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**Running title:** Digestibility of oligosaccharides
ABSTRACT

The utilization of oligosaccharides by oral microorganisms and intestinal enzymes are important factors in determining their effectiveness as alternative sweeteners. In this study, classes of naturally occurring sugars were assessed using in vitro models for oral and intestinal digestibility in order to test the influence of chemical structure on functional properties. Amongst the classes of sucrose isomers, α-glucobioses, β-glucobioses and sucrose-based oligosaccharides, structures were identified that were not utilized by the common oral bacterium Streptococcus mutans, and would therefore contribute to the acariogenic potential of a sweetener. Analysis of the rate and products of digestion by a mammalian glucosidase mixture was used to define the relative intestinal digestibility. The results showed that oligosaccharides containing a (1→6)-β-Glc group, including gentiobiose and gentiobiitol, together with melezitose, a sucrose-derived oligosaccharide containing a α-Glc-(1→3)-Fru moiety were resistant to digestion by both S. mutans and mammalian intestinal enzymes, highlighting their potential as dietary sugar substitutes.

Keywords: alternative sweetener, digestibility, glucobiose, glucosidase, sucrose isomers, sugar
1. INTRODUCTION

Carbohydrates are the most abundant group of natural products, providing a significant proportion of the total energy for the human diet as well as being important biosynthetic precursors. In the food industry, sugars add not only sweetness, but also colour, texture and preservation qualities. Due to increasing health concerns about the effects of dietary sugars, however, there is growing interest in alternative sugars as sweeteners. Desirable properties for an alternative sweetener include low calorific value due to low absorption or digestibility, and acariogenicity as a result of poor metabolism by oral bacteria, while ideally retaining solubility, texture (“mouth-feel”), crystallinity and bulking properties (Crittenden & Playne, 1996).

Currently many alternative sugars exist in the market, including sugars with low calorific value, such as sugar alcohols and sucrose isomers, and non-calorific sugars, such as fructans and sucralose. Many other naturally-occurring sugars that are not currently in commercial production have been described. It is likely that some of these sugars will also display properties that would make them useful alternative sweeteners.

In assessing the value of a potential sweetener, the two major sites of carbohydrate digestion need to be considered. The oral cavity, where breakdown of starch is initiated by salivary enzymes, is most important for dental health, as residual food in the oral cavity can provide an energy source for cariogenic bacteria. Fermentable sugars, such as sucrose in particular, are significant contributors to dental decay (Moynihan & Petersen, 2004). Among the many bacteria present in the oral cavity, *Streptococcus mutans* is the most commonly found species. Production of organic acids by *S. mutans* following fermentation of sugars results in a lowering of pH which promotes tooth decay and also provides a suitable environment to encourage growth of other aciduric bacteria (Hamada, 2002). *S. mutans* is a particularly effective oral colonizer due to its ability to transport a variety of sugar structures (Tao, Sutcliffe, Russell & Ferretti, 1993).
Non-cariogenic sugars are being exploited to reduce the incidence of dental caries, with sugar alcohols such as maltitol and xylitol used commercially in confectionery.

In the second, and major, site of carbohydrate breakdown, the gut, a pancreatic amylase continues the breakdown of starches. The four major digestive enzymes are present in the intestinal brush border as two complexes, sucrase-isomaltase (SI) and maltase-glucoamylase (MGAM), collectively known as the α-glucosidases. These complexes have overlapping activity on starch-related structures containing (1→4)-α-glucose (Nichols, Avery, Sen, Swallow, Hahn & Sterchi, 2003) and together they complete the release of glucose monosaccharides from the starch-related oligosaccharides and also contribute directly to the breakdown of intact starch structures (Ao et al., 2007). The SI complex is also responsible for digestion of the common dietary sugar, sucrose, as well as the (1→6)-α-glucose branches of starch and the (1→6)-α-glucobiose, isomaltose (Gray & Ingelfin, 1965). Because the rate of intestinal digestion of carbohydrates affects glucose homeostasis, the α-glucosidases are targets for inhibition by drugs such as acarbose (Jenkins et al., 1981), or by functional food ingredients including flavonoids (Tadera, Minami, Takamatsu & Matsuoka, 2006). Modification of food components to slow the rate of glucose release is an alternative option, particularly in the case of resistant starches (Zhang & Hamaker, 2009). Such slowly-digested or non-digested carbohydrates may have a positive influence on the gut microflora, as prebiotic agents (Gibson & Roberfroid, 1995).

