Degradation and fermentation of fructo-oligosaccharides by oral streptococci.

Journal of Applied Bacteriology

Hartemink, R.; Quataert, M.C.J.; Laere, K.M.J.; Nout, M.J.R.; Rombouts, F.M.

<https://doi.org/10.1111/j.1365-2672.1995.tb03176.x>

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne. This has been done with explicit consent by the author.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. In this project research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact openscience.library@wur.nl

Degradation and fermentation of fructo-oligosaccharides by oral streptococci

R. Hartemink, M.C.J. Quataert, K.M.J. van Laere, M.J.R. Nout and F.M. Rombouts

Department of Food Science, Wageningen Agricultural University, Wageningen, The Netherlands

5301/04/95: received 3 April 1995, revised and accepted 30 May 1995

R. HARTEMINK, M.C.J. QUATAERT, K.M.J. VAN LAERE, M.J.R. NOUT AND F.M. ROMBOUTS.

1995. Fructo-oligosaccharides (FOS) are claimed to have a positive effect on the intestinal flora. They are being used in functional foods in Japan and Europe. This group have tested the degradation of two commercial FOS preparations by oral streptococci in order to predict the cariogenicity of these products. Both preparations could be fermented to some extent by the species of oral streptococci tested.

The enzymes necessary for the degradation of FOS were inducible. Each strain showed a specific degradation pattern. All strains, particularly *Streptococcus mutans* rapidly produced acid, mainly lactic acid. *Streptococcus mitis* also produced high concentrations of acetic acid. Plaque formation by *Strep. mutans* was similar to the sucrose control.

It is concluded that FOS are cariogenic to a similar extent as sucrose.

INTRODUCTION

Fructo-oligosaccharides (FOS) are a group of oligosaccharides, consisting of several $\beta(1-2)$ - or $\beta(1-6)$ -linked fructose residues (Fm series), which may be linked to a glucose residue (GFn series). Two FOS mixtures are available commercially, 'Profeed' and 'Raftilose'. Profeed is produced enzymatically from sucrose using a fungal fructosyl-transferase. The final product consists of a mixture of linear and branched oligosaccharides (Fig. 1) of the GFn-series. The product has been marketed as 'Neosugar', 'Meiologo', 'Actilight' or 'Nutraflora' (Hidaka *et al.* 1990).

Raftilose is produced enzymatically from inulin using an endo-inulinase. The final product consists of a mixture of linear oligosaccharides from both the GFn series and the Fm series. This product has also been referred to as 'Oligo-fructose' (Roberfroid *et al.* 1993).

FOS are not degraded by the human digestive enzymes and thus reach the colon unaltered (Hidaka *et al.* 1990). It has been shown for both commercial mixtures that a daily intake of FOS results in an increase of faecal bifidobacteria (Mitsuoka *et al.* 1987; Wang and Gibson 1993). An increase in faecal bifidobacteria is claimed to have a beneficial effect

on health. FOS are therefore used as a health-promoting agent in 'functional foods' (Spiegel *et al.* 1994).

As FOS are widespread in nature, they are considered in food legislation as ingredients, rather than additives. In Japan this has resulted in the addition of FOS to a large range of products (Spiegel *et al.* 1994).

As FOS are used in many food products it is likely that some product remains in the oral cavity after consumption. It is therefore possible that FOS are degraded by the oral microflora and that, consequently, they may be cariogenic. It is well known that oral streptococci produce several fructanases, which can degrade inulin and other fructans (Hamada and Slade 1980; Walker *et al.* 1983; Takahasi *et al.* 1985; Burne *et al.* 1987).

Nystose (GF3), one of the components in both Raftilose and Profeed, has been claimed to be non-cariogenic (Hirasawa *et al.* 1984; Ikeda *et al.* 1990). However, Ziesenitz and Siebert (1987) came to the opposite conclusion. Neosugar (Profeed) has been shown to be non-cariogenic, although some acid is produced by oral streptococci (Hirasawa 1987).

Among the genera present in the oral cavity, the streptococci are considered to be the main cariogenic group of bacteria. The authors have therefore tested the degradation of FOS by members of this group, mainly *Streptococcus mutans* to predict the cariogenicity of this group of oligosaccharides.

