Effect of xylitol-containing carbohydrate mixtures on acid and ammonia production in suspensions of salivary sediment

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Abstract – pH changes and the production of lactic acid, acetic acid and ammonia were studied in suspensions of salivary sediment supplemented with mixtures of xylitol and other carbohydrate sweeteners. The only mixtures which increased the pH values of the suspensions were those containing xylitol alone or mixtures of xylitol and sorbitol. Mixtures of xylitol and Lycasin 80/55 caused a relatively small pH reduction. Xylitol was not able to inhibit the acid production from the easily fermented glucose, fructose and Lycasin 05/60. The levels of lactic acid, determined in the incubation mixtures, directly reflected these pH changes. The levels of acetic acid and ammonia were, however, relatively similar in all incubation mixtures. The results suggest that the inhibitory effects of xylitol on acid production of oral flora should be retained, provided that xylitol is used either alone or in mixtures with slowly fermentable carbohydrates, such as sorbitol and Lycasin 80/55.

Key words: acid production; carbohydrates; dental plaque; sweeteners; xylitol.

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The majority of oral bacteria lack the ability to ferment xylitol (1-4). The inability of plaque to metabolize xylitol may be one of the factors leading to a reduction both in the amount of plaque and the incidence of Streptococcus mutans, both of which have been found to result from between-meal intake of xylitol (5,6). The enhancement of remineralization of carious lesions observed in connection with the intake of xylitol seems to be based on the above microbiologic effects and the ability of this polyol to stimulate the flow of saliva without a pH decrease (7). Thus, the nonfermentability and non-acidogenicity of xylitol seem to be behind most of its favorable actions in the oral cavity (8).

Although the beneficial oral effects of xylitol are widely acknowledged, the fact remains that xylitol, partly as a result of its high price and partly as a result of food technologic aspects, is normally used in mixtures with other sweeteners. Only a relatively small number of studies have, however, dealt with the dental effects of mixtures of xylitol and other carbohydrates. The aim of the present study was to elucidate the effects of various mixtures of xylitol and other common carbohydrate sweeteners on the acid and ammonia production by salivary sediment.

Material and methods

Carbohydrates – The following carbohydrates were used: xylitol (Finnish Sugar Co., Helsinki, Finland), fructose, glucose, sorbitol (Sigma Chemical Co., St. Louis, MO, USA), Lycasin 05/60 and Lycasin 80/55 (Roquette, Lille, France). Xylitol (x) and the other carbohydrates (ch) were used in the following ratios: x/ch = 0/100; 50/50; 99/1; 99.5/0.5; 100/0 (wt/wt).

Salivary sediment mixture – The salivary sediment incubations were performed according to KLEINBERG (9). Whole saliva samples were collected from dental students (five women and five men, aged 21-23 yr) 2 h after breakfast, using 10-min paraffin wax stimulation. The samples were pooled and divided into 10-ml aliquots. The aliquots were centrifuged (12 000xg, 10 min, +4°C) and the supernatant fluids and the pellets stored separately at −20°C until used in the incubation experiments within 4 wk.

Incubation conditions and assay methods – Before the incubation each sediment was washed with 10 ml of 0.9% NaCl (10-s Vortex agitation, followed by a 5-s sonication and centrifugation for 10 min at 12 000xg; +4°C). The washed sediments were combined with 4 ml of Millipore-filtered (HAWP 0.45 μm) saliva supernatant. These mixtures were homogenized using Vortex treatment and sonification as above. 300 μl aliquots of the suspensions were mixed with 300 μl of a 20% (w/v) carbohydrate solution (mixtures of xylitol and other carbohydrates mentioned above). The resulting suspensions were incubated for 4 h at 37°C. The final total concentration of the carbohydrates in the incubation mixtures was thus 10% (w/v). For Lycasin 80/55 the amount of dry matter in the syrup was used as a basis for calculations. The pH values were measured electrometrically at the beginning and after the 4-h incubations. After the incubation, the mixtures were centrifuged as above and the supernatants stored at −20°C for the determination of lactic acid, acetic acid (using the Boehringer-Mannheim test kits) and ammonia (10).

Results

Fig. 1 illustrates the pH changes measured for the various x/ch mixtures. The pH drops caused by 10% fructose, glucose or Lycasin 05/60 alone (x/ch = 0/100) were large and of almost identical magnitude. For 10% sorbitol, Lycasin 80/55, and the control containing water instead of added carbohydrates, slight decreases in the pH values were observed. The presence of 10% xylitol alone, however, resulted in a slight pH increase.