Although the major dietary sugars have been well studied, there are still many questions about the oral and intestinal utilization of potential novel sweeteners. In particular, defining the relationship between chemical structure and digestibility by comparing structurally related groups of oligosaccharides has not previously been performed in detail. The results will be
important for predicting the value of naturally occurring sugars as non-calorific sweeteners and may also be used to inform combinatorial chemistry strategies to produce “designer” sweeteners.

2. MATERIALS AND METHODS

2.1 Materials

The carbohydrates leucrose, kojibiose, nigerose, isomaltose, sophorose, gentiobiose, 1-kestose, and panose were sourced from Carbosynth (Berkshire, UK) while erlose, maltotriose and maltotriitol were supplied by Hayashibara Biochemical Laboratories (Okayama, Japan). Gentiobiitol was prepared by Epichem Ltd. (Murdoch, Australia), as described previously (Hodoniczky, Robinson, McGraw & Rae, 2010). All remaining sugars, oligosaccharides and other chemicals were purchased from Sigma-Aldrich (Castle Hill, Australia) unless noted. Stock solutions (200 mM) of carbohydrates were prepared in distilled water (H2O).

2.2 Streptococcus mutans fermentation

An oral isolate of *S. mutans* was obtained from the Australian Collection of Microorganisms (Brisbane, Australia). Culturing of the isolate was routinely performed in 50 mL tubes with brain heart infusion medium (Oxoid, Thebarton, Australia) and static incubation at 37°C for 30 h. In preparation for use in the fermentation assay, based on the method of Imai et al. (1984), *S. mutans* cells (50 mL) were first centrifuged for 5 min at 3 000g. The supernatant was removed and the pellet washed in 5 mL of 0.9% NaCl. After re-centrifugation, the cells were suspended in Stephan’s buffer (3 mL), which mimics the pH, inorganic content and buffering capacity of saliva (Stephan & Hemmens, 1947). Suspensions from several tubes were combined for use in the assay. Aliquots of cells (760 µL) were transferred to 1.5 mL tubes and then 40 µL of a test oligosaccharide solution was added to reach 10 mM final concentration. Samples were incubated
for 90 min at 37°C then centrifuged (13 000g, 5 min). Supernatants were transferred to fresh
tubes and the pH was measured (pH pen; Sper Scientific, Scottsdale, AZ). Additionally 100 µL
of supernatant was combined in a 96 well plate with 10 µL of universal indicator to visually
confirm measured pH changes. For analysis by high performance anion exchange
chromatography (HPAEC), 20 µL of supernatant was diluted with 1 mL H₂O.

2.3 Yeast invertase digestion

Yeast invertase (Megazyme, Co. Wicklow, Ireland; 5 µL of a stock solution prepared by 1 in 10
dilution of supplied enzyme in H₂O) was combined with 25 µL sodium phosphate buffer (100
mM, pH 7.0) and 15 µL H₂O. To this, 5 µL of a test oligosaccharide solution (100 mM) was
added and briefly mixed. Controls with no sugar or without invertase were also prepared, the
total volume being adjusted to 50 µL using H₂O. Reactions were incubated at 37°C for 30 min
then halted by heating at 90°C for 3 min. Samples were then diluted by the addition of 1.2 mL
H₂O and stored at -20°C until HPAEC analysis could be performed.