Correspondence to: Ir R. Hartemink, Department of Food Science, Wageningen Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands.

Fig. 1 Structures of fructo-oligosaccharides

MATERIALS AND METHODS

Bacteria

The following strains of oral streptococci were obtained from Dr F. H. M. Mikx, Department of Oral Microbiology, Catholic University of Nijmegen, The Netherlands: *Strep. mutans* 44 (Nijm. 591), *Strep. oralis* 45 (Nijm. 592), *Strep. mitis* biovar I 46 (Nijm. 593), *Strep. sanguis* biovar I 43 (Nijm. 594) and *Strep. gordonii* biovar II 42 (Nijm. 595). In addition *Strep. mutans* 36 (DSM 20523) was used. For the induction studies a *Streptococcus* sp. was isolated from saliva of a human volunteer. It was identified as *Strep. salivarius*, using the Rapid ID 32 Strep and the APILAB identification program (Bio-Mérieux, Marcy-l'Étoile, France).

Oligosaccharides

Raftilose P95 was obtained from Orafit (Tienen, Belgium). Profeed P95 was obtained from Nutreco (Boxmeer, The Netherlands). Both preparations consisted of at least 95% (P95) oligosaccharides according to the manufacturers. The remaining part consists of glucose, fructose and sucrose.

Screening

FOS agar was used to screen for growth and acid formation of oral isolates. FOS agar consisted of Buffered Peptone Water (Oxoid) to which were added (g l⁻¹): Raftilose P95, 15; agar technical (Oxoid), 15; yeast extract (Oxoid), 5; and

chlorophenol red (Merck), 0.05. Only Raftilose P95 was used, as most components of Profeed P95 are present in Raftilose P95 as well. Appropriate dilutions of the strains, pre-grown in Peptone-Yeast extract broth supplemented with glucose (PY-G) were applied to FOS agar plates. PY-G was prepared according to Holdeman and Moore (1977). Growth was considered positive when the colony size was over 1 mm diameter. Acid production was considered positive when a yellow zone was formed around the colony. Raftilose P95 contains some fermentable mono- and disaccharides. Acid formation due to fermentation of these compounds was neutralized by the buffer in the medium. Any yellow discoloration is therefore due to fermentation of oligosaccharides. The plates were incubated at 37°C anaerobically, using an atmosphere of 80% nitrogen, 10% hydrogen and 10% carbon dioxide (HoekLoos, Rotterdam, The Netherlands).

Induction

Induction was studied using a *Strep. salivarius* strain which showed slow growth and slow acid production on FOS agar (i.e. no yellow zone within 24 h). The strain was cultured in 25 ml of PY-G. After 24 h, 100 µl was inoculated into 25 ml of PY-broth with 0.5% Raftilose P95 (PY-Raf). This strain was subsequently subcultured daily in PY-Raf for 7 d.

Growth curves were determined by adding 25 µl of a 24 h PY-G culture to 250 µl of PY-G and PY-Raf, respectively, in a microtitre plate. Similarly 25 µl of a 24-h-old culture of *Strep. salivarius* subcultured seven times on PY-Raf was

added to PY-Raf. Growth curves were determined by measuring the absorbance at 620 nm every 15 min for 11 h in a microtitre plate reader (EAR 400, SLT Instruments, Groedig, Austria).

Acid formation

Acid formation was studied by culturing the following strains in HPLC-broth: *Strep. mutans* 36, *Strep. mutans* 44, *Strep. oralis* 45, *Strep. mitis* 46, *Strep. sanguis* 43 and *Strep. gordonii* 42. HPLC broth is a medium which minimizes interference on the HPLC, it consists of (g l⁻¹): neutralized bacterial peptone (Difco), 5; yeast nitrogen base (Difco), 6; dipotassium hydrogen phosphate (anhydrous, Merck), 13; Raftilose P95 or Profeed P95, 10. The pH was adjusted to 7.0 using 3 mol l⁻¹ sodium hydroxide before sterilization for 15 min at 121°C. The strains were pre-cultured on PY-G for 16 h. All strains were grown anaerobically for 60 h at 37°C. Samples were taken at 12 h intervals for *Strep. mutans* 36 and after 60 h for the other strains.

