Carbohydrate Polymers 117 (2015) 223–229

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis, characterisation and microbial utilisation of amorphous polysugars from lactose

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ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form
16 September 2014
Accepted 23 September 2014
Available online 2 October 2014

Keywords:
Carbohydrate analysis
Melt polymerisation
Microbial utilisation
Microwave

Chemical compounds studied in this article:
Lactose (PubChem CID: 6134)
Glucose (PubChem CID: 79025)
Galactose (PubChem CID: 6036)
Xylose (PubChem CID: 135191)
Fucose (PubChem CID: 71315513)
Polydextrose (PubChem CID: 71306906)
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).

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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http://dx.doi.org/10.1016/j.carbpol.2014.09.061
0144-8617/

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.

Table 1
Carbohydrate linkage analysis data for polydextrose, polygalactose and "polylactose" polymers from 1:1 glucose:galactose as well as derived from lactose.

Sugar derivative	Relative amount (mol%)					
	PD and PG			Galactose	PL	
	PD	Glc-2[a]	Glc-8[a]		Glc/Gal[b]	Lactose derived[c]
t-Glc*p*	45.2	33.9	36		23	20.4
t-Gal*p*				20.6	15.3	18.7
t-Glc*f*	6.2	8.6	6.6		3.3	3.7
t-Gal*f*				21.9	15.7	13.7
2-Glc*p*	3.9	4.6	4.4		2.1	2
2-Gal*p*				3.7	–	–
2-Glc*f*	0.7	0.6	0.6		*	*
2-Gal*f*				3.8	*	*
3-Glc*p*	6.8	6.4	6.6		6.3	5.6
3-Gal*p*				3.7	3.4	3.9
3-Glc*f*	1.9	1.3	1.1		0.4	0.2
3-Gal*f*				4.8	–	–
4-Glc*f* + 2-Gal*f*[d]	6.9	6.3	6.1		5.6	9.4
4-Gal*p*				5.5	2.4	2.3
6-Glc*p*	16.6	17.3	18.3		6.8	6.1
6-Gal*p*				10.4	3.4	4.9
6-Glc*f*	1.8	3.2	2.5		0.7	0.7
6-Gal*f*				6.9	2.8	2.8
2,3-Glc*p*	0.2	0.4	0.4		0.1	–
2,3-Gal*p*				0.4	*	–
2,4-Glc*p* + 2,3-Gal*p*[d]	0.5	0.9	0.9		0.4	0.5
2,4-Gal*p*				1	0.3	0.2
2,6-Glc*p*	1.3	2.5	2.6		0.5	0.5
2,6-Gal*p*				1.3	0.2	0.2
2,6-Glc*f*	0.3	0.5	0.5		–	–
3,4-Glc*p* + 3,4-Gal*p*[d]	1.3	1.4	1.3		0.9	0.7
3,4-Gal*p*				1.5	*	*
3,6-Glc*p* +4,6-Gal*p*[d]	2.2	3.2	3.6		0.9	0.7
3,6-Gal*p*				4.4	1	0.6
3,6-Glc*f*	0.6	0.8	0.7		0.1	–
4,6-Glc*p*	3	5.6	5.2		0.8	1
4,6-Gal*p*				7.2	2.4	1.6
2,3,4-Glc*p*	–	0.2	0.1			
2,3,4-Gal*p*				0.2		
2,3,6-Glc*p*	–	0.2	0.2			
2,3,6-+2,4,6-Gal*p*				1		
2,3,6-Glc*f*	–	tr	tr			
2,4,6-Glc*p*	0.2	0.8	0.7		–	
3,4,6-Glc*p*	0.4	1.2	1.1		0.1	
3,4,6-Gal*p*				1.7	0.1	0.1
6-OAc-Glc*p*		0.1	0.3			
6-OAc-Gal*p*				0.1		
Total		100	100	100	99	100.5

Not detected. tr: trace. *: cannot be detected in isolation, see footnote – d.
[a] Polyglucose (PG) prepared without sorbitol for 2 and 8 h reaction times.
[b] Melt polymerisation of glucose and galactose monomers.
[c] Microwave-hydrolysed lactose subsequently melt polymerised.
[d] Glucose species only for PD and PG, GCMS peak co-elutes so combined species for PL polymers.

2.7. Utilisation of oligosaccharides measured by HPAEC

Utilisation of oligosaccharides by the bacteria was monitored by determination of the relative proportions of individual oligosaccharides in cell-free culture supernatants (spent media), compared with un-inoculated media. Samples were analysed by HPAEC.