2.4 Mammalian α-glucosidase digestion

Aliquots of rat intestinal glucosidase were prepared by dissolving 50 mg rat intestinal acetone
powder (Sigma) in 1.5 mL of 0.9% NaCl. The mixture was briefly vortexed and then
homogenised by sonicating in ice cold water for 10 min. After centrifugation (10 000g, 30 min)
the supernatant was pooled for use in the assay. Serial dilution of test oligosaccharides in sodium
phosphate buffer (100 mM, pH 7.0) were prepared from 200 mM stocks and triplicate aliquots of
10 µL were transferred to 96 well U-bottom plates. For reactions including the α-glucosidase
inhibitor acarbose, 10 µL of a 2 mg mL⁻¹ solution in phosphate buffer was added (20 mg mL⁻¹
stock in 50% ethanol was stored at 4 °C). Volumes were adjusted to 30 µL with phosphate
buffer, followed by the addition of 20 μL of enzyme preparation. Controls with no enzyme and/or oligosaccharide were included and total volumes also adjusted to 50 μL accordingly. Plates were mixed briefly and incubated at 37°C for up to 90 min. Enzyme digestions were stopped by addition of 75 μL well⁻¹ of 2 M Tris buffer (pH 7.0) and each plate mixed again. Glucose release was measured using a hexokinase reagent according to manufacturer’s instructions (Sigma-Aldrich). The glucose standard (Sigma-Aldrich) was used to generate a standard curve by preparing serial dilutions of the 1 mg mL⁻¹ stock solution. For the hexokinase assay, 30 μL of samples along with standards were transferred in triplicate into a clear flat 96 well plate, and 170 μL of hexokinase reagent was added to each well. Following incubation for 15 min at RT, absorbance was measured at 340 nm (Labsystem iEMS Reader MF, Labsystems, Franklin MA). Enzyme assays for HPAEC analysis were performed with the same reagents in 1.5 mL tubes, and the reactions were stopped by heating (90°C for 3 min), rather than with Tris buffer which interfered in chromatograms. Samples were diluted with 1 mL H₂O before HPAEC analysis.

2.5 Carbohydrate analysis by High Performance Anion Exchange Chromatography (HPAEC)

Samples were filtered using Phenex-RC (Phenomenex, Lane Cove, Australia) syringe filters and transferred to sample vials. A 10 μl aliquot of each sample was injected into a HPLC system (Model 600s controller, 626 pump and 717 autosampler; Waters, Milford, MA, USA). Oligosaccharides were separated on an analytical column (CarboPac PA-100; Dionex, Sunnyvale, CA, USA) protected by a guard column (CarboPac PA-100 guard column; Dionex) using a mobile phase of sodium hydroxide. Eluted sugars were detected by pulsed amperometric detection (Waters 464).
3. RESULTS AND DISCUSSION

3.1 Functional properties of sweeteners

For a compound to be effective as an alternative sweetener, it needs to meet a number of physical, biochemical and sensory parameters. The principal criterion is taste, which includes a complex profile of sweetness, cleanness, and various aftertastes, normally assessed by a trained human panel. Functional properties are also important, especially low calorific yield, due to low digestibility in the human gut. For some applications, low cariogenic potential is also an important attribute. Although these properties have been assessed for individual sweetener products, there are few studies which systematically address the influence of chemical structure on the functional properties of potential sweeteners. In this study, classes of naturally occurring sugars and oligosaccharides were assessed using in vitro models for human oral and intestinal utilization. Oligosaccharides used in the study were chosen to represent a cross section of compounds of dietary significance, focusing on groups with related structures including sucrose isomers, α and β-linked glucobioses, starch-related oligosaccharides, sucrose-containing oligosaccharides and sugar alcohols (Table 1).

3.2 Potential for cariogenic activity

Utilization by the oral bacterium Streptococcus mutans was used as an indicator of the relative cariogenic potential of oligosaccharides. The results confirmed the ability of S. mutans to utilize a wide variety of structures, measured as a change in pH of the medium compared to a control reaction containing no added sugar (Fig. 1). As expected, sucrose caused significant pH reduction (mean ± SD, 29.0 ± 1.4%), while the sucrose isomers, turanose, leucrose and palatinose were not utilized by S. mutans, consistent with their commercial application as non-cariogenic sugar substitutes (Peltroche-Llacshahuanga, Hauk, Kock, Lampert, Lutticken & Haase, 2001; Ziesenitz, Siebert, Schwengers & Lemmes, 1989).
*S. mutans* is known to secrete extracellular enzymes, including invertase (β-fructofuranosidase) (Tanzer, Brown & McInerney, 1973), which would catalyze cleavage of sucrose. When the medium following growth on sucrose was analysed by HPLC and compared to the products obtained by digestion of sucrose with yeast invertase, peaks corresponding to glucose and fructose were identified in both (Fig. 2a). The amount of glucose was reduced in the *S. mutans* supernatant compared to the invertase products, confirming preferential uptake of glucose by the bacterium (Schachtele, Knudson & Loken, 1972). In the *S. mutans* supernatant, there was an additional peak at a retention time of 5 min which could not be identified by comparison to the standards used. It is likely that this peak corresponds to precursors of the extracellular polysaccharides produced by *S. mutans* which contribute to dental plaque formation.

