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ORIGINAL ARTICLE

Effects of Isomalt on enamel de- and remineralization, a combined in vitro pH-cycling model and in situ study

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Abstract Isomalt is a non-cariogenic sweetener, which is widely used in sugar-free candy and chewing gum. Little is known about the effects of Isomalt on de- and remineralization. Binding between calcium and Isomalt has been reported, which could affect the mineral balance. The objective of this study was to examine the effects of Isomalt on de- and remineralization of bovine enamel lesions, both in vitro and in situ. In in vitro study, subsurface enamel lesions were subjected to 3-weeks pH-cycling. Treatments were 5-min rinses with 10% Isomalt solutions daily and 10% Isomalt additions to re- or demineralizing solutions. Standard pH-cycling conditions were used with a 0.2 ppm fluoride background during the remineralization phase. In in situ study, subsurface lesions were exposed 2 months in vivo and brushed three times daily with 10% Isomalt containing toothpaste. Treatment effects were assessed by chemical analysis of the solutions (in vitro) and transversal microradiography (in vitro and in situ). In in vitro study, while 5-min rinses with 10% Isomalt gave slightly increased remineralization, continuous presence of 10% Isomalt (in re- or demineralizing solutions) inhibited both de- and/or remineralization. This lead to significantly smaller overall mineral loss when Isomalt was added during demineralization. In in situ study, remineralization enhancement during short Isomalt treatments was confirmed. Isomalt had a positive effect on the de/remineralization balance when given under conditions relevant to practical use.

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Introduction

The addition of sugar substitutes to foods and drinks is well established, and the use of such products is popular, as most sugar substitutes are not only non-cariogenic but also low-caloric. The large consumption of diet products is probably mainly driven by today's concerns on overweight and obesity, but a favorable side effect is that it does add to a dental awareness. Bulk sweeteners that replace sucrose while mimicking its textural and taste properties are in high demand. In that group, Isomalt is widely used in candy and chewing gum. Isomalt is one of the sugar alcohols that have been tested in vitro for fermentation by oral microorganisms, and it has been classified as non-acidogenic and non-cariogenic [12]. Isomalt is formed by hydrogenation of isomaltulose and consists of a 1:1 mixture of (1-*O*-α-glucopyranosyl-D-mannitol and 6-*O*-α-glucopyranosyl-D-sorbitol). In the literature, it is also called Palatinit®. The mixture has a weak calcium-binding ability due to the presence of nine hydroxyl groups within one molecule [7]. Isomalt has been tested in rat caries studies [8] and with the telemetric plaque pH method [7]. No human caries clinical data is available.

Because of its calcium-binding properties, Isomalt might influence enamel de- and remineralization. This effect could either be positive by facilitating calcium diffusion due to masking of the positively charged calcium ion, as has, e.g., been documented for calcium tartrate [6]. Alternatively, the calcium binding could have a negative effect by decreasing the degree of supersaturation of ambient fluids to hydroxyapatite.

Aim of this study was to assess the effects of Isomalt on de- and remineralization. This was done with the in vitro

pH-cycling model [10] and in an 'in situ study' with enamel lesion placed on intra-oral appliances.

Materials and methods

In vitro study

Study design

This part of the study consisted of a pH-cycling experiment in which the effects of Isomalt in different treatment modes on enamel de- and remineralization were assessed from the changes in the chemical composition of the respective solutions. Moreover, the overall mineral uptake or loss was verified by post pH-cycling microradiographic analyses of the lesions, which also provided depth-specific mineral uptake or loss data.

Lesion formation

Bovine enamel specimens were embedded in acrylic resin. The enamel surfaces were ground flat on 600-grid silicon carbide abrasive paper under running tap water. The specimens were placed, enamel surface up, in a glass tray and immersed in 150 ml of 8% methylcellulose gel. After 24 h, filter paper was placed on top of the gel, and 150 ml of 0.1 M lactic acid buffer adjusted to pH 4.6 was poured over it. The tray was covered and incubated at 37°C for 7 days.

pH-cycling conditions

pH-cycling conditions were used with a daily schedule of six cycles, each of 0.5 h demineralization and 2.5 h of remineralization, followed by a "night" period of 6 h of remineralization [10, 11]. The experiment was run in a dedicated robot developed for pH-cycling experiments.