3. Results and discussion

3.1. Melt polymerisation of monosaccharides

A polyglucose (PG) was synthesised without the "capping agent" sorbitol, as is present in polydextrose (PD), for reaction times of 4 and 8 h, as we wished to maximise molecular weight and minimise the proportion of unreacted monomer to generate a complex-carbohydrate, lower-calorie food ingredient. Citric acid was chosen as the catalyst for direct comparison to the commercial product. Citric acid is a food grade ingredient, and as a "combining acid", through Fischer esterification it is covalently entrained into the polymer, thereby eliminating the requirement of a second step of moderating the pH of the material. PG prepared under melt conditions for either 4 h (Glc-4) or 8 h (Glc-8) had a similar composition to the commercially available PD as determined by linkage analysis (Table 1), excepting the enhanced proportion of terminal glucopyranose (t-glc*p*) in the commercial product (Stumm & Baltes, 1997). The two polymers had lower proportions of terminal residues than the commercial PD and higher proportions of total branched residues (17.7 and 17.3 mol% for Glc-4 and Glc-8, respectively, vs. 10.0 mol% for PD). SEC-HPLC showed that Glc-4

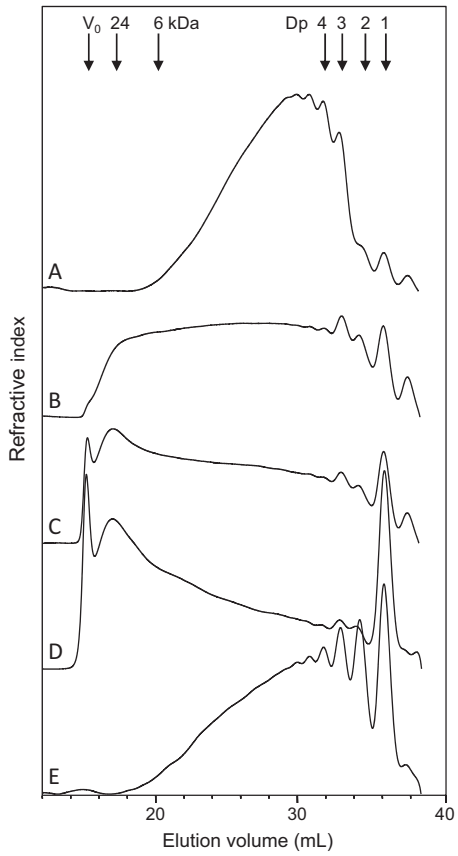

Fig. 1. SEC-HPLC plots for (A) polydextrose, (B) polyglucose reacted for 4 h, (C) polyglucose reacted for 8 h, (D) polygalactose, (E) polymer formed from 50:50 glucose:galactose.

Table 2
Carbohydrate linkage analysis data for polyfucose, polymer derived from 50:50 fucose:galactose, and polyxylose.

Sugar derivative	Relative amount (mol%)		
	Polyfucose	Fuc/Gal[a]	Polyxylose
t-Fuc*p*	24.2	19.5	
t-Gal*p*		7	
t-Xyl*p*			23.9
t-Fuc*f*	13	10.8	
t-Gal*f*		24.1	
t-Xyl*f*			9.8
2-Fuc*p*	10.8	5.6	
2-Xyl*p* + 4-Xyl*p*			30.2
2-Fuc*p*	7	3.9	
2-Gal*f*		0.8	
2-Xyl*f*			2.5
3-Fuc*p*	12.1	7.4	
3-Gal*p*		0.6	
3-Xyl*p*			8.4
3-Fuc*f*	8.2	4.4	
3-Xyl*f*			4.8
4-Fuc*p*	10.5	6.3	
4-Gal*p*		1.1	
5-Xyl*f*			0.9
6-Gal*p*		0.7	
6-Gal*f*		1.1	
2,3-Xyl*p* + 3,4-Xyl*p*			11.1
2,3-Xyl*f*			0.3
2,4-Fuc*p* + 2,3-Fuc*p*	6.9	2.8	
2,4-Gal*p*		0.1	
2,4-Xyl*p*			6.6
3,4-Fuc*p*	4.6	2.1	
3,4-Gal*p*		0.1	
3,5-Xyl*f*			0.6
3,6-Gal*p*		0.3	
4,6-Gal*p*		0.4	
3,4,6-Gal*p*		0.1	
Total	97.3	99.2	99.1

[a] Melt polymerisation of fucose and galactose monosaccharides.

and Glc-8 ranged in size from monosaccharide to >24 kDa (Fig. 1B and C), compared with PD, which ranged from monosaccharide to ~6 kDa (Fig. 1A). Glc-8 showed more material eluting earlier on the SEC-HPLC trace than Glc-4, indicating that it contained a greater proportion of higher molecular weight material, consistent with the longer reaction time. With increased reaction time from 4 h to 8 h the composition of our PD did indicate a subtle shift to an overall higher MW material, especially the portion of material that was recorded eluting at the exclusion limit of the column; however, the degree of caramelisation and colour imparted due to sugar degradation (Claude & Ubbink, 2006) was pronounced for reaction times >4 h. Integration of the area in the SEC trace relevant to monosaccharide peaks showed that Glc-4 and Glc-8 contained >2.5 times more free monomer than PD.