Five related α-glucobioses, varying only in the position of the glycosidic linkage, were also tested for utilization by *S. mutans* (Fig. 1). Amongst this family of oligosaccharides, trehalose ((1→1)-α-glucobiose) caused the largest change to pH (24.6 ± 2.6%). In contrast to the results of this *in vitro* assay, trehalose has been reported to have low cariogenicity following measurement of plaque pH in human patients (Neta, Takada & Hirasawa, 2000) although a much longer incubation time was used in the *in vivo* trial. The two starch-related α-glucobioses ((1→4)-α- and (1→6)-α-) were also utilized by *S. mutans*, consistent with the reported cariogenic potential of starch (Pollard, 1995). The HPLC analysis of the *S. mutans* medium following growth on isomaltose ((1→6)-α-glucobiose) showed that the concentration of isomaltose was reduced without detectable production of glucose (Fig. 2b), suggesting that the isomaltose was not cleaved extracellularly but was taken up directly by the bacteria. A range of uptake systems have been described in *S. mutans*, including ATP-binding cassette transporters and phosphotransferase systems (PTS)(Tao et al., 1993; Ajdic & Pham, 2007). Of the remaining
α-glucobioses, the (1→2)-α and (1→3)-α structures, kojibiose and nigerose, resulted in a negligible pH change, indicating that they were not used by *S. mutans*. The potential of kojibiose and nigerose as non-cariogenic sugars has not previously been reported and this result suggests that inclusion of these linkages in future functional oligosaccharides may be beneficial.

Glucose disaccharides with β-linkages were utilized quite differently from the α-linked family (Fig. 1). The (1→2)-β and (1→3)-β structures, sophorose and laminaribiose, induced significant changes to pH, as did the β-1,4 structure, cellobiose. In a similar way to isomaltose, the HPLC analysis of the supernatants following growth on laminaribiose or sophorose showed no production of glucose, suggesting that the disaccharides are taken up directly (Fig. 2c, d). It has been reported that a PTS is responsible for cellobiose uptake and this may also be responsible for uptake of the other β-glucobioses in this study (Cote, Cvitkovitch, Bleiweis & Honeyman, 2000). The nutrient limiting conditions of the assay system would be likely to promote β-glucoside utilization (Zeng & Burne, 2009). Only the (1→6)-β structure, gentiobiose, was resistant to utilization by *S. mutans*, inducing negligible change in pH of the medium compared to the control.

The starch-related trisaccharides, panose and maltotriose were both utilized by *S. mutans*, generating a change in pH which was greater than the control but less than the change induced by growth of the bacterium on sucrose (Fig. 1). Interestingly, HPLC analysis of the medium following growth on panose revealed the presence of small amounts of maltose (Fig. 2e), suggesting the activity of a secreted α-glucosidase on these starch-related structures.

Dextranases with activity against (1→2)-α-glucose groups have been described in *S. mutans*, but the secreted form of the enzyme has previously been reported to act only on long chain dextrans (Colby & Russell, 1997).
Of the four sucrose-containing trisaccharides tested, 1-kestose was notable for the large change to the pH of the medium (31.6 ± 1.7 %), similar to the change induced by growth on sucrose (Fig. 1). Together with other short fructo-oligosaccharides, 1-kestose is a component of native inulin which is used as a dietary fibre due to its low intestinal digestibility (Roberfroid & Delzenne, 1998). Analysis of the supernatant following growth on 1-kestose suggests that the trisaccharide is cleaved by the secreted invertase, as fructose and sucrose were detected as intermediate products after 30 min (Fig. 2f). Erlose and raffinose were also utilized by S. mutans, however melezitose, in which sucrose is modified by the addition of a (1→3)-α-glucose unit to the fructose moiety, was resistant.

The sugar alcohols maltitol and maltotriitol resulted in minimal change to pH (Fig. 1), a well-characterised property of this oligosaccharide group which is exploited for application as commercial sweetener products (Hamada, 2002). In this study, the β-linked sugar alcohol gentiobiitol was assayed for potential cariogenic activity for the first time. Gentiobiitol was identified in transgenic sugarcane plants engineered to produce sorbitol (Fong Chong, Abeydeera, Glassop, Bonnett, O’She & Brumbley, 2010) and a recent study in an animal model system indicated that it may be sweet-tasting (Hodoniczky et al., 2010). Consistent with the inability of S. mutans to metabolise other sugar alcohols, gentiobiitol resulted in only negligible change to the pH of the medium.