During 3 days before the start of the treatment phase, all specimens were pH-cycled without treatments. Calcium uptake and loss were determined, which data were used to exclude specimen outliers and to match the specimens to form five groups of eight specimens comparable in mineral uptake and loss properties. This method has previously been shown to increase sensitivity of the model to detect differences between treatments [10]. The treatment phase lasted 3 weeks, with 15 days of daily cycling and treatments and storage in the remineralizing solution during the intermittent weekends. The remineralizing solutions contained 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, 0.2 ppm fluoride, and 20 mM 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) adjusted to pH 7.0. The demineralizing solutions contained 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, and 50 mM acetic acid adjusted to pH 4.8. The pH-cycle solutions were refreshed daily. Each 22 mm^2-sized specimen was cycled between individual 3-ml aliquots of the two solutions.

Experimental groups

Test treatments were identified as follows:

1. Control, no treatment (C)
2. Rinse once daily with 10% Isomalt solution before "night" remineralization period (RR)
3. Rinse once daily with 10% Isomalt solution before the first demineralization period (RD)
4. Addition of 10% Isomalt to the remineralizing solutions (AR)
5. Addition of 10% Isomalt to the demineralizing solutions (AD).

For the rinse treatments, the specimens were immersed in 10 wt% Isomalt in deionized water for 5 min, then rinsed for 20 sec under tap water, and followed by rinsing for 10 s under deionized water.

Calcium measurement

Mineral uptake and loss during pH-cycling were assessed by sampling the individual de- and remineralizing solutions for changes in the calcium content. To this end, 200 μl samples were taken to which 3 ml of 53 mM $La(NO_3)_3$ in 50 mM HCl was added. The solutions were analyzed by atomic absorption spectroscopy (Perkin Elmer 372) at 423 nm. The cumulated calcium loss and uptake data were calculated by adding the daily data.

Transversal microradiography

At the end of the pH-cycling period, a section was cut from the middle of each specimen and perpendicular to the surface, using a water-cooled diamond-coated wire saw model 3242 (Well, Le Locle, Switzerland). The sections were ground down to 100 μm by Al_2O_3-slurry with 3 μm particles. The sections, together with a 12-step aluminum step wedge, were microradiographed on high-resolution plates (Kodak Type 1A), with a nickel filtered Cu–Kα source operated at 20 mA and 20 kV for 10 min. The plates were developed according to the instructions of the manufacturer (AGFA Gevaert AG, FRG). Mineral content vs depth profiles were measured with a microscope with CCD camera-computer set-up with software (TMR1.25e, Inspektor Research Systems, Amsterdam, The Netherlands) for transversal microradiography (TMR). Profiles were also determined for baseline lesions, taken from a separate group of specimens immediately after lesion formation. Previous experiments have shown that this procedure provides reliable data (e.g., [11]). The tracings

were expressed as mineral content profiles, integrated mineral loss (IML) and lesion depth, using the established parameter definitions [4].

In situ study

A randomized crossover in situ study was designed and approved by the Institutional Review Board. Twelve healthy volunteers (mean age, 31±3 years) agreed to participate and signed informed consent letters. Each participant was provided an intra-oral appliance with two demineralized enamel specimens [14]. The subjects were instructed to brush with either the experimental toothpaste or the control toothpaste three times per day for 2 months. Brushing was done with the appliances in place and ensuring that the specimens were not brushed. The experimental toothpaste contained 0.11% sodium fluoride and 10% Isomalt in a silica base. The control toothpaste had an identical composition although without Isomalt. After 1 and 2 months, respectively, specimens were collected and analyzed by microradiography, as outlined above. The data were expressed as the relative remineralization (in %), which was calculated $(IML_{ini}-IML_{rem})/IML_{ini}$. IML_{ini} refers to the IML of the baseline lesions and IML_{rem} of this parameter after remineralization. A wash-out period of 1 month was installed between the two legs of the experiment.

