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Synthesis, characterisation and microbial utilisation of amorphous polysugars from lactose

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Citric acid (PubChem CID: 311)

ABSTRACT

The melt polymerisations of glucose, galactose, xylose and fucose with citric acid, and mixtures of sugars therein are reported. Characterisation of the citric-acid catalysed reaction products indicated similar degrees of branched polymerisation but differences in the overall molecular weight of the polymers produced. The dairy by-product lactose could not be polymerised in a similar fashion but was shown to be readily hydrolysed using microwave radiation and a polymer generated from the melt condensation of the resultant glucose and galactose monosaccharides. A preliminary assessment of the bifido-bacterial utilisation of the lactose-derived polymerised products demonstrated a significantly different growth profile compared to commercially utilised galactooligosaccharides (GOS).

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1. Introduction

The demand for food and industrial ingredients that may be manufactured in a renewable manner commands serious attention for manufacturers with a growing, discerning consumer population. In the 2011/12 season, dairy companies in New Zealand processed more than 19 billion litres of milk (www.dairynz.co.nz/dairystatistics) which, at ~50 g/L, represents almost 1 million tonnes of lactose. New Zealand is the largest producer of milk powder *per capita*, manufacturing 1.25 million tonnes of milk powder *per annum* and a related product of over 20,000 tonnes of lactose, recovered from whey during cheese manufacture. Lactose is recovered from the whey produced as a by-product of the cheese and casein industries and purified for food and pharmaceutical uses.

Galactooligosaccharides (GOS) are synthesised by the reverse action of β-galactosidase on lactose at high concentration in solution (≥40%, w/v) to yield predominantly linear tri-, tetra- and penta-saccharides with mostly β1 → 4 or β1 → 6 linked galactopyranosyl residues, although low proportions of β1 → 2 or β1 → 3 linkages may also be present (Coulier et al., 2009; Gosling, Stevens, Barber, Kentish, & Gras, 2010). Monomeric glucose can make up a significant (~20%, w/w) proportion of GOS syrups, or it may be removed chromatographically to give GOS powders with >90% oligosaccharides (Playne & Crittenden, 2009). Polydextrose (PD), a polymer of glucose, is a well understood food bulking ingredient with some dietary fibre properties (Lahtinen et al., 2010). Both in vitro and in vivo studies have shown that PD is partially fermented and stimulates the growth of bifidobacteria, which are generally considered as beneficial to human health (Jie et al., 2000; Probert, Apajalahti, Rautonen, Stowell, & Gibson, 2004). PD is a highly branched oligomeric material prepared by heating glucose under reduced pressure at 150–160 °C for about 20 min in the presence of sorbitol and catalytic amounts of citric acid (Murray, 1988).

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The average degree of polymerisation (DP) of PD is reported to be about 12 (average molecular weight 2000 Da), with 30% having a DP ≤ 4 and more than 90% DP ≤ 30 (Lahtinen et al., 2010). Despite the relatively simple preparation, the melt condensation of other simple sugars is not widely reported; general reference to the feasibility of preparing such oligomers is often cited in the patent literature but not exemplified (Shah, Craig, Morrill, & Wuesthoff, 2003; Shah, Gros, & Lindholm, 2004)

The preparation of oligomeric or polymeric materials from lactose using a process similar to that used in the manufacture of polydextrose raises the interesting possibility of generating new food ingredients with novel functional properties from an abundant non-food ingredient: a recent report of utilising extrusion technology to generate polymeric material from lactose is a good example (Tremaine, Reid, Tyl, & Schoenfuss, 2014). Thus, our attention turned to the potential to rapidly and directly prepare a polymer from lactose that demonstrates comparable utility to the enzymatically derived GOS that enjoy significant use as food additives (Lamsal, 2012).

In this paper we exemplify the utility of melt polymerisation to generate amorphous polysugars from a series of monosaccharides including not only glucose, but galactose, fucose and xylose. We also describe a method, utilising microwave technology, which provides a mechanism for the conversion of lactose into a polymeric species. We detail analysis on the size and branching of these polysugars and show their potential application as a food ingredient by comparing the relative utilisation by bifidobacteria of commercial GOS with our new polymeric species.

2. Materials and methods

2.1. Materials

D-Glucose was purchased from Sigma, D-galactose, D-xylose and L-fucose were from Carbosynth, and α-lactose-monohydrate and citric acid were from BDH. Polydextrose was purchased from IngredientStop (Auckland, New Zealand). All other solvents and reagents used were reagent grade and used as received without further purification. For a table of bacterial strains used for growth experiments please refer to the supplementary information (Table S1).