The samples were centrifuged for 15 min at 10 000 rev min⁻¹ to precipitate the cells. Lactic and acetic acids were determined using HPLC with an Aminex HPX-87H column and 0.01 mol l⁻¹ sulphuric acid as the mobile phase (Voragen *et al.* 1986).

Rapid acidification

For the rapid acidification experiments *Strep. mutans* 36 was precultured for 16 h in PY-G. Cells were harvested by centrifuging 100 ml of culture and washed twice with reduced peptone physiological salt solution (RPS). RPS consisted of (g l⁻¹): neutralized bacterial peptone (Difco), 1.0; sodium chloride (Merck), 8.5; and cysteine. HCl (Sigma), 0.5. The pH was adjusted before sterilization to 6.7 with 3 mol l⁻¹ potassium hydroxide. After the second washing the cells were resuspended in 5 ml of RPS. For acidification tests 500 µl of carbohydrate or carbohydrate/RPS mixture was added to 5 ml of RPS. The mixture was placed in an incubator and the pH was measured continuously. When the pH was stabilized 500 µl of cell suspension was added and the pH was measured continuously for at least 30 min.

Fermentation patterns

Fermentation patterns were determined by growing all strains anaerobically at 37°C in HPLC-broth supplemented with 20 g l⁻¹ Raftilose P95 for 60 h. Profeed P95 was not used in this experiment, as most components of Profeed P95 are present in Raftilose P95. After 60 h a sample (1 ml) was taken and centrifuged at 10 000 rev min⁻¹ to precipitate the cells. Residual Raftilose P95 was analysed using High Performance Anion Exchange Chromatography (HPAEC). HPAEC was

performed using a BIO-LC system (Sunnyvale, CA, USA), equipped with a Dionex Carbopac PA-100 (4 × 250 mm) column and a Dionex pulsed electrochemical detector in the pulsed amperometric detection method (Voragen *et al.* 1986). The oligosaccharides were analysed using a gradient of 0–1.0 mol l⁻¹ sodium acetate in 100 mmol l⁻¹ sodium hydroxide.

Artificial plaque formation

Plaque formation was studied using *Strep. mutans* 36. The strain was pre-cultured anaerobically at 37°C for 24 h in PY-G broth. Plaque formation was determined using the procedure described by Hirasawa *et al.* (1984). PY supplemented with 10% Profeed, Raftilose or sucrose was used. In a second experiment 0.1 mol l⁻¹ potassium dihydrogen phosphate was added and the pH adjusted to 7.0 using 3 mol l⁻¹ sodium hydroxide.

RESULTS

Screening

All oral streptococci were able to grow on FOS agar. Good growth was observed within 24 h for all strains except *Strep. sanguis* 43, which showed good growth only after 48 h (Table 1). Acid production was evident within 24 h for *Strep. mutans* 36 and *Strep. mitis*. All strains showed acid production after 3 d, except for *Strep. oralis* which showed only weak acid production.

Similarly, FOS agar plates inoculated with samples of saliva from 40 healthy volunteers and four healthy piglets, all showed acid production and good growth within 48 h.

Table 1 Growth of oral streptococci on fructo-oligosaccharide agar, containing 1.5% Raftilose P95

Streptococcus strain	Growth and acid production after					
	Day 1		Day 2		Day 3	
	Growth*	Acid†	Growth	Acid	Growth	Acid
<i>gordonii</i> 42	++	+	++	++	++	++
<i>mitis</i> 46	++	+	++	++	++	++
<i>mutans</i> 36	++	+	++	++	++	++
<i>mutans</i> 44	++	+	++	+	++	++
<i>oralis</i> 45	+-	--	++	--	++	+
<i>sanguis</i> 43	--	--	++	+	++	+

*Growth: ++, colony size over 1 mm diam.; +, colonies small but clearly visible; +-, colonies barely visible; --, no visible colonies.