The mixtures of fructose or glucose with xylitol behaved identically. The decreases in the pH values directly reflected the amounts of fermentable fructose or glucose present in these incubation mixtures. The presence of various amounts of xylitol had no or only a slight effect on the decrease of the pH values. With these two carbohydrates, the only mixture which did not show a large pH drop was the x/ch = 0.5/99.5 mixture. The mixture x/ch = 50/50 of Lycasin 05/60 and xylitol decreased the pH values similarly to the corresponding mixtures of fructose or glucose with xylitol. For the x/ch = 99/1 mixtures of Lycasin 05/60 and xylitol, however, a smaller pH drop was observed as compared to fructose or glucose. With all combinations of sorbitol and xylitol not an increase but a decrease in the pH values was observed. Mixtures of Lycasin 80/55 and xylitol showed a relatively small pH drop.

The amount of lactic acid produced during the incubations reflected the results observed in the pH measurements (Fig. 2). The lowest amounts of acetic acid were, however, observed in mixtures containing xylitol as the only added carbohydrate. The increase

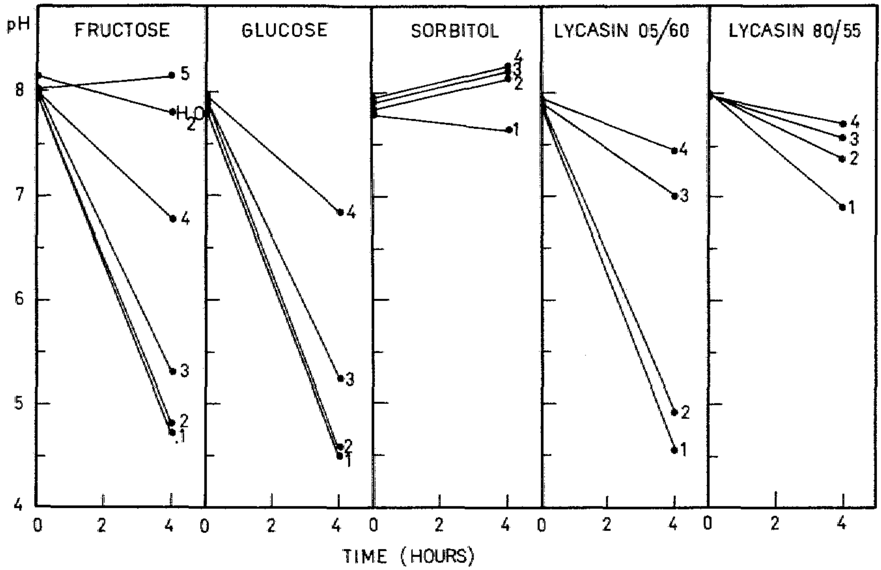

Fig. 1. pH-changes in suspensions of salivary sediment for 10% (w/v) mixtures of xylitol (x) and other carbohydrates (ch). The pH measurements were made at the start of the incubation, and after 4 h. 1: x/ch = 0/100; 2: x/ch = 50/50; 3: x/ch = 99/1; 4: x/ch = 99.5/0.5; 5: x/ch = 100/0.

in the relative amount of xylitol in the sorbitol-xylitol mixtures resulted in decreased amounts of acetic acid. This effect exerted by xylitol was also observed with mixtures of xylitol and Lycasin 80/55 (not shown), but not with the other x/ch mixtures studied. The acetic acid levels of the control mixtures incubated for 4 h in an ice-bath showed values which were about half of those found after incubation at 37°C (not shown).

Changes in the x/ch ratio had no clear effect on the amounts of ammonia produced (Fig. 2). The ammonia levels of the controls kept in an ice-bath were about 50% lower than those found after incubation at 37°C (not shown). The presence of fructose seemed to result in a lower production of ammonia, the ammonia levels detected were the smallest in the presence of fructose only. The production of ammonia increased when the relative portion of fructose was decreased in the x/ch mixtures. The acid and ammonia levels observed in the presence of mixtures of xylitol and Lycasin 80/55 closely resembled those mixtures of xylitol and sorbitol; the levels of lactic acid were only slightly higher in the former.

Discussion

Several studies have demonstrated that xylitol does not cause any pH-reduction in plaque either in vivo or in vitro (2, 11-13). In pure cultures, xylitol inhibits the growth and acid production of some oral bacteria (14, 15). The results of studies on acid production with mixtures of xylitol and other carbohydrates are partly controversial, however (12, 16-20). For example, xylitol decreased the acid production in plaque when used simultaneously with glucose or fructose (16). Other studies have demonstrated a