2.2. General methods

Microwave experiments were carried out using a CEM Discover Microwave reactor. Size exclusion high-performance liquid-chromatography (SEC-HPLC) was carried out on a Waters Alliance HPLC with refractive index detection, using 2 Superdex Peptide columns in series, eluted with 0.1 M $NaNO_3$ at 0.5 mL min^{-1}. Proton and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker Avance 500 spectrometer at 27 °C in D_2O using an inverse probe. Glycosyl linkage compositions were determined by gas chromatography–mass spectrometry (GC–MS) of partially methylated alditol acetates. Samples (~0.5 mg) were methylated as previously described (Ciucanu & Kerek, 1984). After extraction into chloroform, the methylated oligosaccharides were hydrolysed with TFA and the products were reduced and acetylated before analysis by GC–MS (Carnachan, Bootten, Mishra, Monro, & Sims, 2012). Identifications were based on peak retention times and on comparisons of electron impact spectra with the spectra obtained from reference compounds. High-performance anion exchange chromatography (HPAEC) was completed in duplicate on all samples using a CarboPac PA-100 (4 × 250 mm, operating temperature 30 °C) column equilibrated in 150 mM NaOH, operating a Dionex ICS 3000 (Dionex Corp., Sunnyvale, CA, USA). Samples (1 μg) were injected onto the column in distilled water (0.1 mg mL^{-1}) and eluted with a linear gradient of NaOAc (0–250 mM) in 150 mM NaOH from 5 to 60 min after injection. The eluant was monitored by pulsed amperometric detection. Differential scanning calorimetry was completed on a Mettler DSC1 STAR^e system with a GC200 gas controller and autosampler. Samples (0.5–1.5 mg) in pierced aluminium crucibles (40 μL, PN ME-26763) were assessed using three contiguous repeat cycles from −20 to 160 at a ramp rate of 5 °C/min under a constant atmosphere of nitrogen (30 mL min^{-1}). Samples assessed with acid were from freeze-dried solutions. The conditions the sugar-acid aqueous mixture experienced in the freeze drying process did not modify the sugar as determined by NMR.

2.3. Melt polymerisation of monomers

A typical method used for melt polymerisation is as follows. Galactose (50 g), glucose (50 g) and citric acid (1.28 g) were ground together to a fine powder in a mortar and pestle. The powder was heated in a round-bottom flask (170 °C, 5 h) under reduced pressure (10–0.1 mBar) during which time the melting sugar material expelled water and set into a glassy foam. Recovery of the cooled foam and repeat grind by mortar and pestle afforded a pale yellow powder which was analysed by NMR, SEC-HPLC and linkage analysis. Other polymerisations were carried out in a similar manner, with the same proportions of monosaccharide to catalyst, but differing reaction conditions as described in the text.

2.4. Microwave polymerisation of lactose

Lactose (2 g) and 5% aqueous citric acid solution (250 μL) were placed in a 10 mL microwave reactor vessel, the vessel capped and irradiated in a microwave reactor at 200 W and 170 °C for 5 min. The pale yellow oil which resulted became viscous upon cooling. This was analysed by NMR and HPLC without any further purification.

2.5. Microwave hydrolysis of lactose

Lactose (500 mg) was placed into a 10 mL microwave reactor vessel with water (5 mL) and phosphoric acid (30 μL), the vessel capped and irradiated at 100 W and 100 °C for 2 × 6 min periods before cooling, freeze drying and the resulting powder analysed by NMR and HPLC.

2.6. Screening bifidobacterial strains for utilisation of carbohydrates as growth substrates

The growth of 19 bifidobacterial strains (4 *Bifidobacterium longum* subspecies *infantis*, 5 *Bifidobacterium bifidum*, 5 *Bifidobacterium breve*, 5 *B. longum* subspecies *longum*) was tested (triplicate cultures for each strain/substrate) in medium containing 0.2% (w/v) of the test substrate. The substrate was added to a glucose/carbohydrate (CHO) free MRS medium (per litre: proteose peptone 10.0 g, beef extract 10.0 g, yeast extract 5.0 g, Tween 80 1.0 mL, ammonium citrate 2.0 g, sodium acetate 5.0 g, magnesium sulphate 0.1 g, manganese sulphate 0.05 g, di-potassium phosphate 2.0 g). The media were inoculated with 1% (v/v) of starter bacterial culture that had been incubated anaerobically (18 h, 37 °C) in MRS medium containing glucose (1%, w/v). The cultures were incubated at 37 °C and optical density ($A_{600\,nm}$) readings were made after 24 h incubation. Aliquots of cultures were centrifuged (17,000 × *g*, 5 min, 5 °C), the supernatants filtered (0.2 μm) and stored at −20 °C prior to carbohydrate analysis. Controls (in triplicate) for each strain were also completed using CHO-free MRS medium for comparison of growth with media containing test substrates.

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