†Acid: ++, yellow zone around colonies; +, orange to yellow zone; --, no zone around colonies.

Fig. 2 Induction of growth of *Streptococcus salivarius* on Raftilose P95. *Streptococcus salivarius* was grown on PY-G. After 24 h the strain was subcultured in PY-Raf. This procedure was repeated every 24 h. Growth of the following cultures in PY-Raf was measured in a microtitre reader with absorbance at 620 nm: ■, pre-grown on PY-G only; ●, subcultured once on PY-Raf; ▲, subcultured four times on PY-Raf

Induction

The induction experiments with a slow-growing *Strep. salivarius* strain showed that the production of FOS-degrading enzymes is inducible. Figure 2 shows that the main effect is a reduced lag-phase, indicating the induction of the necessary enzymes. The growth rate was unaffected by induction.

Acid formation

All strains showed production of both lactic and acetic acids (Table 2). Lactic acid was generally present in higher concentrations than acetic acid. Most acid was produced by *Strep. gordonii*, *Strep. mutans* 36 and 44 and *Strep. mitis*. These strains also showed the fastest production of acid in the screening experiment. *Streptococcus mitis* also produced a relatively high concentration of acetic acid, indicating heterofermentative fermentation.

Streptococcus mutans 36 showed slower growth on Profeed, which resulted in lower concentrations of acid after 60 h. *Streptococcus mutans* 36 produced similar amounts of acid on Raftilose as on sucrose, indicating a similar and rapid fermentation.

Rapid acidification

Acid formation was very rapid as could be concluded from this experiment. A critical pH for dental caries of 5.5 was reached within 15 min for glucose as well as both FOS preparations (Fig. 3). With a lower concentration of Raftilose

Table 2 Acid formation on 1.0% Raftilose P95 and Profeed P95 by oral streptococci in HPLC broth

<i>Streptococcus</i> strain	Time of incubation (h)	Lactic acid (mmol l ⁻¹)	Acetic acid (mmol l ⁻¹)
<i>gordonii</i> 42	60	107.0	6.9
<i>mitis</i> 46	60	49.6	38.4
<i>mutans</i> 36	12	1.1	9.7
	24	58.4	13.6
	36	105.3	12.9
	48	115.4	12.4
	60	123.7	10.3
<i>mutans</i> 44	60	124	8.9
<i>oralis</i> 45	60	38.2	10.3
<i>sanguis</i> 43	60	41.2	11.8

Acid production was determined using HPLC. See Materials and Methods for details.

(2.3 mmol l⁻¹) the critical pH was reached within 30 min. No acid was produced in the absence of a fermentable carbohydrate.

Oligosaccharide degradation and fermentation pattern

The fermentation patterns of the different strains are shown in Fig. 4. It is clear that the strains degrade the FOS mixture differently. The only common factor is a complete fermentation of sucrose (GF). *Streptococcus mutans* 44, *Strep. gordonii* and *Strep. mitis* showed the most extensive degra-

Fig. 3 Rapid acidification by *Streptococcus mutans* 36. Cells of *Strep. mutans* 36 pre-grown in PY-G for 16 h were washed twice with RPS and added to 500 µl of RPS containing 23 mol l⁻¹ carbon sources: ■, no carbon source added; ●, glucose; ▲, Raftilose P95; ▾, Profeed P95; ○, 2.3 mol l⁻¹ Raftilose P95

Fig. 4 Change in composition of Raftilose P95 upon fermentation by oral streptococci. Composition patterns were determined by HPLC. For experimental conditions see Materials and Methods. A, Raftilose P95; B, Raftilose P95 after fermentation by *Streptococcus gordonii* 42; C, Raftilose P95 after fermentation by *Strep. mitis* 46

dation. This is in accordance with both the screening and the acid formation experiments.

Streptococcus gordonii degraded the higher oligosaccharides, both Fm and GF_n series (Table 3), thereby producing fructose, F2 and GF2. The intermediate structures F3 and GF3 seemed to be unaffected, or their degradation and formation rates are in equilibrium. Similarly, glucose levels seemed to be unaffected. This may also be due to a preference for fructose by *Strep. gordonii*.