Although many factors contribute to development of dental caries, the assay for utilization by S. mutans was useful in identifying structures least likely to be cariogenic. In addition to the known non-cariogenic groups of sucrose isomers and sugar alcohols, the results showed that (1→2)-α–, (1→3)-α– and (1→6)-β–glucobiose structures were less likely to support the growth of cariogenic bacteria.
3.3 Utilization by intestinal enzymes

The human gut contains a complex of enzymes with α-glucosidase activities against a variety of glycosidic linkages. Since single enzyme activities would not be effective analogues for the digestion process, a mammalian intestinal extract was selected for the in vitro assay of the relative digestibility of oligosaccharides. Digestibility was measured by the release of glucose over time, using a range of substrate concentrations, and for some substrates, the products were analysed by HPLC to add further information on the intermediates of digestion. Substrates were also assayed in the presence of the α-glucosidase inhibitor acarbose.

Structures that resulted in detection of glucose after incubation with the enzyme mixture are shown in Fig. 3. Sucrose was digested at a slower rate than disaccharides such as maltose, shown by the reduced yield of glucose, even when the 0.5 molar ratio of glucose in the starting substrate is taken into account. The sucrose isomers, turanose and palatinose were digested extremely slowly by the intestinal enzyme mixture, however leucrose was digested at a similar rate to sucrose. This result highlights the importance of using an enzyme that mimics the in vivo digestion, since an earlier report characterized leucrose as more slowly digested than sucrose in an assay with a yeast α-glucosidase (Ziesenitz et al., 1989) Analysis of the reaction rate for these substrates over a range of substrate concentrations up to 40 mM (Fig. 4a) confirmed the preference of the enzyme for the (1→2)-α and (1→5)-α glucose-fructose linkages in sucrose and leucrose, compared to the (1→3)-α and (1→6)-α linkages. For turanose and palatinose, the rate showed little change in response to increasing substrate concentrations.

As expected, the rat intestinal enzymes did not digest any of the β-glucobioses while the α-glucobioses were digested at various rates. The (1→4)-α linked glucobiose, maltose, was digested most rapidly, followed by the (1→3)-α (nigerose), (1→2)-α (kojibiose) and (1→6)-α
(isomaltose) glucobioses (Fig. 3). The (1→1)-α–glucobiose, trehalose was not digested by the mammalian glucosidases. This is consistent with the substrate preferences of other mammalian α-glucosidases, including those from porcine liver and rabbit muscle (Matsui & Chiba, 1983; Matsui, Sasaki, Takemasa, Kaneta & Chiba, 1984). Analysis of the supernatant after enzymic digestion of maltose confirmed that the disaccharide was quickly broken down to glucose (Fig 5a). Although no residual maltose was detected after 30 min incubation, the yield of glucose was seen to increase after 2 h incubation. It is possible that residual maltose is tightly bound to the enzyme complex, with gradual release of glucose monosaccharides. The relationship between the rate of glucose release and substrate concentration (Fig. 4b) confirmed the strong preference for maltose and showed linear increases in activity against nigerose and kojibiose at the lower substrate concentrations.

Some differences in the kinetic properties of the isomaltase enzyme were detected. Isomaltose was not only digested more slowly than the other glucobioses, but the rate showed little increase when the substrate concentration was doubled (Fig. 3). High substrate concentrations of isomaltose (40 mM) appeared to inhibit the enzyme, resulting in lowered reaction rate (Fig. 4b). Furthermore, glucose release from isomaltose continued at a slow rate even in the presence of acarbose, an observation shared with the (1→6)-α-glucose containing trisaccharide, panose, suggesting that the isomaltase activity is not inhibited completely by acarbose. This hypothesis was supported by the HPLC analysis of the products of panose digestion in the presence of acarbose, which showed that the monosaccharide glucose was derived from the α-1,6 linked group, as the maltose intermediate remained intact (Fig 5b).

Amongst the sucrose-containing oligosaccharides, only erlose was digested by the mammalian α-glucosidases. Raffinose, 1-kestose and melezitose did not yield any glucose in the assay,
demonstrating that the addition of specific side groups rendered the sucrose moiety resistant to digestion.