Statistic analysis

Differences between the groups were tested for significance at the $p<0.05$ level by Duncan's multiple range test (in vitro study) and paired t test (in situ study) using SPSS-PC software.

Table 1 Cumulated calcium loss and calcium uptake of the enamel specimens ($n=8$)

Group	Calcium loss	Diff.[a]	Calcium uptake	Diff.	Net calcium[b]	Diff.
C	−99.9±6.7	a	75.1±5.0	b	−24.7±4.9	B
RR	−95.4±4.0	a	80.5±4.3	c	−14.8±5.6	C
RD	−98.4±3.9	a	81.9±2.4	c	−16.5±4.2	C
AR	−89.7±6.8	b	48.7±2.2	a	−41.0±6.6	A
AD	−66.0±5.8	c	72.1±4.6	b	6.0±3.5	D

[a] Data in the same column indicated with the same character are not statistically significant different (at $p=0.05$).
[b] "–"indicates loss and "+" indicates uptake
Data are expressed as µmol/cm^2.
C Control; *RR* rinse once daily with 10% of Isomalt solution before long remineralization period; *RD* rinse once daily with 10% of Isomalt solution before the first demineralization period; *AR* addition of 10% Isomalt to the remineralizing solutions; *AD* Addition of 10% Isomalt to the demineralizing solutions

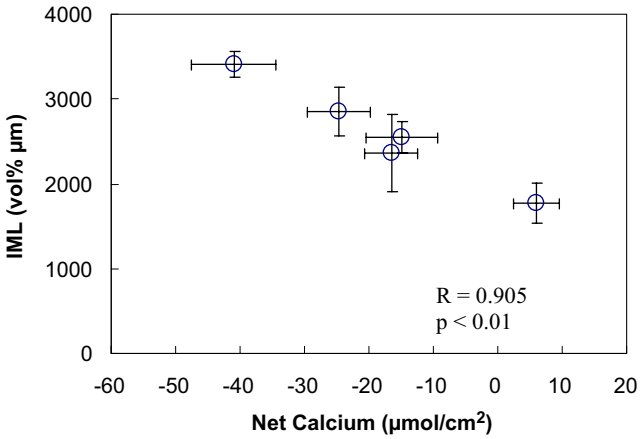

Fig. 1 Correlation of microradiographic assessment of lesion severity increments (expressed as Integrated Mineral Loss, *IML*) vs net calcium uptake/loss (measured with chemical analysis of the de-/remineralization solutions)

Results

In vitro pH-cycling: calcium measurements

The conditions of this experiment were chosen to result in net demineralization of the enamel specimens in the control group. The cumulated calcium uptake and loss data for all groups are given in Table 1. A short 10% Isomalt rinses, given at either moment during pH-cycling (RR, RD) resulted in increased calcium uptake, whereas calcium loss was not changed. Isomalt added to the demineralizing solution (AD) resulted in a decreased calcium loss but did not influence calcium uptake. Isomalt added to the remineralizing solution (AR) resulted not only in a small decrease in calcium loss, but also in a lower calcium uptake. Taking the rinse effects on demineralization and remineralization together (groups RR, RD), as shown in the net calcium values (Table 1), showed a small although significant inhibition of demineralization, when compared with the control group. In contrast, continuous presence of Isomalt led to increased overall demineralization (AR) or substantial inhibition of demineralization leading to net remineralization of the specimens (AD).

In vitro pH-cycling: microradiography (TMR)

TMR assessment of the lesions produces IML data and mineral content vs depth profiles. The IML values may be directly compared with the chemical net calcium data to check for consistency in the overall data set. Doing so, we found a good correlation between IML and net calcium uptake/loss (Fig. 1: $R=0.905$; $p<0.01$).