Streptococcus sanguis showed only limited degradation of Raftilose. Only GF5 and F4 were degraded completely. In contrast to *Strep. gordonii*, no fructose accumulation was observed. On the other hand an accumulation of F3 was observed, probably due to hydrolysis of a fructose residue from F4.

Streptococcus mutans 44 showed no production of GF2,

Table 4 Changes in carbohydrate pattern after incubation of 2% Raftilose P95 by *Streptococcus mutans* 36

Component	Time (h)			
	0	24	36	42
G (glucose)	100	0	113	100
F (fructose)	100	0	1409	200
GF (sucrose)	100	0	6	0
GF2 (kestose)	100	19	29	0
F2	100	0	0	0
GF3	100	23	4	0
F3	100	0	0	0
GF4	100	24	1	0
F4	100	0	0	0
GF5	100	27	0	0

Values were determined using HPLC. For experimental details see Materials and Methods. Values are relative percentages as compared to $T = 0$.

Increases are due to degradation of larger oligosaccharides and incomplete fermentation.

which may be due to a better, more extensive degradation. Similarly no glucose could be detected. Large amounts of fructose and F2 were detected.

The degradation with time by *Strep. mutans* 36 differed from the pattern of strain 44, in that GF3 was degraded as rapidly as the other components. Similarly no increase in F3 was observed. From the data (Table 4) it can be seen that all components were degraded rapidly, the Fm series somewhat faster. Increases in fructose and glucose were only observed after 36 h. *Streptococcus oralis* showed very limited degradation. Only glucose, sucrose and GF2 were degraded. Surprisingly no changes in fructose were observed.

Streptococcus mitis showed again extensive degradation. In contrast to *Strep. gordonii* and *Strep. mutans* 44 no degradation of GF3 or higher was observed. All linear fructose oligomers

Table 3 Changes in carbohydrate pattern after incubation of 2% Raftilose P95 by oral streptococci

Strain*	G	GF	GF2	GF3	GF4	GF5	F	F2	F3	F4	Fm
42	/	---	+	/	-	--	+++	++	/	--	--
43	/	---	/	/	/	-	-	/	+	-	/
44	-	-	--	/	-	--	+++	++	/	--	-
45	-	---	-	/	/	/	/	/	/	/	/
46	---	---	/	-	/	/	+	---	--	-	/

*42, *Streptococcus gordonii*; 43, *Strep. sanguis*; 44, *Strep. mutans*; 45, *Strep. oralis*; 46, *Strep. mitis*.

/, No visible changes; -, some degradation (<20%); --, degradation (20–75%); ---, complete degradation; +, slight increase (<20%); ++, clear increase (20–100%); +++, considerable increase (>100%).

Increases are due to degradation of larger oligosaccharides and incomplete fermentation.

Table 5 Artificial plaque formation by *Streptococcus mutans* 36

Carbon source (10%)	Day 1 (mg)	Day 2 (mg)	Day 3 (mg)
Sucrose	11	14	16
Sucrose + buffer*	15	16	25
Raftilose P95	12	10	18
Raftilose P95 + buffer	18	22	34
Profeed P95	8	5	13

Values are average of duplicate measurements.

*Buffer was 0.1 mol l⁻¹ KH₂PO₄, pH adjusted to 7.0.

Plaque formation was determined by growing *Strep. mutans* 36 on the carbon source in pre-weighed plastic tubes. After growth the tubes were emptied, dried and weighed again.

(F to F4) were degraded completely, as were sucrose and GF2. No accumulation of fructose could be detected. Glucose concentrations remained unaffected, similarly as in *Strep. gordonii*.

Artificial plaque formation

Plaque formation was determined using *Strep. mutans* 36. The results (Table 5) show that Raftilose induces the formation of plaque as rapidly as does sucrose. Growth and formation of plaque on Profeed was considerably slower. However, after 3 d of incubation, the difference was negligible. Use of a phosphate buffer, which was added to imitate the conditions in the mouth more accurately, clearly influenced the formation of plaque. The amount of plaque formed with Raftilose as a substrate even surpassed the amount formed with sucrose.