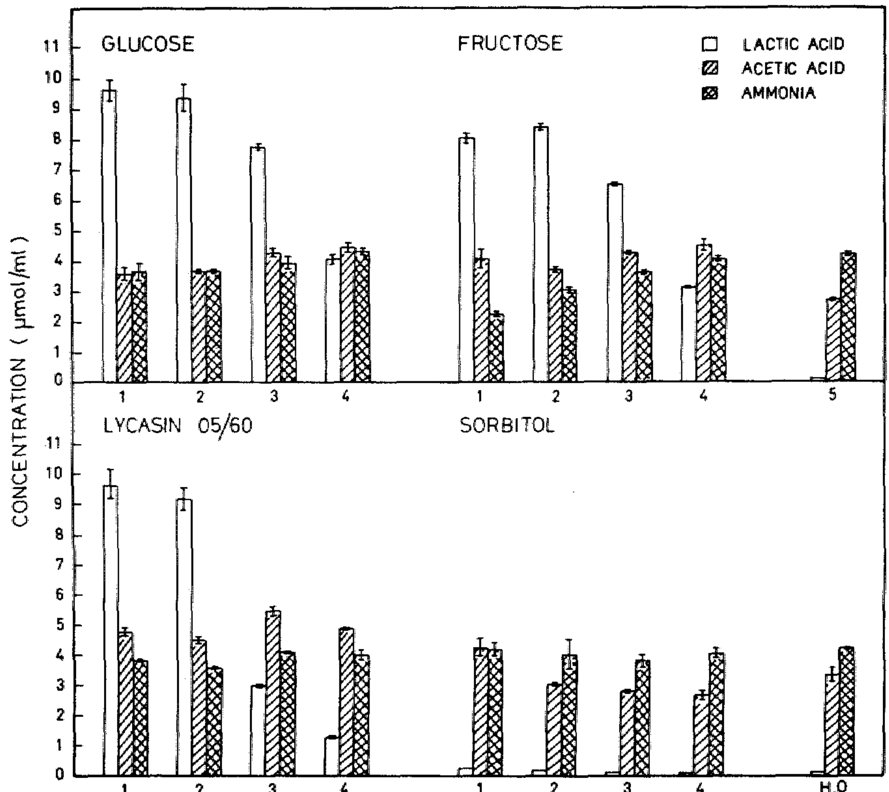

Fig. 2. The levels of lactic acid, acetic acid, and ammonia detected in the suspended salivary sediment mixtures after 4 h incubation in the presence of 10% (w/v) mixtures of xylitol and other carbohydrates. The columns show the means and ranges of duplicate determinations. For other details, see Fig. 1.

very slight or no inhibitory effect on acid production by xylitol in mixtures of sucrose and xylitol (12, 17, 20). Our results are in agreement with the latter ones, suggesting that xylitol exerts no clear inhibitory effect on acid production of mixed oral flora when used in combination with easily fermentable carbohydrates. The ratio of xylitol to the fermentable carbohydrate in the mixture was of little importance.

It has been previously shown that the slow acid production from sorbitol in plaque is further decreased by the presence of xylitol (18). This decrease has been attributed to the formation of xylitol-5-phosphate in some oral bacteria, for example S. mutans, this compound being toxic to these bacteria (19). In the present study, an increase in pH values in the control mixtures containing no added carbohydrate or xylitol alone was found only in the xylitol-sorbitol mixtures, independent of the ratio of xylitol to sorbitol involved. Thus, out of the five sucrose substitutes and their mixtures tested in this study, only those of sorbitol and xylitol were associated with an increase of the pH values of mixed oral flora.

The lactic acid production alone seemed to explain the pH decreases observed in the mixed oral flora, a result which is in agreement with earlier findings (21, 22). The relatively similar levels of acetic acid found with most carbohydrates and the control mixtures suggest that this acid was mainly generated from endogenous sources. Also, the similar levels of ammonia detected after all incubations reflected the urea concentration of the whole saliva used in the incubations, rather than a specific effect of the carbohydrates on the base metabolism of the mixed oral flora. Though acetic acid has been proposed to have buffering properties under the conditions prevailing in the oral cavity (23), the ratio of acetic acid to lactic acid seemed to be of minor importance as a pH-regulating factor under the conditions of this study. The ratio of ammonia to acids (lactic acid and acetic acid) being above one, however, resulted in an increase in the pH values observed in the three xylitol-sorbitol mixtures, in those containing xylitol alone, and in the controls containing no added carbohydrate.

The beneficial effects of xylitol on oral health are mainly based on the nonfermentability of this polyol by most oral bacteria (8), although physicochemical effects may also contribute to the final outcome (24, 25). Thus, according to the present and other studies (17, 18), one of the most important mechanisms of the action of xylitol will be abolished if xylitol is mixed with easily fermentable carbohydrates, such as fructose, glucose or sucrose. In mixtures with Lycasin 80/55, which is fermented slowly but to a greater extent than sorbitol (11), addition of xylitol did not lead to an increase in the pH values during incubation; however, the pH drops were very small. Thus, in mixtures with sorbitol and Lycasin 80/55, xylitol should promote remineralization as efficiently as when used alone. However, the possible adaptation of the oral flora must be taken into consideration when using slowly fermentable carbohydrates in mixtures with xylitol (26). For xylitol alone such adaptation has not been reported (27).

Although maximization of the beneficial pH effects of xylitol would naturally presuppose that these polyols should not be mixed with other carbohydrates in consumer products, it appears that those effects should be retained in mixtures of xylitol and certain slowly fermented carbohydrates, such as sorbitol and Lycasin 80/55.

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