The trisaccharides, erlose, panose and maltotriose were all digested by the rat intestinal enzymes but yielded glucose at a slower rate than expected when compared to maltose on a per mole glucose basis (Fig. 3). Analysis of the products of digestion of maltotriose confirmed the presence of a maltose intermediate after 30 min which was no longer detectable after 2 h (Fig 5c). The analysis of the products of erlose digestion suggested that the (1→4)-α linked glucose unit was released quickly, while the remaining sucrose was digested more slowly, yielding detectable fructose only after 2 h (Fig 5d). Erlose and maltotriose showed similar kinetics in response to increasing substrate concentrations (Fig. 4c). The increase in rate of glucose release was reversed at substrate concentrations of 10 mM or higher. These results suggest that feedback inhibition of the enzyme occurred at higher substrate concentrations. An inhibitory effect of maltotriose has been reported for starch digestion by MGAM (Quezada-Calvillo et al., 2008) although the other oligosaccharides were not previously tested. In contrast to maltotriose, breakdown of panose continued at higher substrate concentrations. This is also consistent with the observations of starch digestion, since the SI complex, responsible for activity against the (1→6)-α glucose unit in panose, was not inhibited (Quezada-Calvillo et al., 2007)

The breakdown of maltotriitol demonstrated that addition of a sugar alcohol group to a starch-related oligosaccharide did not prevent utilization by intestinal enzymes. In contrast, maltitol, without the additional glucose unit, was resistant to digestion (Fig. 3). This was confirmed by HPLC analysis of the products of maltotriitol digestion which showed that maltitol remained intact after 2 h (Fig. 5e). Higher concentrations of maltotriitol appeared to suppress enzyme activity in a similar way to maltotriose (Fig. 4c).
The β-linked sugar alcohol, gentiobiitol, which has not previously been tested for its utilization by gut enzymes, was shown to be resistant in this assay.

### 3.4 Implications for activity of potential sweeteners

The breakdown of a range of sugars by *S. mutans* or by mammalian α-glucosidases has been tested (Fig. 6). The assay systems used in this study as analogues of oral and intestinal utilization confirmed that groups of oligosaccharides used as commercial sweeteners, such as sucrose isomers and sugar alcohols were generally resistant or slowly utilized. By application of the assays to structurally related groups of sugars that have not previously been analyzed, some insights into the value of particular linkage patterns have been revealed. For example, oligosaccharides containing (1→2)-α, (1→3)-α and (1→6)-β linkages were not utilized by *S. mutans*, suggesting that they would be useful in non-cariogenic sweeteners. Although the (1→2)-α- and (1→3)-α-glucobioses were digested by the intestinal glucosidases, the rate of breakdown was slower than that of maltose. The (1→6)-β linked structure was resistant to glucosidase activity when it occurred in both gentiobiose and gentiobiitol (Fig. 6). Structures containing these linkages would therefore fulfil some of the requirements for an alternative sweetener. Although there are few comprehensive reports on the relative sweetness of alternative sugars, kojibiose, nigerose and gentiobiitol were all rated as likely to be sweet-tasting by an assay based on preferential feeding by fruit flies (Hodoniczky et al., 2010). Oligosaccharides that are undigested or poorly digested may have a further effect on health by stimulating growth of beneficial gut microorganisms. Gentio-oligosaccharides containing gentiobiose have been shown to have prebiotic activity (Sanz, Cote, Gibson & Rastall, 2006) and further analysis may reveal similar results with the (1→2)-α- and (1→3)-α-glucobioses.
The major dietary sugars, including sucrose and starch-derived sugars such as maltose, were utilized by both *S. mutans* and the α-glucosidases. Modified sucrose compounds have been considered as potential sweeteners, since many retain a high degree of sweet taste (Godshall, 2007). Most of the sucrose isomers and sucrose-based oligosaccharides tested here were utilized either by *S. mutans* or the gut enzymes to some extent, although palatinose and turanose were digested extremely slowly. An exception was melezitose, a glucosyl-sucrose containing a modified fructosyl unit, which was resistant to both oral and intestinal digestion (Fig. 6). Melezitose is one of a number of sucrose isomers and sucrose-based oligosaccharides found naturally in aphid honeydew, and is thought to be important in reducing the osmotic potential of the high sucrose food source (nectar) in the aphid gut (Douglas, 2006).