DISCUSSION

Fermentation of fructo-oligosaccharides is very common among oral streptococci as can be concluded from these experiments. The presence of FOS-fermenting streptococci is widespread among the population, as saliva of 40 volunteers and four piglets contained strains which were able to degrade FOS (data not shown). None of the volunteers or the piglets had ever consumed commercial FOS, as at this moment no products with added FOS are on the Dutch market.

It has been shown previously (Walker *et al.* 1983) that oral streptococci possess fructanases. These fructanases are able to degrade levan- as well as inulin-type fructans. As the main fraction of FOS are oligomers of the inulin type, it is not surprising that all the strains tested were able to degrade FOS.

The FOS-degrading enzymes may be inducible, as was shown for a strain of *Strep. salivarius*. This has also been

demonstrated previously for *Strep. mutans* using D-fructans as a substrate (Burne *et al.* 1987).

Various streptococci degraded FOS in a different way. *Streptococcus mutans* and *Strep. gordonii* especially showed extensive degradation of all oligomers. *Streptococcus mitis*, however, showed mainly degradation of the Fm series, and hardly any degradation of the GFn series. This was surprising, as in a previous study no strain of *Strep. mitis* seemed to produce a fructanase, when pre-grown on glucose (Walker *et al.* 1983). However, the latter studied only extracellular fructanases using fructan as a substrate. It is possible that fructanases are not able to degrade oligosaccharides. It is also possible that the FOS-degrading enzymes are not necessarily extracellular.

The GF3 compound, nystose, seems to be relatively stable towards degradation by most strains. Only *Strep. mutans* 36 rapidly degraded nystose. *Streptococcus mutans* 44 degraded nearly all substrates except nystose. This would also explain the results obtained by other researchers. Ikeda *et al.* (1990) showed that nystose was slowly degraded or fermented by five strains of *Strep. mutans*, among them *Strep. mutans* NCTC 10449, which is the same as this group's strain 36. On the other hand Ziesenitz and Siebert (1987) showed that nystose was degraded rapidly by their strain of *Strep. mutans* NCTC 10449 and also with human mixed dental plaque bacteria. Walker *et al.* (1983) reported that fructanase activities ranged from 0 to 944 units l⁻¹. In the same study they showed that the production of fructanase was dependent on pre-cultivation as well as medium conditions. Finally, fructanases produced by different strains showed different preferences towards fructans (Walker *et al.* 1983). These factors may have contributed to the observed differences towards degradation of nystose.

All species produced acid from FOS, both from Raftilose and from Profeed. Acid production on Raftilose was similar to that on sucrose, about 0.11 mol l⁻¹. Profeed apparently is a less suitable substrate for *Strep. mutans* 36, as could be determined from the lower acid production and slower growth rate.

Acidification studies with *Strep. mutans* 36 showed that acidification is as rapid on glucose as on FOS. The critical pH for dental caries (5.5–5.7) was reached within 15 min with 23 mmol l⁻¹ FOS as substrate. This is somewhat longer than found with mixed dental plaque (Ziesenitz and Siebert 1987), which is probably due to the lower cell concentration in these experiments. Even with a reduced concentration of Raftilose (2.3 mmol l⁻¹) the critical pH was reached within half an hour.

Artificial plaque formation was similar on Raftilose and on sucrose: about 17 mg was formed after 3 d. Plaque formation on Profeed was somewhat less, 13 mg after 3 d. This data is in contrast with the results of Hirasawa *et al.* (1984), who could not detect any plaque after 3 d incubation with Neo-

sugar (Profeed). Even if only the sucrose unit of the GFn series were utilized in plaque formation, it is to be expected that Neosugar, which consists entirely of the GFn series, would be a good substrate for plaque formation. The slower production of plaque on Profeed may again be due to the slower growth rate of *Strep. mutans* 36 on Profeed.

