year old.[60)] There are also serum-derived antibodies such as IgG isotype in whole saliva. These IgG antibodies to oral microbes have been detected in 1 year old[61,62)] and enter into whole saliva through the gingival crevice.[63)] Particularly, saliva has been reported to increase serum IgG retention on *S. mutans* cells among caries-resistant people,[64)] which means that serum-derived IgG antibodies to oral pathogens could also have a significant role in host defense.

(2) Lysozyme

Salivary lysozyme is derived from major salivary glands, minor salivary glands, phagocytic cells, and gingival crevicular fluid. The epithelial cells lining the intralobular and intercalated ducts secrete and synthesize lysozyme in major salivary glands, while lysozyme is detected at the intralobular and intercalated ducts as well as in serous acinar cells in minor salivary glands. Lysozyme mainly kills gram-positive bacteria with surface-exposed peptidoglycan, because lysozyme can hydrolyze β-1,4-glycosidic bonds between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine of bacterial cell wall peptidoglycan.[10] Digestion of peptidoglycan structures via salivary lysozyme may also assist antimicrobial cationic peptides of saliva to get across peptidoglycan layers of bacterial membranes. Lysozyme plays an important role in membrane-permeable property, which leads to non-enzymatic antimicrobial activity against both gram-positive and gram-negative bacteria as well as fungi.[65,66] Lysozyme can bind to bacterial lipopolysaccharide responsible for tissue destructive inflammatory reactions.[67] Besides the above, lysozyme also exerts antiviral properties and may also induce lysis of tumor cells.[68,69]

(3) Peroxidase

There are two types of peroxidase system in saliva, namely lactoperoxidase and myeloperoxidase. Lactoperoxidase is produced by salivary glands, while myeloperoxidase is produced by neutrophil granulocytes entering the oral cavity and also derived from gingival crevicular fluid. Salivary peroxidase catalyze the oxidation of thiocyanate (SCN^-) by hydrogen peroxide, which leads to the production of much more bactericidal and fungicidal hypothiocyanite ($OSCN^-$).[70] Duox-1 is localized in the luminal plasma

membrane of epithelial cells of terminal collecting ducts of major salivary glands, and provides hydrogen peroxide for salivary peroxidase just prior to delivery into the oral cavity.[71)]

(4) Glucose oxidase-mediated peroxidase

The actual supplements for the peroxidase system in oral health care products are GO-PO system which consists of bovine lactoperoxidase (bLPO), glucose oxidase, and SCN^-. Glucose oxidase convert glucose into oxidative glucono-lactone and generates hydrogen peroxide which serves as a substrate for bLPO and peroxidase in saliva during formation of antimicrobial $OSCN^-$ from of SCN^-.[13)] Glucose oxidase could hamper the carbohydrate metabolism of glycolytic cariogenic bacteria by the depleting glucose.

(5) Clinical and commercial use

Lysozyme and lactoferrin purified from bovine colostrum can be added into oral health care products, such as toothpastes and mouth-rinses. Biotene®, Oralbalance®, BioXtra® and Zendium Saliva® are commercial products used currently. These products can contain growth factors, such as IGF-1, TGF-β1 and TGF- β2.[72)] However, it is not remarkable if these have any effects on mucosal tissues. Human lactoferrin, or its antimicrobial 25-residue peptide fragment lactoferricin B, has recently been cloned by fermentation of *Aspergillus niger*. Recombinant human lactoferrin will be more preferable than bovine milk derived lactoferrin for future, because it has been reported to be active and safe for human clinical use. It is difficult to purify human salivary peroxidase in large scale because of unfavorable cost effect. Thus, bLPO purified from milk or colostrum has been used commercially, because it is structurally and catalytically close to human salivary peroxidase. Hydrogen peroxide can be produced in situ in the mouth, when oral hygiene products with

glucose and glucose oxidase system are used. Biotene®, BioXtra®, Zendium Saliva® and Oralbalance® are commercial products incorporating lactoperoxidase system. These products comprise toothpaste, non-alcoholic mouth-rinse, xylitol-flavored chewing gum, moisturizing gels and denture adhesives for patients with dry mouth problems. All commercialized products containing lactoperoxidase system are non-foaming because detergents inactivate the enzymes.

3. Molecular interactions

There is no clear evidence that any one of antimicrobial agents in saliva is more important than the others. Meanwhile, it is more important that many salivary antimicrobial agents interact each other simultaneously in solution and on surface phase, that their concentrations tend to be correlated, and that they depend on pathophysiological condition age, salivary flow rate, acidic pH and so on. Many of their interactions are synergistic or additive way in most cases. For example, such interactions between sIgA and peroxidase,[18] lactoferrin and peroxidase,[19,20] lactoferrin and lysozyme,[21] and lysozyme and histatins[22] were reported. Although these observations are from in vitro experiments, it seems that these concerted effects extend into in vivo systems, where all the components are simultaneously present. Therefore, salivary antimicrobial agents form a network where single component may be insufficient for the overall antimicrobial capacity of the host's defense system. Lysozyme and lactoferrin have been incorporated in some of these products to further mimic the in vivo situation, and that fluoride ions in these products have an additive effect with $OSCN^-$ against *S. mutans*.[76] Specific antibodies of *S. mutans* in bovine immune whey inhibit extracellular polysaccharide formation by *S. mutans*, aggregate both *S. mutans* and *Streptococcus sobrinus* cells, prevent the

adherence of *S. mutans* to saliva-coated hydroxyapatite, and support the phagocytosis and killing of *S. mutans* by human leukocytes.[77] In addition, immune whey and $OSCN^-$ act in an additive way against *S. mutans*.[78]

4. Xerostomia

(1) Prevalence

The prevalence of xerostomia in general population ranges from 0.9% to 64.8%, depending on population-based samples.[79] However, patients with Sjögren's syndrome and those who are receiving radiation therapy for head and neck cancer almost suffer from xerostomia without exception.[80] The prevalence increases with age, thus about 30% of the population older than 65 experience dry mouth.[80] Xerostomia is more prevalent in postmenopausal women compared to men. Especially, those who had taken antidepressants showed 22 times higher risk for xerostomia.

(2) Etiology

The most common medications causing hyposalivation are anticholinergics, sympatomimetics and benzodiazepines ant they are the most commonly prescribed medications in geriatric population.[81] Salivary gland hypofunction and chronic xerostomia can also be derived from the side effect of autoimmune disorders such as Sjögren's syndrome,[82] rheumatoid disorders, scleroderma and lupus;[83] endocrine disorders such as uncontrolled diabetes, adrenal gland diseases[84] and graft versus host disease (GVHD);[85] advanced stages of human immunodeficiency virus (HIV) infection,[86] chronic or neurogenic pain, malnutrition, smoking,[87] alcohol or caffeine-containing fluids, mouth breathing and iatrogenic procedures and regimens.[88-90] The subjective symptom of dry mouth can also result from the change in cognitive abilities of the central

nervous system following a stroke.[91] Xerostomia and salivary gland hypofunction are major complications of head and neck radiation therapy and chemotherapy. Irradiation and cytostatic drugs result in sialoadenitis following irreversible damage of acinar cells of salivary glands.[88]

(3) Clinical signs and symptoms

Dry mouth is a subjective feeling. Xerostomia is not equal to hyposalivation because it may occur with the changes in the quality and without the changes of the amount of saliva. Dry mouth is one of the most common and unpleasant symptom which adversely affects all oral functions and compromise oral health. Patients with xerostomia usually experience difficulties while speaking, chewing, swallowing and wearing dentures.[80,88-91] Oral mucosa is prone to injuries, fungal infection and inflammation and readily experience painful burning sensations.[92] Taste is also altered and even halitosis is present. The parotid glands become visibly enlarged in those with Sjögren's syndrome. Hyposalivation increases the risk of developing caries, enamel erosions and periodontal diseases.[88,89] The lack of lubrication can cause traumatic ulcerations of the mucosa and increased susceptibility to candidiasis in patient with dentures.

(4) Treatment

Treatment modalities are divided into etiologic, stimulative, symptomatic or palliative approach depending on the cause and the severity of diseases. The combination of chewing gums or solid food or acidic fruits can be effective in stimulating salivary flow for patients with intact salivary function. Acupuncture have focused mainly on a curative approach when the salivary gland tissues are already damaged.[93] However, it is considered to be efficient as preventive approach in the management of patients with head and neck cancer undergoing

radiotherapy recently. Only four sialagogues such as bromhexine, anetholetrithione, pilocarpine hydrochloride (HCl), and cevimeline HCl among systemic stimulants, have been studied extensively, but results were controversial.[94] Bromhexine has not proven to be beneficial to salivary function, yet it may stimulate lacrimal function in patients with Sjögren's syndrome. Anetholetrithione has been suggested to up-regulate muscarinic receptors and increased saliva flow in patients with mild salivary gland hypofunction. Pilocarpine HCl is a parasympathomimetic agent and causes stimulation of cholinergic receptors on the surface of acinar cells. Cevimeline is another parasympathomimetic agonist. A number of saliva substitutes have been developed for the palliative care of patients with salivary hypofunction. These agents, in liquid, spray, or gel form have moistening and lubricating properties, and are aimed to provide prolonged wetness of the oral mucosa.

5. Development of artificial saliva

(1) Current issues

There is no choice except palliative treatment, if the salivary secretory cells is damaged irreversibly and impaired completely. The best remedies available today are only symptomatic and alleviate discomfort and pain resulting from xerostomia like artificial saliva. Patients with irreversible xerostomia should be instructed to maintain adequate hydration of the oral cavity by taking plenty of fluids throughout the day and using artificial saliva preparations.