Mineral content vs depth profiles per group are depicted in Fig. 2, showing that lesions progressed beyond the baseline lesion in all experimental groups. Generally, net mineral

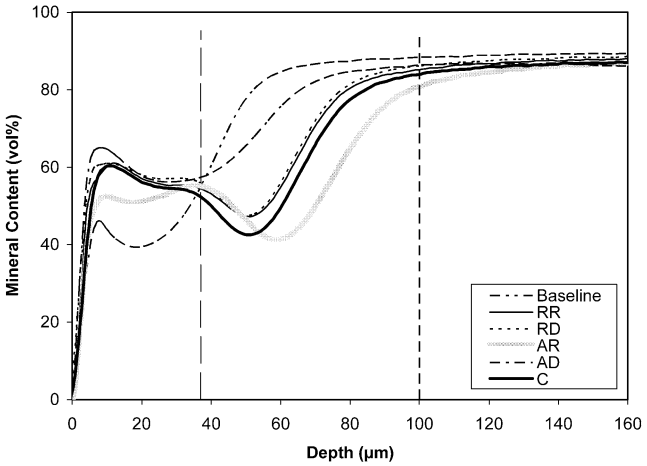

Fig. 2 Mineral content-depth profiles in each groups; *C* control; *RR* rinse once daily with 10% of Isomalt solution before long remineralization period; *RD* rinse once daily with 10% of Isomalt solution before the first demineralization period; *AR* addition of 10% Isomalt to the remineralizing solutions; *AD* addition of 10% Isomalt to the demineralizing solutions

deposition was observed over the depth corresponding with the original lesion, while secondary lesions had formed beyond the former lesion depth (Fig. 2). These two phenomena occurred below and beyond a depth of about 37 μm, respectively. To discriminate between deposition in the original lesion and secondary lesion formation, separate IML values were calculated for the two zones: 0–37 μm covering the original lesion and 38–100 μm covering the newly formed lesions (Table 2).

Data for the original lesion (IML0–37) showed significantly ($p<0.05$) smaller IML values for the group with Isomalt added to demineralization solution (AD) and significant ($p<0.05$) larger IML values for the group with Isomalt added to remineralization solution (AR), both in comparison with the control group. Both rinse groups (RR, RD) were affected only marginally in the 0–37 μm region. Similarly, there were statistically significant differences in IML38–100 between the control group and the four treatment groups (Table 2). Isomalt given as rinse or added to the demineralization solutions resulted in significant smaller secondary lesions (RR, RD, AD), while Isomalt added to the remineralizing solution led to more demineralization in the deeper regions (AR).

Table 3 Relative remineralization (in% with SD) in in situ study

Group	1 month (%)	Diff.	2 month (%)	Diff.
Isomalt toothpaste	21.9±15.7	a	33.3±21.9	*NS*
Control toothpaste	9.9±14.7		25.2±19.0	

a Statistic significant difference; *NS* not significant

In situ study

A statistically significant difference was found between the relative remineralization in the Isomalt toothpaste group vs the control group after the first month period (Table 3). After 2 months, the relative remineralization in the experimental toothpaste group was still higher than in the control group. However, this difference was no longer statistically significant.

Discussion

This study convincingly demonstrates that Isomalt has an effect on enamel de- and remineralization. The results from both rinse groups (RR, RD) showed a small but statistically significant enhancement of remineralization. We note that this was observed irrespective the timing of the Isomalt rinse, suggesting that the Isomalt is to some degree retained by the specimens.