In conjunction with other tests of physical and sensory properties, knowledge of the structures that are non-cariogenic and/or non-calorific will be useful in the selection and design of alternative sweeteners. Advances in controlled carbohydrate synthesis through improved enzyme engineering techniques or use of microbial synthesis may enable the development of novel functional food ingredients based on these principles.

4. ROLE OF THE FUNDING SOURCE

Funding for this study was provided by the Cooperative Research Centre for Sugar Industry Innovation through Biotechnology. The sponsors had no role in study design, collection of data, analysis and interpretation of data, writing of the report and submission of the paper.
5. REFERENCES


6. FIGURE CAPTIONS

Figure 1. Utilization of oligosaccharides by Streptococcus mutans. The production of acid as a result of fermentation of individual oligosaccharides by S. mutans was measured and displayed as the % change in pH of the growth medium. Bars represent ± standard error from at least 3 independent experiments.

Figure 2. Analysis of growth media following incubation of Streptococcus mutans with oligosaccharides. Supernatants from S. mutans assays were analyzed for their content of oligosaccharides and monosaccharides following incubation for 90 min with 10 mM concentration of the substrates: (a) sucrose, (b) isomaltose, (c) laminaribiose, (d) sophorose, (e) panose and (f) 1-kestose. Peaks were identified by comparison with retention times (min) of standards; an unidentified compound is marked with an asterisk. In control experiments, the medium was not inoculated with S. mutans. For sucrose and 1-kestose, the products following incubation with yeast invertase were also analysed for comparison.

Figure 3. Time course of digestion of oligosaccharides by mammalian α-glucosidases. Concentration of glucose (mmol) was measured at three time points (30, 60, and 90 min) during incubation of individual oligosaccharides (5 mM and 10 mM) with solubilized intestinal powder. In control experiments, the α-glucosidase inhibitor, acarbose was added to 10 mM substrate. Results are displayed only for oligosaccharides which released measureable amounts of glucose. Triplicate reactions were analysed. Error bars (± SEM) are smaller than the shapes used for data points.
Figure 4. Kinetic analysis of digestion of oligosaccharides by mammalian α-glucosidases. The concentration-dependent release of glucose was measured following incubation of solubilized intestinal powder with a range of substrate concentrations (1 - 40 mM) for the substrates: (a) sucrose and its isomers turanose, leucrose and palatinose; (b) the α-glucobioses, kojibiose, nigerose, maltose and isomaltose; (c) erlose, panose, maltotriose and maltotriitol. Data points result from triplicate assays. Error bars (± SEM) are smaller than the shapes used for the data points.

Figure 5. Analysis of growth media following incubation with mammalian α-glucosidases. Supernatants from assays with solubilized intestinal powder were analyzed for their content of oligosaccharides and monosaccharides following incubation for 30 min or 2 h with 10 mM concentration of the substrates: (a) maltose, (b) panose, (c) maltotriose, (d) erlose and (e) maltotriitol. Supernatants were also analysed following reactions in the presence of the α-glucosidase inhibitor, acarbose. Peaks were identified by comparison with retention times (min) of standards.

Figure 6. Summary of oligosaccharides utilized by Streptococcus mutans and intestinal α-glucosidases. The diagram indicates structures that were utilized only by S. mutans on the left hand side, structures utilized only by α-glucosidases on the right hand side and structures utilized by both in the central overlap region. Gentiobiose, melezitose, maltitol and gentiobiotol were not utilized by either S. mutans or α-glucosidases.
**Table 1.** Oligosaccharides used in the current study, organized by structural relationships.