(2) Clinical and commercial use

There has been attempts to replace normal saliva with a variety of different solutions. The simplest form is water, but it is too short lived. Thus, Carboxy-methy-cellulose (CMC) is added for obtaining proper viscosity, and it makes

salivary substitute last for a longer period than water alone.[95] Buffered solutions include calcium, phosphate and fluoride ion for improving the qualities of artificial saliva. Polyethylene oxide is added for modifying rheological properties. Mucin containing solution often dilute with artificial saliva. Commercially available products are divided into five classes;[96] aqueous-ion solution as liquid form; aqueous-ion CMC solution as spray, aerosol and liquid form; mucin-containing preparation as spray, lozenge and gum type; glycoprotein-containing solution as liquid form; enzyme-containing solution as gel type. CMC and mucin-containing saliva substitute are lower contact angle and better wetting property than water.[97] The rheological property affects the coating and lubrication of oral surface and the consistency of ingested food. Dry mouth reduces influx of fluid. Therefore, it is necessary to add adequate rheological properties to artificial saliva. Facilitating lubrication and reducing sensation of dry mouth improve chewing, swallowing and speech.[98] The addition of calcium, phosphate and fluoride ions into artificial saliva is beneficial for the prevention of dental caries and the remineralization of enamel.

(3) Future aspects

It is necessary to consider the design and composition of salivary substitutes that disease-oriented as well as function-oriented. Customized artificial saliva can be applied with site-specific monoclonal antibodies against individual levels of molecules in saliva and tooth pellicles, because adequate amounts of molecules could be included in this replacement therapy. The advanced techniques in computer modelling to design relevant functional domain and use of peptide engineering to synthesize this domain, permit the development of composite salivary molecules with greater substantivity, biocompatibility and selectivity for precise localization.

III. MATERIALS AND METHODS

1. Collection of human saliva

Saliva samples were collected from 4 healthy adult participants (2 men and 2 women) between 8 am and 12 pm to minimize variability in salivary composition. The participants kept from eating, drinking, and tooth brushing for at least 1 h before saliva collection. Unstimulated whole saliva was collected by the spitting method.[99] Saliva was placed in a chilled centrifuge tube in which phenylmethylsulphonylfluoride (PMSF) was added immediately to final concentrations of 1.0 mM. The saliva sample was centrifuged at 3,500xg for 15 min at 4°C, and the resulting clarified supernatant fluid was used immediately for assays. The research protocol was approved by the Institutional Review Board of the Seoul National University Dental Hospital (#CRI13005).

2. Lysozyme, peroxidase, the peroxidase system, the GO-PO system, and sugar alcohols

Reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless stated otherwise.

Hen egg-white lysozyme (HEWL, final concentration of 30 μg/mL) and bLPO (final concentration of 25 μg/mL) served as the sources of lysozyme and peroxidase, respectively, in all experiments. For candidacidal assays, the peroxidase system included final concentrations of 25 μg/mL bLPO, 1 mM potassium thiocyanate (KSCN), and 50 μM hydrogen peroxide (H_2O_2). The GO-PO system included final concentrations of 25 μg/mL bLPO, 1 mM KSCN, 50 μg/mL GO from *A. niger*, and 0.03 mg/mL glucose. Among sugar alcohols, xylitol and sorbitol were used. All components were dissolved in simulated

salivary buffer (SSB, 0.021 M Na_2HPO_4/NaH_2PO_4, pH 7.0, containing 36 mM NaCl and 0.96 mM $CaCl_2$.[100)]

3. Measurement of enzymatic activities of lysozyme, peroxidase, and GO-PO

Lysozyme activity was determined by the turbidimetric method,[101)] in which the degradation rate of lyophilized cell suspension of *Micrococcus lysodeikticus* ATCC 4698 was measured. Peroxidase activity was determined by the NbsSCN assay which measures the rate of oxidation of 5-thio-2-nitrobenzoic acid (Nbs) to 5,5-dithiobis(2-nitrobenzoic acid) $(Nbs)_2$.[102)] The enzymatic activity of GO-PO was measured using a glucose assay kit, which measures oxidized o-dianisidine production.

4. Influences of xylitol and sorbitol on the enzymatic activity of lysozyme in the solution phase

The effects of xylitol and sorbitol on the enzymatic activity of HEWL and whole salivary lysozyme in the solution phase were examined by incubating 500 μL of xylitol or sorbitol at different concentrations (final concentrations of 32.5, 65, 130, and 260 mM) with 500 μL of HEWL (final concentration of 30 μg/mL) or clarified whole saliva for 10 min at room temperature (RT). Incubated mixture was placed in a suspension of *M. lysodeikticus*, and incubated buffer with HEWL or clarified whole saliva was a control. An incubated mixture of xylitol or sorbitol with the buffer or an incubated buffer alone was used as a blank. The experiment was duplicated and performed 6 times for HEWL. For whole saliva, saliva samples were collected twice in different days from 4 participants. The experiment was also duplicated and performed 8 times.

5. Influences of xylitol and sorbitol on the enzymatic activity of lysozyme on the HA surface phase

To determine the influences of xylitol and sorbitol on the enzymatic activity of lysozyme adsorbed to a HA surface, experiments were performed in 2 ways; In assay I, the effects of adsorbed xylitol or sorbitol on subsequent adsorption of lysozyme were examined. In assay II, HA-adsorbed lysozyme activity was examined after pre-incubation of xylitol or sorbitol with lysozyme. Ceramic HA beads (Macro-prep, HA type I) were obtained from Bio-Rad (Hercules, CA, USA) and used a solid phase. Ten milligrams of HA beads were used in each assay.

In assay I, HA beads were coated with 300 μL of xylitol or sorbitol (final concentrations of 130 and 260 mM) for 30 min at RT. After coating, the beads were washed 5 times with the buffer. The xylitol- or sorbitol-coated HA beads were then incubated with 300 μL of HEWL or clarified whole saliva samples for 30 min at RT. Unbound HEWL or salivary molecules were removed by 5 washes. The beads were incubated with a suspension of *M. lysodeikticus*, and lysozyme activity was determined as described above. The lysozyme activities of these samples were compared with those of the bare HA surfaces coated with HEWL or clarified whole saliva.

In assay II, 300 μL of xylitol or sorbitol (final concentrations of 130 and 260mM) was incubated with 300 μL of HEWL solution or clarified whole saliva samples for 10 min at RT. HA beads were incubated with 600 μL of the mixture for 30 min at RT, then washed 5 times with the buffer to remove unbound molecules. Lysozyme activities of these samples were compared with those of the HA samples coated with the pre-incubated mixture of HEWL or clarified whole saliva with buffer.

All the experiments was duplicated and performed 6 times for HEWL and 8 times for whole saliva. Equal amounts of HA beads incubated with xylitol or sorbitol, or an incubated buffer alone were used as blanks.

6. Influences of xylitol and sorbitol on the enzymatic activity of peroxidase in the solution phase

The effects of xylitol and sorbitol on the enzymatic activity of bLPO or whole salivary peroxidase in the solution phase were examined by incubating 500 μL of xylitol or sorbitol (final concentrations of 32.5, 65, 130, and 260 mM) with 500 μL of bLPO (final concentration of 25 μg/mL) or clarified whole saliva for 10 min at RT. The influences of different mixtures of xylitol and sorbitol (3:1, 2:2, and 1:3 in molar concentrations) on the enzymatic activity of bLPO were also examined. For NbsSCN assay, 15 μL of KSCN (final concentration of 4.2 mM SCN^-) and 15 μL of sample solution were added to 300 μL of reaction mixture, and reaction was initiated by the addition of 15 μL of H_2O_2 (final concentrations were 25 μM for bLPO and 100 μM for whole saliva). An incubated mixture of buffer with either bLPO or clarified whole saliva was used as a control. For the blank reaction, an incubated mixture of xylitol or sorbitol with buffer, or an incubated buffer alone was used. All the experiments was duplicated and performed 6 times for HEWL and 8 times for whole saliva.

7. Influences of xylitol and sorbitol on the enzymatic activity of peroxidase on the HA surface phase

Like the lysozyme assays, experiments were also performed in 2 ways; In assay I, the effects of adsorbed xylitol or sorbitol on subsequent adsorption of

peroxidase were examined. In assay II, HA-adsorbed peroxidase activity was examined after pre-incubation of xylitol or sorbitol with peroxidase.

In assay I, HA beads were coated with 300 μL of xylitol or sorbitol (final concentrations of 130 and 260 mM) for 30 min at RT. After coating, the beads were washed 5 times with the buffer. The xylitol- or sorbitol-coated HA beads were then incubated with 300 μL of bLPO or clarified whole saliva samples for 30 min at RT. Unbound bLPO or salivary molecules were removed by 5 washes. The beads were used for the NbsSCN assay. The peroxidase activities of these samples were compared with those of the bare HA surfaces coated with bLPO or clarified whole saliva.

In assay II, 300 μL of xylitol or sorbitol (final concentrations of 130 and 260 mM) was incubated with 300 μL of bLPO solution or clarified whole saliva samples for 10 min at RT. HA beads were incubated with 600 μL of the mixture for 30 min at RT, then washed 5 times with the buffer to remove unbound molecules. Peroxidase activities of these samples were compared with those of the HA samples coated with the pre-incubated mixture of bLPO or clarified whole saliva with buffer.

All the experiments was duplicated and performed 6 times for bLPO and 8 times for whole saliva. Equal amounts of HA beads incubated with xylitol or sorbitol, or an incubated buffer alone were used as blanks.