The potential of Isomalt in affecting the de-/remineralization processes is further illustrated in the two groups where Isomalt was added to the de- or remineralization solutions. With this experimental condition, we presume that the longer contact time will result in substantial Isomalt deposition inside the lesions and presumably also at greater depth. The observed significant inhibition of remineraliza-

Table 2 Lesion depth (Ld) and integrated mineral loss (IML; $n=8$)

Group	Ld (μm)	Diff.[a]	IML (vol% μm)	Diff.	IML0–37 (vol% μm)	Diff.	IML38–100 (vol% μm)	Diff.
Baseline	54±2	a	2,027±100	ab	1,835± 83	d	350±48	a
C	87±9	d	2,855±292	d	1,409±101	b	1,539±194	d
RR	84±4	cd	2,552±187	cd	1,359±124	ab	1,313±122	c
RD	77±7	c	2,368±457	bc	1,275±133	a	1,254±309	c
AR	105±7	e	3,411±157	e	1,537± 96	c	1,915±140	e
AD	66±5	b	1,768±237	a	1,248±104	a	695±139	b

The depth of 37 μm was chosen from the microradiographs as the point where the secondary lesions formed beyond the original lesions.
Baseline Lesion parameters of the original lesion; *C* control; *RR* rinse once daily with 10% of Isomalt solution before long remineralization period; *RD* rinse once daily with 10% of Isomalt solution before the first demineralization period; *AR* addition of 10% Isomalt to the remineralizing solutions; *AD* addition of 10% Isomalt to the demineralizing solutions
[a] Data in the same column indicated with the same character are not statistically significant different (at $p=0.05$).

tion (in AR group) might be explained by binding of Isomalt onto the enamel crystallites, decreasing the surface available for crystal growth. Although a condition with continuous exposure to high concentrations of Isomalt would not occur in practical usage, it does illustrate what effects an accumulation of Isomalt might have [2].

Calcium phosphate precipitation studies using hydroxyapatite powder seeds in supersaturated calcium phosphate solutions with various Isomalt concentrations (0–20%) confirmed an inhibition of crystal growth in the higher Isomalt concentration range and indicated that higher calcium concentrations were required to reach HAP saturation (data not shown).

While chemical analysis during pH-cycling provides kinetic data on mineral uptake and loss and how this is affected by therapeutic agents, the post pH-cycling TMR assessment gives depth-specific information. The current dataset shows good agreement in total calcium uptake/loss values obtained by these two independent methods.

The TMR mineral content vs depth profiles (Fig. 2) show patterns similar to those observed for specimens pH-cycled with short fluoride treatments [11]. Also in the latter case, remineralization of the initial lesion is found in combination with the formation of a new ('secondary') lesion at greater depth.

Although sugar substitutes were studied extensively in clinical trials, animal studies, and microbiological studies [13, 15], few data are available concerning their direct effects on de-/remineralization. Arends et al. [3] compared high level additions of fluoride and xylitol on enamel demineralization and found similar degree of inhibition from 6 ppm fluoride and 2.63 M xylitol (equivalent to 40% *w/v*). These effects were additive when both agents were combined. Amaechi et al. [1] showed that in vivo tolerable levels of xylitol did not have a significant effect on remineralization. Autoradiographic assessment of 14C xylitol showed penetration after a short (5 min) treatment throughout the 400-μm deep lesions [2], but we note that data could have been corrupted by the long specimen 'storage' during the autoradiographic exposure.

From the current pH-cycling data and the HAP seeding experiments, we conclude that binding to calcium ions and hydroxyapatite crystals will be the main mode of action of Isomalt, as has also been proposed for xylitol and sorbitol [5, 9].

The in situ study confirmed that Isomalt, when provided by tooth brushing from an Isomalt containing toothpastes, enhances remineralization. This difference was significant during the first month of in vivo brushing. Lack of significance after the second month may be due to plateauing of the remineralization or insufficient group size partly due to increased coefficient of variation. Nevertheless, we qualify the in vivo data as convincing.

With the current study, we demonstrated that Isomalt can strongly interfere with both de- and remineralization. Unlike in previous studies, our experiments show that an effect of Isomalt on enamel de-/remineralization is also observed after short rinses, rather than just during long-term presence during either demineralization or remineralization. Moreover, we note that the experimental groups were diverging from the control group in spite of a low level of fluoride being present. This could suggest an additive effect of Isomalt when given as an adjunct to fluoride, presumably due to a different mode of action.

We conclude that Isomalt affects de-/remineralization and might be an effective agent as an additional agent in caries prevention.

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