Addition of buffer increased the plaque formation considerably to about 30 mg. Surprisingly plaque formation on Raftilose surpassed plaque formation on sucrose. As sucrose is the preferred substrate for *Strep. mutans* glycosyltransferases (Hamada and Slade 1980), it is unlikely that Raftilose would be a better substrate. However, it has been described that oligosaccharides may act as a primer for *Strep. mutans* glycosyltransferase (Hamada and Slade 1980) and this may be the explanation for the increased plaque formation. As growth and acid production on Profeed was slower than on Raftilose, it is likely that the Fm series are degraded more rapidly by the streptococci. This was confirmed with the degradation experiments. *Streptococcus mutans* 36 degraded the Fm series faster than the GFn series, and after 60 h considerably more degradation of the Fm series could be detected with the other strains.

As all strains grow and produce acid rapidly from Fm and GFn oligosaccharides, it can be predicted that both substrates are cariogenic. Furthermore, artificial plaque formation occurs and this will enhance cariogenicity. Definite proof of cariogenicity, however, has to come from animal studies.

Comparison of both fructo-oligosaccharide preparations would lead to the conclusion that Raftilose is somewhat more cariogenic than Profeed, due to the slower growth rate and acid formation by the most cariogenic species, *Strep. mutans*, on Profeed.

REFERENCES

- Burne, R.A., Schilling, K., Bowen, W.H. and Yasbin, R.E. (1987) Expression, purification, and characterization of an exo- β -D-fructosidase of *Streptococcus mutans*. *Journal of Bacteriology* **169**, 4507–4517.
- Hamada, S. and Slade, H.D. (1980) Biology, immunology and cariogenicity of *Streptococcus mutans*. *Microbiological Reviews* **44**, 331–384.
- Hidaka, H., Hirayama, M., Tokunaga, T. and Eida, T. (1990) The effects of undigestible fructooligosaccharides on intestinal microflora and various physiological functions on human health. In *New Developments in Dietary Fiber* ed. Furda, I. and Brine, C.J. pp. 105–117. New York: Plenum Press.
- Hirasawa, M., Kurita, M. and Ikeda, T. (1984) Cariogenicity of fructooligosaccharides. In *Proceedings of the 2nd Neosugar Research Conference*. pp. 21–27. Tokyo: Meiji-Seika Publications.
- Holdeman, L.V. and Moore, W.E.C. (1977) *Anaerobic Laboratory Manual*, 4th edn. Blacksburg: Virginia Polytechnic Institute and State University.
- Ikeda, T., Kurita, T., Hidaka, H., Michalek, S.M. and Hirasawa, M. (1990) Low-cariogenicity of the tetrasaccharide nystose. *General Pharmacology* **21**, 175–179.
- Mitsuoka, T., Hidaka, H. and Eida, T. (1987) Effect of fructooligosaccharides on intestinal microflora. *Die Nahrung* **31**, 427–436.
- Roberfroid, M., Gibson, G.R. and Delzenne, N. (1993) The biochemistry of oligofructose, a nondigestible fiber: an approach to calculate its caloric value. *Nutrition Reviews* **51**, 137–146.
- Spiegel, J.E., Rose, R., Karabell, P., Frankos, V.H. and Schmitt, D.F. (1994) Fructooligosaccharides as food ingredients. *Food Technology* **48**, 85–89.
- Takahashi, N.F., Mizuno, F. and Talamori, K. (1985) Purification and preliminary characterisation of exo- β -D-fructosidase in *Streptococcus salivarius* KTA-19. *Infection and Immunity* **47**, 271–276.
- Voragen, A.G.J., Schols, H.A., Searle-Van Leeuwen, M.F., Beldman, G. and Rombouts, F.M. (1986) Analysis of oligomeric and monomeric saccharides from enzymatically degraded polysaccharides by high-performance liquid chromatography. *Journal of Chromatography* **370**, 113–120.
- Walker, G.J., Hare, M.D. and Morrey-Jones, J.G. (1983) Activity of fructanase in batch cultures of oral streptococci. *Carbohydrate Research* **113**, 101–112.
- Wang, X. and Gibson, G.R. (1993) Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *Journal of Applied Bacteriology* **75**, 373–389.
- Ziesenitz, S.C. and Siebert, G. (1987) *In vitro* assessment of nystose as a sugar substitute. *Journal of Nutrition* **117**, 846–851.