8. Influences of xylitol and sorbitol on the enzymatic activities of GO-PO

The GO-PO reagent in the glucose assay kit was divided into two parts, one dissolved in SSB and the other dissolved in SSB containing xylitol or sorbitol (final concentration of 130 mM), and pre-incubated for 30 min at RT. Enzymatic activity of the two different GO-PO reagents was measured using samples with known glucose concentrations (0.02, 0.04, and 0.06 mg/mL). Oxidized o-

dianisidine production, measured by the ODs at 540 nm, reflected the enzymatic activity of GO-PO reagents. The influences of different mixtures of xylitol and sorbitol (3:1, 2:2, and 1:3 in molar concentrations) on the enzymatic activities of GO-PO were also examined. Experiments were duplicated and performed 8 times.

9. Influences of xylitol and sorbitol on the candidacidal activities of lysozyme, the peroxidase system and the GO-PO system

One colony of *Candida albicans* (ATCC 18804, 10231, and 11006) grown on Sabouraud dextrose agar (SDA) was inoculated into 10 mL Sabouraud dextrose broth and incubated with shaking at 37℃ for 18 h. Cells were harvested, washed, and resuspended to 1 x 10^5 cells per mL in SSB. Twenty microliters of xylitol or sorbitol (final concentration of 130 mM) solution was added to 20 μL of HEWL, or the peroxidase system, or the GO-PO system and incubated with shaking at 37℃ for 30 min. The mixture was added to 20 μL of the cell suspension and incubated with shaking at 37℃ for 1 h. After the incubation, the sample was diluted 10-fold, and 50 μL of the diluted cells was plated onto SDA plates in triplicate and grown overnight at 37℃. Candidacidal activity was determined by comparing the number of colonies (colony forming units, CFUs) between plates containing mixtures of sugar alcohols and antimicrobials and ones containing only antimicrobials. The percent loss of cell viability was calculated as 1 minus the ratio of the number of colonies on the test and control (no sugar alcohols and antimicrobials) plates. Experiments were triplicated and performed 8 times.

10. Statistics

The Wilcoxon signed rank test and Mann-Whitney U-test were used to analyze statistical differences between two variables. The Kruskal-Wallis test was also used to analyze statistical differences and the Mann-Whitney U-test with Bonferroni's correction was used as post-hoc analysis. For each test, a *P*-value less than 0.05 was treated as statistically significant.

IV. RESULTS

1. Influences of xylitol and sorbitol on the enzymatic activity of lysozyme in the solution phase

Both xylitol and sorbitol did not affect the enzymatic activities of HEWL, but significantly inhibited the enzymatic activities of salivary lysozyme ($P < 0.05$) in the solution phase. At 2 time-increase of xylitol and sorbitol concentrations (final concentrations of 260 mM), the enzymatic activities of HEWL were not affected either (data not shown). The effects of xylitol and sorbitol on the enzyme activities of salivary lysozyme were concentration-dependent significantly ($P < 0.05$). The effect of sorbitol on the enzymatic activities of salivary lysozyme was greater than that of xylitol and there was a significant difference at 65 mM (Table 1).

2. Influences of xylitol and sorbitol on the enzymatic activity of lysozyme on the HA surface phase

Both xylitol and sorbitol did not affect the adsorption and subsequent enzymatic activity of HEWL and salivary lysozyme on the HA surfaces when xylitol or sorbitol was adsorbed first or was pre-incubated with HEWL or whole saliva (Table 2). At 2 time-increase of xylitol and sorbitol concentrations (final concentrations of 260 mM), the results were the same (data not shown).

3. Influences of xylitol and sorbitol on the enzymatic activity of peroxidase in the solution phase

Both xylitol and sorbitol enhanced the enzymatic activity of bLPO and salivary peroxidase significantly ($P < 0.05$) in the solution phase and the effects were greater in salivary peroxidase. The effects of xylitol and sorbitol on the enzyme activities of bLPO and salivary peroxidase were concentration-dependent significantly ($P < 0.05$). There were no significant differences between the effects of xylitol and sorbitol at the same concentration on the enzyme activities of bLPO and salivary peroxidase (Table 3).

As expected, the mixtures of xylitol and sorbitol with different ratios also enhanced the enzymatic activities of bLPO significantly ($P < 0.05$). However, the relative ratios of the mixtures did not affect the effects on the enzyme activities of bLPO significantly (Table 4).

4. Influences of xylitol and sorbitol on the enzymatic activity of peroxidase on the HA surface phase

Both xylitol and sorbitol did not affect the adsorption and subsequent enzymatic activity of bLPO and salivary peroxidase on the HA surfaces when

xylitol or sorbitol was adsorbed first or was pre-incubated with bLPO or whole saliva (Table 5). At 2 time-increase of xylitol and sorbitol concentrations (final concentrations of 260 mM), the results were the same (data not shown).

5. Influences of xylitol and sorbitol on the enzymatic activity of GO-PO

Xylitol did not affect the enzymatic activity of GO-PO, while the enzymatic activity was inhibited by increasing the relative amounts of sorbitol. Sorbitol at 130 mM and a 1:3 mixture of xylitol and sorbitol (at 32.5 mM xylitol and 97.5 mM sorbitol) inhibited the enzymatic activity of GO-PO significantly ($P < 0.05$) and the effects increased as the glucose level increased (Table 6).

6. Influences of xylitol and sorbitol on the candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system

The antimicrobial enzymes showed significant candidacidal activities in the order of the GO-PO system (%killing, 18.0 - 40.1%), the peroxidase system (18.6 - 33.8%), and lysozyme (13.9 - 27.8%). All the three antimicrobials showed the highest candidacidal activities in ATCC 11006 and the lowest activities in ATCC 10231. The pre-incubated mixtures of xylitol or sorbitol with lysozyme, the peroxidase system, or the GO-PO system did not show any additive candidacidal activities compared with the antimicrobial enzyme or the systems alone (Figs. 1 and 2).

V. DISCUSSION

A large number of previous studies showed concerted interactions between antimicrobial host proteins and potential base molecules in artificial saliva as well as just between antimicrobial host proteins, both in solution and on surface phases. The peroxidase system enhanced the enzymatic and canididacidal activity of lysozyme.[24)] Hyaluronic acid which might be included in artificial saliva as a base molecule had inhibitory effects on the candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system.[25,29)] The presence of animal mucins in artificial saliva affected the enzymatic activity of lysozyme on HA surfaces as well as in solution.[26)] Yam tuber mucilage enhanced the enzymatic activity of lysozyme on HA surfaces like animal mucin.[30,31)]

Sugar alcohols have been used frequently in artificial saliva and oral health care products for their anticariogenic properties, but there has been no research about the influences of sugar alcohols on the enzymatic and candidacidal effects of antimicrobials. For this reason, we tried to investigate the effects of xylitol and sorbitol, the most popular sugar alcohols, on the enzymatic and candidacidal activities related with lysozyme and peroxidase in the present study.

All sugar alcohols have common polyol properties such as absence of reducing carbonyl group, complex formation with polyvalent metal cations, and competition with water molecules.[1)] On the other hand, xylitol consists of five carbon atoms with hydroxyl group and sorbitol six carbon atoms, so that xylitol is differentiated from sorbitol in many biological reactions because of shorter structure.[103)] Phosphorylated xylitol which is xylitol-5-phosphate can easily participate in glycolytic pathway of *S. mutans* owing to less steric hindrance, and suppress bacterial growth and acid production.[4)] Different chemical structure of xylitol from sorbitol could not only contribute to decrease dental caries and adhesive potential of dental plaque more efficiently, but also provide systemic

merits such as appropriate regulation of blood glucose level with insulin elevation,[2] retardation of osteoporosis,[104] and prevention of otitis media.[105]

Both xylitol and sorbitol inhibited the enzymatic activities of salivary lysozyme, while they enhanced the enzymatic activities of bLPO and salivary peroxidase in this study. Lysozyme is known to hydrolyze polysaccharides of bacterial cell wall and destruct the microorganisms.[106] Both xylitol and sorbitol are considered to interact with water molecule through hydrogen bonding and lower water activity, which may lead to hampering the hydrolytic ability of lysozyme.[107] Furthermore, mucinous components in saliva could restrict the diffusion-controlled mobility of water molecules,[108] which might result in inhibitory effects in salivary lysozyme significantly rather than HEWL.

On the contrary, both xylitol and sorbitol have been suggested to make complex formation with polyvalent cations in the active site of peroxidase. The desolvation of the cations governs the thermodynamic properties to be favorable, which could increase the enzymatic activities of bLPO and salivary peroxidase.[109]

The enzymatic activity of GO-PO was not affected significantly by xylitol, but inhibited remarkably by sorbitol, though both xylitol and sorbitol enhanced the enzymatic activity of bLPO in this study. This means the critical point of catalytic activity should be GO rather than peroxidase. It would be possible that sugar alcohols affects the generation of H_2O_2 by regulating GO activity, as if methylglyoxal prevents the production of H_2O_2 by modifying the protein structure of GO through crosslinking.[110] Furthermore, GO is known to lose thermodynamic stability when flavin adenine dinucleotide (FAD), a cofactor, is released. Some evidences that KCl, $CaCl_2$, and xylitol were efficient at preventing from the release of FAD,[111] might support the differential effects between xylitol and sorbitol.

The effects of sugar alcohols on the enzymatic activities of lysozyme and peroxidase were significant in the solution phase, but not on the surface phase in

this study. Once sugar alcohols adsorbed onto the hydroxyapatite surfaces through hydrogen bonding, their structures would be reconstituted to surface-oriented conformation and confined to the surface eventually.[112,113] Therefore, it would be harder for sugar alcohols to interact with lysozyme or peroxidase and regulate enzymatic activities of them on the HA surfaces.

The candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system were neither enhanced nor inhibited significantly by xylitol and sorbitol in the present study.