<table>
<thead>
<tr>
<th>Structural group</th>
<th>Name</th>
<th>Structure</th>
</tr>
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<tbody>
<tr>
<td><strong>Sucrose isomers</strong></td>
<td>sucrose</td>
<td>$\beta$-Fru-$\left(2\rightarrow1\right)$-$\alpha$-Glc</td>
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<tr>
<td></td>
<td>turanose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow3\right)$-Fru</td>
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<td></td>
<td>leucrose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow5\right)$-Fru</td>
</tr>
<tr>
<td></td>
<td>palatinose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow6\right)$-Fru</td>
</tr>
<tr>
<td><strong>$\alpha$-Glucobioses</strong></td>
<td>trehalose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow1\right)$-$\alpha$-Glc</td>
</tr>
<tr>
<td></td>
<td>kojibiose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow2\right)$-Glc</td>
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<tr>
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<td>nigerose</td>
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<tr>
<td><strong>$\beta$-Glucobioses</strong></td>
<td>sophorose</td>
<td>$\beta$-Glc-$\left(1\rightarrow2\right)$-Glc</td>
</tr>
<tr>
<td></td>
<td>laminaribiose</td>
<td>$\beta$-Glc-$\left(1\rightarrow3\right)$-Glc</td>
</tr>
<tr>
<td></td>
<td>cellobiose</td>
<td>$\beta$-Glc-$\left(1\rightarrow4\right)$-Glc</td>
</tr>
<tr>
<td></td>
<td>gentiobiose</td>
<td>$\beta$-Glc-$\left(1\rightarrow6\right)$-Glc</td>
</tr>
<tr>
<td><strong>Sucrose-containing</strong></td>
<td>raffinose</td>
<td>$\alpha$-Gal-$\left(1\rightarrow6\right)$-$\alpha$-Glc-$\left(1\rightarrow2\right)$-$\beta$-Fru</td>
</tr>
<tr>
<td>oligosaccharides</td>
<td>1-kestose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow2\right)$-$\beta$-Fru-$\left(1\rightarrow2\right)$-$\beta$-Fru</td>
</tr>
<tr>
<td></td>
<td>melezitose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow3\right)$-$\beta$-Fru-$\left(2\rightarrow1\right)$-$\alpha$-Glc</td>
</tr>
<tr>
<td></td>
<td>erlose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc-$\left(1\rightarrow2\right)$-$\beta$-Fru</td>
</tr>
<tr>
<td><strong>Starch-related</strong></td>
<td>panose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow6\right)$-$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc</td>
</tr>
<tr>
<td>oligosaccharides</td>
<td>maltotriose</td>
<td>$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc</td>
</tr>
<tr>
<td><strong>Sugar alcohols</strong></td>
<td>maltotriitol</td>
<td>$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc-$\left(1\rightarrow4\right)$-$\alpha$-Glc-$\left(1\rightarrow6\right)$-Glc-ol</td>
</tr>
<tr>
<td></td>
<td>maltitol</td>
<td>$\alpha$-Glc-$\left(1\rightarrow4\right)$-Glc-ol</td>
</tr>
<tr>
<td></td>
<td>gentiobiotol</td>
<td>$\beta$-Glc-$\left(1\rightarrow6\right)$-Glc-ol</td>
</tr>
</tbody>
</table>
FIGURE 1

[Graph showing the % change pH for various saccharides: sucrose, turanose, leucrose, palatinose, trehalose, kojibiose, nigerose, maltose, isomaltose, sophorose, laminaribiose, cellobiose, gentiobiose, raffinose, 1-kestose, melezitose, erlose, panose, maltotriose, maltotriitol, maltitol, gentiobioitol, trehalose, palatinose, leucrose, and succrose.]
FIGURE 2

(a) Control S. mutans
(b) Control S. mutans
(c) Control S. mutans
(d) Control S. mutans
(e) Control S. mutans
(f) Control S. mutans

Time (min)
FIGURE 3

[Graph showing glucose release over time for different carbohydrates and treatments: Sucrose, Turanose, Leucrose, Palatinose, Kojibiose, Nigerose, Maltose, Isomaltose, Erlose, Panose, Maltotriose, Maltotriitol, 10 mM + acarbose, 5 mM, 10 mM]
FIGURE 4

(a) Glucose released (umol/min) vs. sucrose, leucrose, turanose, and palatinose concentrations.

(b) Glucose released (umol/min) vs. maltose, nigerose, kojibiose, and isomaltose concentrations.

(c) Glucose released (umol/min) vs. erlose, maltotriose, panose, and maltotriitol concentrations.
FIGURE 5

(a) maltose

(b) panose

(c) maltotriose

(d) sucrose

(e) maltotriitol
Streptococcus mutans

- trehalose
- sophorose
- laminaribiose
- cellobiose
- raffinose
- 1-kestose

Mammalian α-glucosidases

- sucrose
- maltose
- isomaltose
- erlose
- panose
- maltotriose

- turanose
- leucrose
- palatinose
- kojibiose
- nigerose
- maltotriitol

gentiobiose, melezitose, maltitol, gentiobiitol