Instead, *C. albicans* cultured in a variety of carbohydrate supplement media showed significant difference in the susceptibility to lysozyme. Xylitol as supplement media provided more susceptibility to lysozyme by the regulation of polysaccharides and proteins in yeast cell walls than other dietary carbohydrates.[114] The epithelial adhesion of *C. albicans* grown in xylitol demonstrated significant decrease and marginal increase in sorbitol, while *C. albicans* grown in other dietary carbohydrates enhanced adhesion significantly.[115]

This means that the effect of sugar alcohol on candidacidal activities would not be associated with antimicrobial enzymes, but depend on other factors such as as adhesive capacity and cell wall components of fungus.

The present study includes some limitations to extend the *in vitro* results into *in vivo* situations. There are more enzymes and other biological molecules, and more complicated interactions among them in human saliva than we have focused. The oral cavity is a dynamic open system composed of the different types of soft and hard tissues communicating with outer environment and inner gastrointestinal and respiratory systems, and it is different from a minimized closed system containing solution and synthetic HA surfaces. However, the results of the present study could help understand the molecular interactions in oral health care products and possibly in the oral cavity.

In conclusion, both xylitol and sorbitol inhibited the enzymatic activity of salivary lysozyme, while enhanced the enzymatic activity of bLPO and salivary peroxidase in solution. On the contrary, neither xylitol nor sorbitol has effect on the enzymatic activities of lysozyme and peroxidase on the HA surface. Sorbitol inhibited the enzymatic activity of GO-PO. The candidacidal activities of lysozyme, the peroxidase system, and the GO-PO system were not additive by xylitol and sorbitol.

VI. CONCLUSIONS

Xylitol and sorbitol are the most widely used sugar alcohols as sweeteners and well known to anticariogenic property. Thus, they are often included in many commercialized oral health care products. It has been suggested that there must be concerted interactions between antimicrobial host proteins and sugar alcohols. Xylitol and sorbitol inhibited salivary lysozyme activity, but enhanced both bLPO and salivary peroxidase activities significantly in solution phase. Sorbitol, not xylitol, inhibited the enzymatic activity of GO-PO significantly. Both xylitol and sorbitol were not additive to the candidacidal activities of HEWL, the bLPO system, and the GO-PO system.

REFERENCES

1. Mäkinen KK. Xylitol-based caries prevention: is there enough evidence for the existence of a specific xylitol effect? Oral Dis 1988;4:226-230.

2. Islam MS, Indrajit M. Effects of xylitol on blood glucose, glucose tolerance, serum insulin and lipid profile in a type 2 diabetes model of rats. Ann Nutr Metab 2012;61:57-64.

3. Maguire A, Rugg-Gunn AJ. Xylitol and caries prevention--is it a magic bullet? Br Dent J 2003;194:429-436.

4. Söderling EM. Xylitol, mutans streptococci, and dental plaque. Adv Dent Res 2009;21:74-78.

5. Olsson H, Spak CJ. The effect of a chewing gum on salivary secretion, oral mucosal friction, and the feeling of dry mouth in xerostomic patients. Acta Odontol Scand 1991;49:273-279.

6. Turner MD, Ship JA. Dry mouth and its effects on the oral health of elderly people. J Am Dent Assoc 2007;138:15S-20S.

7. Cassolato SF, Turnbull RS. Xerostomia: clinical aspects and treatment. Gerodontology 2003;20:64-77.

8. Tenovuo J. Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia: efficacy and safety. Oral Dis 2002;8:23-29.

9. Jollès P, Jollès J. What's new in lysozyme research? Always a model system, today as yesterday. Mol Cell Biochem 1984;63:165-189.

10. Laible NJ, Germaine GR. Bactericidal activity of human lysozyme, muramidase-inactive lysozyme, and cationic polypeptides against *Streptococcus sanguis* and *Streptococcus faecalis*: inhibition by chitin oligosaccharides. Infect Immun 1985;48:720-728.

11. Ibrahim HR, Matsuzaki T, Aoki T. Genetic evidence that antibacterial activity of lysozyme is independent of its catalytic function. FEBS Lett 2001;506:27-32.

12. Ihalin R, Loimaranta V, Tenovuo J. Origin, structure, and biological activities of peroxidases in human saliva. Arch Biochem Biophys 2006;445:261-268.

13. Hannig C, Hannig M, Attin T. Enzymes in the acquired enamel pellicle. Eur J Oral Sci 2005;113:2-13.

14. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000;14: 40-47.

15. Pruitt KM, Caldwell RC, Jamieson AD, Taylor RE. The interaction of salivary proteins with tooth surface. J Dent Res 1969;48:818-823.

16. Sandwick RK, Schray KJ. Conformational states of enzymes bound to surfaces. J Colloid Interface Sci 1988;121:1-12.

17. Stayton PS, Drobny GP, Shaw WJ, Long JR, Gilbert M. Molecular recognition at the protein-hydroxyapatite interface. Crit Rev Oral Biol Med 2003;14:370-376.

18. Tenovuo J, Moldoveanu Z, Mestecky J, Pruitt KM, Rahemtulla BM. Interaction of specific and innate factors of immunity: IgA enhances the antimicrobial effect of the lactoperoxidase system against *Streptococcus mutans*. J Immunol 1982;128:726-731.

19. Lassiter MO, Newsome AL, Sams LD, Arnold RR. Characterization of lactoferrin interaction with *Streptococcus mutans*. J Dent Res 1987;66:480-485.

20. Soukka T, Lumikari M, Tenovuo J. Combined inhibitory effect of lactoferrin and lactoperoxidase system on the viability of *Streptococcus mutans*, serotype c. Scand J Dent Res 1991;99:390-396.

21. Soukka T, Lumikari M, Tenovuo J. Combined bactericidal effect of human lactoferrin and lysozyme against *Streptococcus mutans* serotype c. Microbial Ecol Health Dis 1991;4:259-264.

22. Murakami Y, Nagata H, Amano A, Takagaki M, Shizukuishi S, Tsunemitsu A. Inhibitory effects of human salivary histatins and lysozyme on coaggregation between *Porphyromonas gingivalis* and *Streptococcus mitis*. Infect Immun 1991;59:3284-3286.

23. Lenander-Lumikari M, Månsson-Rahemtulla B, Rahemtulla F. Lysozyme enhances the inhibitory effects of the peroxidase system on glucose metabolism of *Streptococcus mutans*. J Dent Res 1992;71:484-490.

24. Lee JY, Kim YY, Chang JY, Park MS, Kho HS. The effects of peroxidase on the enzymatic and candidacidal activities of lysozyme. Arch Oral Biol 2010;55:607-612.

25. Cho MA, Kim YY, Chang JY, Kho HS. Interactions between hyaluronic acid, lysozyme, and the glucose oxidase-mediated lactoperoxidase system in enzymatic and candidacidal activities. Arch Oral Biol 2013;58:1349-1356.

26. Park WK, Chung JW, Kim YK, Chung SC, Kho HS. Influences of animal mucins on lysozyme activity in solution and on hydroxyapatite surfaces. Arch Oral Biol 2006;51:861-869.

27. Lee SG, Jeon EH, Kho HS. Influences of animal mucins on peroxidase activity in solution and on the surface of hydroxyapatite. J Oral Med Pain 2008;33:229-240.

28. Park MS, Chang JY, Kang JH, Park KP, Kho HS. Rheological properties of hyaluronic acid and its effects on salivary enzymes and candida. Oral Dis 2010;16:382-387.

29. Kang JH, Kim YY, Chang JY, Kho HS. Influences of hyaluronic acid on the anticandidal activities of lysozyme and the peroxidase system. Oral Dis 2011;17:577-583.

30. Park MS, Chang JY, Kim YY, Kang JH, Kho HS. Physical and biological properties of yam as a saliva substitute. Arch Oral Biol 2010;55:177-183.

31. Kho HS, Park MS, Chang JY, Kim YY. Yam tuber mucilage as a candidate substance for saliva substitute: in vitro study of its viscosity and influences on lysozyme and peroxidase activities. Gerodontology 2014;31:34-41.

32. Mäkinen KK, Tenovuo J, Scheinin A. Xylitol-induced increase of lactoperoxidase activity. J Dent Res 1976;55:652-660.

33. Schiweck H, Bar A, Vogel R. Ullmann's encyclopedia of industrial chemistry. Wiley-VCH, 2011, pp. 1-28.

34. Mäkinen KK. Sugar alcohol sweeteners as alternatives to sugar with special consideration of xylitol. Med Princ Pract 2011;20:303-320.

35. Mäkinen KK. Sugar alcohols, caries incidence, and remineralization of caries lesions: a literature review. Int J Dent 2010;2010:1-23.

36. Mäkinen KK. Can the pentitol-hexitol theory explain the clinical observations made with xylitol? Med Hypotheses 2000;54:603-613.

37. Bradshaw DJ, Marsh PD. Effect of sugar alcohols on the composition and metabolism of a mixed culture of oral bacteria grown in a chemostat. Caries Res 1994;28:251-256.

38. Talbot JM, Fisher KD. The need for special foods and sugar substitutes by individuals with diabetes mellitus. Diabetes Care 1978;1:231-240.

39. Peters R, Lock RH. Laxative effect of sorbitol. Br Med J 1958;2:677-678.

40. Kartal A, Björkqvist M, Lehto VP, Juppo AM, Marvola M, Sivén M. Compatibility of chewing gum excipients with the amino acid L-cysteine and stability of the active substance in directly compressed chewing gum formulation. J Pharm Pharmacol 2008;60:1131-1138.

41. Livesey G. Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. Nutr Res Rev 2003;16:163-191.

42. Scheinin A, Mäkinen KK. Turku Sugar Studies I–XXI. Acta Odontol Scand 1975;33:1-351.

43. Scheinin A, Mäkinen KK, Ylitalo K. Turku Sugar Studies V. Final report on the effect of sucrose, fructose and xylitol diets on the caries incidence in man. Acta Odontol Scand 1976;34:179-216.

44. Isokangas P, Alanen P, Tiekso J, Mäkinen KK. Xylitol chewing gum in caries prevention: a field study in children. J Am Dent Assoc 1988;117:315-320.

45. Mäkinen KK, Bennett CA, Hujoel PP, Isokangas PJ, Isotupa KP, Pape HR Jr, Mäkinen PL. Xylitol chewing gums and caries rates: a 40-month cohort study. J Dent Res 1995;74:1904-1913.

46. Isokangas P, Söderling E, Pienihäkkinen K, Alanen P. Occurrence of dental decay in children after maternal consumption of xylitol chewing gum, a follow-up from 0 to 5 years of age. J Dent Res 2000;79:1885-1889.

47. Thorild I, Lindau B, Twetman S. Caries in 4-year-old children after maternal chewing of gums containing combinations of xylitol, sorbitol, chlorhexidine, and fluoride. Eur Arch Paediatr Dent 2006;7:241-245.

48. Leach SA, Green RM. Effect of xylitol-supplemented diets on the progression and regression of fissure caries in the albino rat. Caries Res 1980;14:16-23.

49. Shyu KW, Hsu MY. The cariogenicity of xylitol, mannitol, sorbitol, and sucrose. Proc Natl Sci Counc ROC 1980;4:21-26.

50. Havenaar R, Huis in‘t Veld JH, de Stoppelaar JD, Dirks OB. Anti-cariogenic and remineralizing properties of xylitol in combination with sucrose in rats inoculated with *Streptococcus mutans*. Caries Res 1984;18:269-277.

51. Mäkinen KK. Dietary prevention of dental caries by xylitol - clinical effectiveness and safety. J Appl Nutr 1992;44:16-28.

52. Kandelman D, Bär A, Hefti A. Collaborative WHO xylitol field study in French Polynesia. I. Baseline prevalence and 32-month caries increment. Caries Res 1988;22:55-62.

53. Scheinin A, Bánóczy J, Szöke J, Esztári I, Pienihäkkinen K, Scheinin U, Tiekso J, Zimmermann P, Hadas E. Collaborative WHO xylitol field studies in Hungary. I. Three- year caries activity in institutionalized children. Acta Odontol Scand 1985;43:327-347.

54. Gales MA, Nguyen TM. Sorbitol compared with xylitol in prevention of dental caries. Ann Pharmacother 2000;34:98-100.

55. Twetman S. Consistent evidence to support the use of xylitol- and sorbitol-containing chewing gum to prevent dental caries. Evid Based Dent 2009;10:10-11.

56. Tenovuo J, Lehtonen OP, Aaltonen AS, Vilja P, Tuohimaa P. Antimicrobial factors in whole saliva of human infants. Infect Immun 1986;51:49-53.

57. Kirstilä V, Häkkinen P, Jentsch H, Vilja P, Tenovuo J. Longitudinal analysis of the association of human salivary antimicrobial agents with caries increment and cariogenic micro-organisms: a two-year cohort study. J Dent Res 1998;77:73-80.

58. Närhi TO, Tenovuo J, Ainamo A, Vilja P. Antimicrobial factors, sialic acid, and protein concentration in whole saliva of the elderly. Scand J Dent Res 1994;102:120-125.

59. Nair PN, Schroeder HE. Duct-associated lymphoid tissue (DALT) of minor salivary glands and mucosal immunity. Immunology 1986;57:171-180.

60. Smith DJ, King WF, Taubman MA. Salivary IgA antibody to oral streptococcal antigens in predentate infants. Oral Microbiol Immunol 1990;5:57-62.

61. Luo Z, Smith DJ, Taubman MA, King WF. Cross-sectional analysis of serum antibody to oral streptococcal antigens in children. J Dent Res 1988;67:554-560.

62. Tenovuo J, Lehtonen OP, Aaltonen AS. Caries development in children in relation to the presence of mutans streptococci in dental plaque and of serum antibodies against whole cells and protein antigen I/II of *Streptococcus mutans*. Caries Res 1990;24:59-64.

63. Challacombe SJ, Russell MW, Hawkes JE, Bergmeier A, Lehner T. Passage of immunoglobulins from plasma to the oral cavity in rhesus monkeys. Immunology 1978;35:923-931.

64. Riviere GR, Bresnick SD, Miller JN. Saliva increases serum IgG retention on *Streptococcus mutans*. Oral Microbiol Immunol 1988;3:129-133.

65. Ibrahim HR, Thomas U, Pellegrini A. A helix-loop-helix peptide at the upper lip of the active site cleft of lysozyme confers potent antimicrobial activity with membrane permeabilization action. J Biol Chem 2001;276:43767-43774.

66. Marquis G, Garzon S, Strykowski H, Auger P. Cell walls of normal and lysozyme-damaged blastoconidia of *Candida albicans*: localization of surface factor 4 antigen and vicinal-glycol staining. Infect Immun 1991;59:1312-1318.

67. Choi S, Baik JE, Jeon JH. Identification of *Porphyromonas gingivalis* lipopolysaccharide-binding proteins in human saliva. Mol Immunol 2011;48:2207-2213.

68. Lee-Huang S, Huang PL, Sun Y, Huang PL. Lysozyme and RNases as anti-HIV components in β-core preparations of human chorionic gonadotropin. Proc Natl Acad Sci USA 1999;96:2678-2681.

69. Sava G, Benetti A, Ceschia V, Pacor S. Lysozyme and cancer: Role of exogenous lysozyme as anticancer agent (review). Anticancer Res 1989;9:583-591.

70. Ashby MT. Inorganic chemistry of defensive peroxidases in the human oral cavity. J Dent Res 2008;87:900-914.

71. Geiszt M, Witta J, Baffi J, Lekstrom K. Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J 2003;17:1502-1504.

72. Wei H, Loimaranta V, Tenovuo J, Rokka S, Syväoja EL, Korhonen H, Joutsjoki V, Marnila P. Stability and activity of specific antibodies against *Streptococcus mutans* and *Streptococcus sobrinus* in bovine milk fermented with *Lactobacillus rhamnosus* strain GG or treated at ultra-high temperature. Oral Microbiol Immunol 2002;17:9-15.

73. Headon DR. Human lactoferrin: production at large scale, characterization and applications. In: Shimazaki K, Tsuda H, Tomita M, Kuwata T, Perraudin JP, eds. Lactoferrin: structure, function and applications. Elsevier Science B.V. 2000, pp. 415-427.

74. Mansson-Rahemtulla B, Rahemtulla F, Baldone DC, Pruitt KM, Hjerpe A. Purification and characterization of human salivary peroxidase. Biochemistry 1988;27:233-239.

75. Hoogendoorn H. Activation of the salivary peroxidase antimicrobial system: clinical studies. In: Pruitt KM, Tenovuo J, eds. The lactoperoxidase system:

chemistry and biological significance. Marcel Dekker, New York, 1985, pp. 217-227.

76. Lenander-Lumikari M, Loimaranta V, Hannuksela S, Tenovuo J, Ekstrand J. Combined inhibitory effect of fluoride and hypothiocyanite on the viability and glucose metabolism of *Streptococcus mutans*, serotype c. Oral Microbiol Immunol 1997;12:231-235.

77. Loimaranta V, Carlén A, Olsson J, Tenovuo J, Syväoja EL, Korhonen H. Concentrated bovine colostral whey proteins from *Streptococcus mutans*/*Strep. sobrinus* immunized cows inhibit the adherence of *Strep. mutans* and promote aggregation of mutans streptococci. J Dairy Res 1998;65:599-607.

78. Loimaranta V, Tenovuo J, Korhonen H. Combined inhibitory effect of bovine immune whey and peroxidase-generated hypothiocyanite against glucose uptake by *Streptococcus mutans*. Oral Microbiol Immunol 1998;13:378-381.

79. Orellana MF, Lagravère MO, Boychuk DG, Major PW, Flores-Mir C. Prevalence of xerostomia in population-based samples: a systematic review. J Public Health Dent 2006;66:152-158.

80. Ship JA, Pillemer SR, Baum BJ. Xerostomia and the geriatric patient. J Am Geriatr Soc 2002;50:535-543.

81. Närhi TO. Prevalence of subjective feelings of dry mouth in the elderly. J Dent Res 1994;73:20-25.

82. Pijpe J, Kalk WW, Bootsma H, Spijkervet FK, Kallenberg CG, Vissink A. Progression of salivary gland dysfunction in patients with Sjögren's syndrome. Ann Rheum Dis 2007;66:107-112.

83. Vincent C, Agard C, Barbarot S, N'guyen JM, Planchon B, Durant C. Orofacial manifestations of systemic sclerosis: A study of 30 consecutive patients. Rev Stomatol Chir Maxillofac 2010;111:128-134.

84. Chavez EM, Taylor GW, Borrell LN, Ship JA. Salivary function and glycemic control in older persons with diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:305-311.

85. Larsen J, Nordström G, Ljungman P, Gardulf A. Factors associated with poor general health after stem-cell transplantation. Support Care Cancer 2007;15:849-857.

86. Navazesh M, Mulligan R, Karim R, Mack WJ, Ram S, Seirawan H. Effect of HAART on salivary gland function in the Women's Interagency HIV Study (WIHS). Oral Dis 2009;15:52-60.

87. Versteeg PA, Slot DE, van der Velden U, van der Weijden GA. Effect of cannabis usage on the oral environment: a review. Int J Dent Hyg 2008;6:315-320.

88. Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc 2003;134:61-69.

89. Scully C, Felix DH. Oral medicine -- update for the dental practitioner: dry mouth and disorders of salivation. Br Dent J 2005;199:423-427.

90. Turner M, Jahangiri L, Ship JA. Hyposalivation, xerostomia and the complete denture: a systematic review. J Am Dent Assoc 2008;139:146-150.

91. Fox PC. Xerostomia: recognition and management. Dent Assist 2008;77:18, 20, 44-48.

92. Borgnakke WS, Taylor GW, Anderson PF, Shannon MC. Oral and general health - exploring the connection. Dry mouth (xerostomia): diagnosis, causes, complications and treatment. Research Review, DDPA, 2011:1-35.

93. Braga FP, Lemos Junior CA, Alves FA , Migliari DA. Acupuncture for the prevention of radiation-induced xerostomia in patients with head and neck cancer. Braz Oral Res 2011;25:180-185.

94. Fox PC, Ship JA. Salivary gland diseases. In: Greenburg MS, Glick M, Ship JA, eds. Burket's Oral medicine 11th edition. Hamilton, BC Decker, 2008, pp. 191-222.

95. Ferguson MM. Management of patients with xerostomia. Compend Suppl 1989;13:S470-S475.

96. Sreebny LM. Recognition and treatment of salivary induced conditions. Int Dent J 1989;39:197-204.

97. Vissink A, De Jong HP, Busscher HJ, Arends J, Gravenmade EJ. Wetting properties of human saliva and saliva substitutes. J Dent Res 1986;65:1121-1124.

98. Schwarz WH. The rheology of saliva. J Dent Res 1987;66:660-666.

99. Birkhed D, Heintze U. Salivary secretion rate, buffer capacity, and pH. In : Tenovuo JO, ed. Human saliva: clinical chemistry and microbiology, Vol. I. CRC Press Inc, Boca Raton, 1999, pp. 25-73.

100. Bennick A, Cannon M. Quantitative study of the interaction of salivary acidic proline-rich proteins with hydroxyapatite. Caries Res 1978;12:159-169.

101. Grossowicz N, Ariel M. Methods for determination of lysozyme activity. Methods Biochem Anal 1983;29:435-446.

102. Mansson-Rahemtulla B, Baldone DC, Pruitt KM, Rahemtulla F. Specific assays for peroxidases in human saliva. Arch Oral Biol 1986;31:661-668.

103. Angyral SJ, Greeves D, Mills JA. Complexes of carbohydrates with metal cations. Conformations of alditols in aqueous solution. Aust J Chem 1974;27:1447-1456.

104. Mattila PT, Kangasmaa H, Knuuttila ML. The effect of a simultaneous dietary administration of xylitol and ethanol on bone resorption. Metabolism 2005;54:548-551.

105. Danhauer JL, Johnson CE, Corbin NE, Bruccheri KG. Xylitol as a prophylaxis for acute otitis media: systematic review. Int J Audiol 2010;49:754-761.

106. Masschalck B, Michiels CW. Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. Crit Rev Microbiol 2003;29:191-214.

107. Larreta-Garde V, Xu ZF, Lamy L, Mathlouthi M, Thomas D. Lysozyme kinetics in low water activity media. A possible hydration memory. Biochem Biophys Res Commun 1988;155:816-822.

108. Lamy L, Portmann MO, Mathlouthi M, Larreta-Garde V. Modulation of egg-white lysozyme activity by viscosity intensifier additives. Biophys Chem 1990;36:71-76.

109. Rongère P, Morel-Desrosiers N, Morel JP. Thermodynamics of the weak interaction between samarium cation and xylitol in water : A chemical Model. J Solution Chem 1994;23:351-362.

110. Majtan J, Bohova J, Prochazka E. Methylglyoxal may affect hydrogen peroxide accumulation in manuka honey through the inhibition of glucose oxidase. J Med Food 2014;17:290-293.

111. Cioci F, Lavecchia R. Effect of polyols and sugars on heat-induced flavin dissociation in glucose oxidase. Biochem Mol Biol Int 1994;34:705-712.

112. Copeland JR, Shi XR, Sholl DS, Sievers C. Surface interactions of C and C(3) polyols with γ-Al_2O_3 and the role of coadsorbed water. Langmuir 2013;29:581-593.

113. Shen JW, Wu T, Wang Q, Pan HH. Molecular simulation of protein adsorption and desorption on hydroxyapatite surfaces. Biomaterials 2008;29:513-532.

114. Samaranayake YH, MacFarlane TW, Aitchison TC, Samaranayake LP. The in vitro lysozyme susceptibility of *Candida albicans* cultured in carbohydrate-supplemented media. Oral Microbiol Immunol 1993;8:177-181.

115. Pizzo G, Giuliana G, Milici ME, Giangreco R. Effect of dietary carbohydrates on the in vitro epithelial adhesion of *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. New Microbiol 2000;23:63-71.

Figure Legends

Fig. 1. Influences of xylitol on the candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase system.
C, control (no antimicrobial, no xylitol); L, hen egg-white lysozyme (30 μg/mL); P, the peroxidase system (25 μg/mL bLPO, 1 mM potassium thiocyanate, and 50 μM hydrogen peroxide); G, the glucose oxidase-mediated peroxidase system (25 μg/mL bLPO, 1 mM potassium thiocyanate, 10 units/mL GO, and 0.03 mg/mL glucose); X, xylitol (130 mM)
Experiments were performed 8 times.

Fig. 2. Influences of sorbitol on the candidacidal activities of lysozyme, the peroxidase system, and the glucose oxidase-mediated peroxidase system.
C, control (no antimicrobial, no sorbitol); L, hen egg-white lysozyme (30 μg/mL); P, the peroxidase system (25 μg/mL bLPO, 1 mM potassium thiocyanate, and 50 μM hydrogen peroxide); G, the glucose oxidase-mediated peroxidase system (25 μg/mL bLPO, 1 mM potassium thiocyanate, 10 units/mL GO, and 0.03 mg/mL glucose); S, sorbitol (130 mM)
Experiments were performed 8 times.

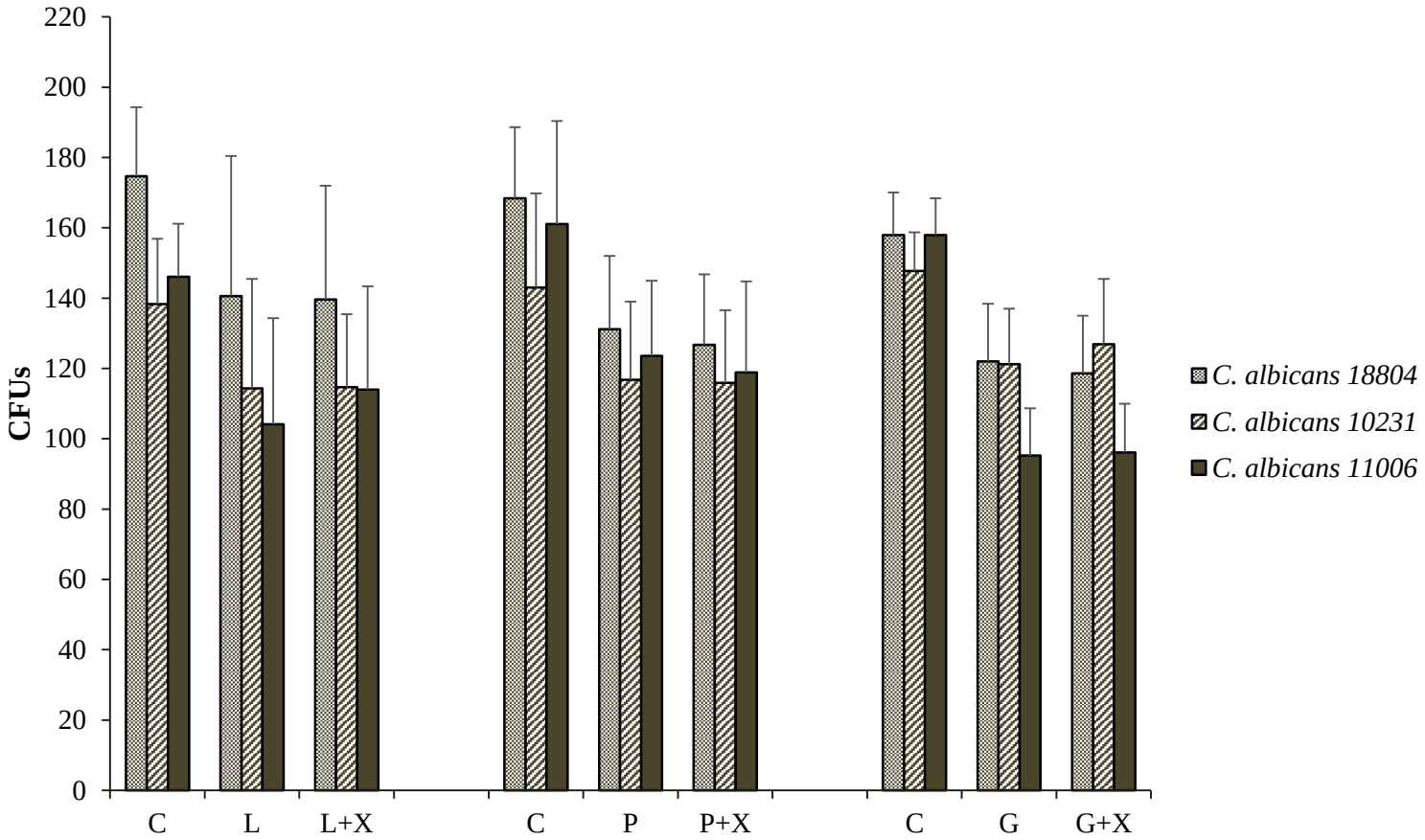
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Fig. 1.

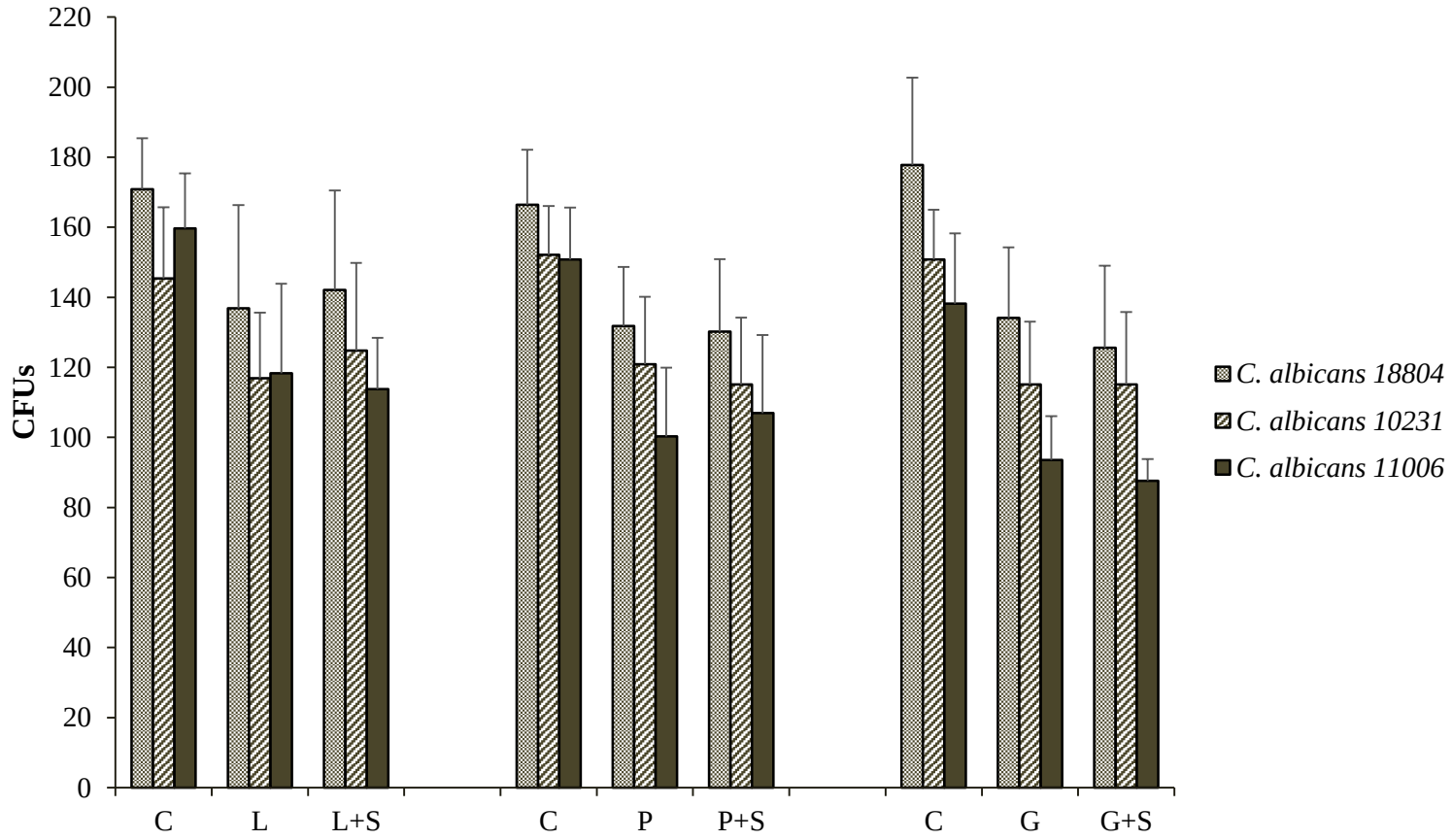
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Fig. 2

Table 1. Influences of xylitol and sorbitol on the enzymatic activities of HEWL and whole salivary lysozyme in the solution phase

Source of lysozyme	Xylitol (mM)	Lysozyme activity (Units/mL)			Sig.	Sorbitol (mM)	Lysozyme activity (Units/mL)			Sig.
		Sample only	with Xylitol	%Diff‡			Sample only	with Sorbitol	%Diff‡	
HEWL (n=6)	130	1009.0 ± 76.9	958.7 ± 102.9	-5.1±5.1	0.080	130	920.0 ± 29.2	918.7 ± 66.6	-0.2±5.6	0.917
Human saliva (n=8)	32.5	450.5 ± 290.6	427.8 ± 279.3	-4.3 ± 5.9^{b,c}	0.021*	32.5	445.5 ± 310.1	406.4 ± 286.6	-8.7 ± 4.5^{a,b,c}	0.012*
	65§	370.6 ± 243.1	334.0 ± 201.8	-8.2 ± 4.6^{d,e}	0.011*	65§	425. 9 ± 235.8	242.8 ± 136.3	-39.4 ± 21.6^{a}	0.012*
	130	483.1 ± 279.9	376.0 ± 248.1	-25.1 ± 9.1^{b,d}	0.012*	130	441.9 ± 282.4	337.5 ± 254.0	-32.4 ± 26.3^{b}	0.012*
	260	393.3 ± 221.9	299.6 ± 195.2	-28.0 ± 12.0^{c,e}	0.012*	260	487.8 ± 238.8	316.0 ± 235.9	-40.9 ± 21.8^{c}	0.012*

HEWL, hen egg-white lysozyme (30 μg/mL)
Sig., Significance
%Diff, percent changes of enzymatic activities in samples with sugar alcohol compared with samples without sugar alcohol
The Wilcoxon signed rank test was used to analyze differences between the enzyme activities of samples with and without sugar alcohol.*$P < 0.05$
The Kruskal-Wallis test was used to analyze differences of %Diff in human saliva according to the concentration of sugar alcohols. ‡$P < 0.05$
The Mann-Whitney U-test with Bonferroni's correction was used as post hoc analysis. Pair of the same alphabet denotes a significant difference. $P < 0.0125$
The Mann-Whitney U-test was used to analyze differences between %Diff of xylitol and sorbitol at the same molar concentrations. §$P < 0.05$

Table 2. Influences of xylitol and sorbitol on the enzymatic activities of HEWL and whole salivary lysozyme on the surface phase

Source of lysozyme	Assay	Xylitol (mM)	Lysozyme activity (Units)		Sig.	Sorbitol (mM)	Lysozyme activity (Units)		Sig.
			Sample only	with Xylitol			Sample only	with Sorbitol	
HEWL (n=6)	I	130	4.7 ± 0.4	4.7 ± 0.7	0.752	130	4.5 ± 0.6	4.4 ± 0.6	0.753
	II	130	2.5 ± 1.1	2.1 ± 0.3	0.343	130	2.9 ± 0.5	2.9 ± 0.6	0.674
Human Saliva (n=8)	I	130	44.2 ± 29.9	46.6 ± 32.5	0.161	130	49.3 ± 30.4	47.9 ± 30.5	0.362
	II	130	15.8 ± 7.9	14.2 ± 8.0	0.327	130	20.3 ± 15.7	21.8 ± 15.1	0.161

HEWL, hen egg-white lysozyme (30 μg/mL)
Sig., Significance
Assay I, Sugar alcohols were first adsorbed to hydroxyapatite beads and HEWL or human saliva was adsorbed next.
Assay II, Sugar alcohols were pre-incubated with HEWL or human saliva, and the mixtures were adsorbed to hydroxyapatite beads.
The Wilcoxon signed rank test was used to analyze differences between the enzyme activities of samples with and without sugar alcohol. $^{*}P < 0.05$

Table 3. Influences of xylitol and sorbitol on the enzymatic activities of bLPO and whole salivary peroxidase in the solution phase

Source of peroxidase	Xylitol (mM)	Peroxidase activity (mUnits/mL)			Sig.	Sorbitol (mM)	Peroxidase activity (mUnits/mL)			Sig.
		Sample only	with Xylitol	%Diff[†,‡]			Sample only	with Sorbitol	%Diff[†,‡]	
bLPO (n=6)	32.5	4.759 ± 1.011	5.088 ± 0.891	7.5 ± 6.3^{b,c}	0.046*	32.5	4.504 ± 0.428	5.057 ± 0.929	11.7 ± 11.6^{c}	0.075
	65	5.155 ± 0.788	5.684 ± 0.744	11.4 ± 14.1	0.141	65	4.351 ± 0.377	4.844 ± 0.422	11.5 ± 6.7^{e}	0.028*
	130	5.355 ± 0.916	6.980 ± 1.073	31.4 ± 16.5^{b}	0.028*	130	4.844 ± 0.424	5.782 ± 0.595	19.8 ± 13.4	0.027*
	260	5.051 ± 1.426	6.907 ± 1.326	39.1 ± 14.5^{c}	0.028*	260	4.139 ± 0.244	5.629 ± 0.452	36.2 ± 9.5^{c,e}	0.028*
Human saliva (n=8)	32.5	1.376 ± 0.298	1.522 ± 0.349	10.6 ± 6.0^{b,c}	0.018*	32.5	1.862 ± 0.815	2.018 ± 0.824	10.2 ± 10.7^{b,c}	0.018*
	65	1.362 ± 0.362	1.604 ± 0.382	20.7 ± 25.8	0.050	65	1.337 ± 0.433	1.625 ± 0.402	24.6 ±13.0^{e}	0.012*
	130	1.438 ± 0.467	2.004 ± 0.664	39.0 ± 13.4^{b}	0.012*	130	1.369 ± 0.429	1.942 ± 0.678	42.0 ± 22.0^{b}	0.012*
	260	1.428 ± 0.595	2.328 ± 0.850	65.9 ± 38.0^{c}	0.012*	260	1.456 ± 0.618	2.266 ± 0.679	65.4 ± 32.5^{c,e}	0.012*

bLPO, bovine lactoperoxidase (25 μg/mL)
Sig., Significance
%Diff, percent changes of enzymatic activities in samples with sugar alcohol compared with samples without sugar alcohol
The Wilcoxon signed rank test was used to analyze differences between the enzyme activities of samples with and without sugar alcohol. $^{*}P < 0.05$
The Kruskal-Wallis test was used to analyze differences of %Diff according to the concentration of sugar alcohols. $^{\dagger}P < 0.05$ in bLPO, $^{\ddagger}P < 0.05$ in human saliva
The Mann-Whitney U-test with Bonferroni's correction was used as post hoc analysis. Pair of the same alphabet denotes a significant difference. $P < 0.0125$
The Mann-Whitney U-test was used to analyze differences between %Diff of xylitol and sorbitol at the same molar concentrations. $^{\S}P < 0.05$

Table 4. Influence of the mixture of xylitol and sorbitol on the enzymatic activities of bLPO in the solution phase

mM ratio of Xylitol : Sorbitol (n=8)	Peroxidase activity (mUnits/mL)			Sig.
	Sample only	With mixture	%Diff	
130 :0	5.355 ± 0.916	6.980 ± 1.073	31.4 ± 16.5	0.028*
97.5 : 32.5	5.356 ± 0.337	6.828 ± 0.780	27.3 ± 8.8	0.028*
65 : 65	5.727 ± 0.474	7.114 ± 0.538	24.8 ± 12.1	0.028*
32.5 : 97.5	5.782 ± 0.869	7.266 ± 1.085	25.7 ± 4.5	0.027*
0 : 130	4.844 ± 0.424	5.782 ± 0.595	19.8 ± 13.4	0.027*

bLPO, bovine lactoperoxidase (25 μg/mL)
Sig., Significance
%Diff, percent changes of enzymatic activities in samples with sugar alcohol compared with samples without sugar alcohol
The Wilcoxon signed rank test was used to analyze differences between the enzyme activities of samples with and without sugar alcohol. $^{*}P < 0.05$
The Kruskal-Wallis test was used to analyze differences of %Diff according to the different ratios of sugar alcohols. $^{\dagger}P < 0.05$

Table 5. Influences of xylitol and sorbitol on the enzymatic activities of bLPO and whole salivary peroxidase on the surface phase

Source of peroxidase	Assay	Xylitol (mg/mL)	Peroxidase activity (mUnits)		Sig.	Sorbitol (mg/mL)	Peroxidase activity (mUnits)		Sig.
			Sample only	with Xylitol			Sample only	with Sorbitol	
bLPO (n=6)	I	130	0.057 ± 0.005	0.057 ± 0.005	0.833	130	0.061 ± 0.016	0.056 ± 0.011	0.463
	II	130	0.054 ± 0.007	0.058 ± 0.004	0.345	130	0.062 ± 0.011	0.065 ± 0.007	0.416
Human Saliva (n=8)	I	130	0.021 ± 0.009	0.021 ± 0.007	0.865	130	0.030 ± 0.009	0.028 ± 0.006	0.528
	II	130	0.012 ± 0.004	0.011 ± 0.005	0.147	130	0.020 ± 0.005	0.018 ± 0.004	0.139

bLPO, bovine lactoperoxidase (25 μg/mL)
Sig., Significance
Assay I, Sugar alcohols were first adsorbed to hydroxyapatite beads and bLPO or human saliva was adsorbed next.
Assay II, Sugar alcohols were pre-incubated with bLPO or human saliva, and the mixtures were adsorbed to hydroxyapatite beads.
The Wilcoxon signed rank test was used to analyze differences between the enzyme activities of samples with and without sugar alcohol. $^{*}P < 0.05$

Table 6. Influence of the mixture of xylitol and sorbitol on the enzymatic activity of glucose oxidase-mediated peroxidase system

mM ratio of Xylitol : Sorbitol (n=8)	GO-PO activity (OD) in glucose 0.02 mg/mL			Sig.	GO-PO activity (OD) in glucose 0.04 mg/mL			Sig.	GO-PO activity (OD) in glucose 0.06 mg/mL			Sig.
	Sample only	With sugar alcohol	%Diff		Sample only	With sugar alcohol	%Diff[†]		Sample only	With sugar alcohol	%Diff[†]	
130 : 0	0.354 ± 0.154	0.355 ± 0.121	0.2 ± 2.4	0.933	0.681 ± 0.111	0.686 ± 0.117	0.7 ± 1.6[c,d]	0.400	0.974 ± 0.343	0.978 ± 0.027	0.5 ± 1.0[d]	0.208
97.5 : 32.5	0.366 ± 0.014	0.358 ± 0.007	-2.0 ± 3.0	0.123	0.686 ± 0.028	0.682 ± 0.224	-0.5 ± 2.8	0.779	0.967 ± 0.064	0.960 ± 0.084	-0.9 ± 2.8	0.623
65 : 65	0.361 ± 0.123	0.359 ± 0.004	-1.0 ± 3.8	0.944	0.690 ± 0.005	0.673 ± 0.295	-2.8 ± 4.4	0.236	0.979 ± 0.031	0.965 ± 0.043	-1.6 ± 2.2	0.115
32.5 : 97.5	0.358 ± 0.012	0.348 ± 0.010	-2.8 ± 2.2	0.017[*]	0.684 ± 0.005	0.658 ± 0.009	-3.8 ± 1.6[c]	0.012[*]	0.964 ± 0.382	0.913 ± 0.523	-5.2 ± 5.1	0.025[*]
0 : 130	0.361 ± 0.016	0.355 ± 0.009	-1.5 ± 2.7	0.092	0.691 ± 0.100	0.673 ± 0.147	-2.5 ±2.0[d]	0.012[*]	0.969 ± 0.572	0.904 ± 0.131	-7.1 ± 9.4[d]	0.012[*]

GO-PO, glucose oxidase-mediated peroxidase system
%Diff, percent changes of enzymatic activities in samples with sugar alcohol compared with samples without sugar alcohol
The Wilcoxon signed rank test was used to analyze differences between the enzyme activities of samples with and without sugar alcohol. [*]$P < 0.05$
The Kruskal-Wallis test was used to analyze differences of %Diff according to the different ratios of sugar alcohols. [†]$P < 0.05$
The Mann-Whitney U-test with Bonferroni's correction was used as post hoc analysis. Pair of the same alphabet denotes a significant difference. $P < 0.01$
The Kruskal-Wallis test was used to analyze differences of %Diff according to glucose concentrations. [§]$P < 0.05$

국문초록

Xylitol과 Sorbitol이 Lysozyme과 Peroxidase에 관련된 효소활성 및 Candidacidal 활성에 미치는 영향

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(지도교수 **고 홍 섭**)

Xylitol과 sorbitol은 감미제로 널리 사용되며 구강건강용품에 포함될 경우 타액 내 여러 항균물질들과 반응할 것으로 추정된다. 이에 본 연구의 목적은 xylitol과 sorbitol이 lysozyme, peroxidase 및 glucose oxidase-mediated peroxidase의 효소활성과 candidacidal 활성에 관한 영향을 평가하기 위한 것이다.

Xylitol과 sorbitol을 hen egg-white lysozyme, bovine lactoperoxidase, glucose oxidase-mediated peroxidase 및 비자극성 전타액에 첨가하여 용액상태 및 hydroxyapatite 표면상태에서 효소활성을 측정하였다. Lysozyme 효소활성은 *Micrococcus lysodeikticus* 현탁액의 혼탁도 변화로 측정하였고, peroxidase 효소활성은 hypothiocyanite에 의한 5,5′-dithiobis-2-nitrobenzoic acid의 산화율 변화로 측정하였으며, glucose oxidase-mediated peroxidase 효소

활성은 o-dianisidine 산화물 생성 정도로 측정하였다. Xylitol과 sorbitol이 hen egg-white lysozyme, bovine lactoperoxidase, glucose oxidase-mediated peroxidase의 candidacidal 활성에 미치는 영향은 *Candida albicans* ATCC 10231, 11006, 18804를 이용하고 군집 형성 단위를 계수하여 측정하였다.

Xylitol과 sorbitol은 용액상태와 hydroxyapatite 표면상태에서 hen egg-white lysozyme의 효소활성과 hydroxyapatite 표면상태에서 타액 lysozyme의 효소활성에 영향을 미치지 않았으나, 용액상태에서 타액 lysozyme의 효소활성을 저해하였다. Xylitol과 sorbitol은 용액상태에서 bovine lactoperoxidase와 타액 peroxidase의 효소활성을 모두 증가시켰으며, 이는 농도에 비례하였다. 하지만, 이러한 증가 현상은 hydroxyapatite 표면상태에서는 관찰되지 않았다. Sorbitol은 glucose oxidase-mediated peroxidase의 효소활성을 유의하게 저해하였다. Xylitol과 sorbitol은 lysozyme, peroxidase 및 glucose oxidase-mediated peroxidase의 candidacidal 활성에 영향을 미치지 않았다.

결론적으로, xylitol과 sorbitol은 타액 lysozyme의 효소활성을 억제하였고 bovine lactoperoxidase와 타액 peroxidase의 효소활성을 증가시켰다. 이와 달리 lysozyme과 peroxidase의 candidacidal 활성에는 부가적인 효과를 보이지 않았다.

주요어 : Xylitol, Sorbitol, Lysozyme, Peroxidase, Glucose oxidase, Candidacidal 활성

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