With the desired oligomeric species generated from glucose, we now turned to homopolymers of galactose, xylose (a pentose), fucose (a deoxy sugar) and sialic acid. In addition, co-polymers of glucose and galactose, and fucose and galactose were prepared to assess a likely outcome of a "polylactose" and galacto-fucane species, respectively. Upon heating under vacuum sialic acid decomposed to a black tar, but the polymerisations utilising all other sugars progressed to give oligomeric species (Tables 1 and 2).

Carbohydrate linkage analysis of the resulting polymers showed that fucose containing polymers contained a higher proportion of furanose moieties than other polymers (Table 2). The xylose polymer was found to be highly branched (similar to the glucose and galactose polymers), whereas the fucose and fucose/galactose polymers were less so (similar to commercial polydextrose). A peak in the GCMS trace was recorded during analysis of poly-fucose (1.7 mol%) that displayed a fragmentation pattern which did not correlate with any of the standard sugar alditol acetates on file. This species is currently unidentified and the subject of further analysis. Melt-polymerisation of galactose resulted in a polymer with a greater proportion of higher molecular weight material (≥24 kDa) and more monosaccharide (~2×) than PG or PD (Fig. 1). Glycosyl linkage analysis showed the presence of highly branched polymers, but interestingly, excepting the terminal residues that may represent free monomer, there were a greater proportion of furanosyl residues than observed for the glucose polymers (Table 1).

A "polylactose"-like product (PL) derived from melt-copolymerisation of the lactose constituents glucose and galactose, displayed a polymer distribution of overall smaller molecular mass (Fig. 1E) than the poly-galactose (Fig. 1D) or PG species (Fig. 1B and C), but was more reminiscent of the commercial PD (Fig. 1A). The linkage analysis suggested a similar distribution of species as would be achieved by combining a mixture of polyglucose and polygalactose (Table 1).

All of the polymers prepared were also analysed by NMR spectroscopy (see supplementary information for spectra). Upon polymerisation the sharp resolved signals observed in the ^{1}H and ^{13}C spectra for the monomeric species are replaced with a series of broad overlapping signals consistent with the generation of a polydisperse and heterogeneous mixture of oligomers. Broad resonances in the ^{13}C spectra observed at ~108–110 ppm and ~96–105 ppm were consistent with furanose and pyranose moieties, respectively. This is consistent with the complex mixture of furanose and pyranose residues determined in the linkage analysis

data. The ^{1}H spectrum of the reaction product displayed a pair of sharp doublets (δ 2.90 and 3.07 ppm) that are assigned to citric acid, further evidenced by the observation of a peak at δ 43.3 ppm in the corresponding ^{13}C NMR spectrum.

All of the polysugars were pale yellow solids and demonstrated the very high water solubility exhibited by polydextrose (>50%, w/w). As proof in principal for generating a "polylactose" type material had been demonstrated with the successful co-polymerisation of glucose and galactose our attention turned to the direct polymerisation of the disaccharide.

3.2. Direct polymerisation of lactose

Initial experiments to synthesise polymers directly from lactose using conventional melt polymerisation under vacuum, in the presence of citric acid and sorbitol, were markedly unsuccessful. The reaction of lactose under standard conditions (160 °C for 2 h), at elevated temperatures (>180 °C), or for extended periods of time, gave rise to significant decomposition before any polymerised material could be detected. Differential scanning calorimetry (DSC) demonstrated lactose mono-hydrate exhibited a water-dissociation endotherm at 145–148 °C and a sharp endotherm at 210–212 °C attributed to a combined decomposition/melt event. With increasing citric acid (1.28, 2.6 or 5.2%, w/w) this decomposition endotherm broadened, but did not reduce the peak maxima below 200 °C (5.2%, w/w citric acid). In comparison, a melting point depression of 12 °C was achieved with just 1.28% (w/w) citric acid for glucose at a significantly lower temperature (see supplementary data). Making the assumption that minimal reaction is taking place in the solid state, if decomposition of lactose occurs prior to, or concurrent with the melt phase-change, then the polymerisation reaction cannot occur before decomposition and so oligomeric products cannot be generated directly from the disaccharide lactose.

To overcome the recalcitrance of lactose to polymerise directly, the reaction was attempted in a microwave reactor. Performing the reactions under a vacuum with microwave irradiation to drive the polymerisation via the removal of water was not possible. These conditions led to microwave plasma formation, caused by the superheating of gaseous molecules, promoted by low-pressure conditions (Karney, 2012). However, heating lactose with sorbitol, citric acid and a small amount of water (9.5%, w/w) with microwave irradiation for just 3 min lead to a free flowing amber-coloured clear gel that increased in viscosity on standing. Analysis by HPLC showed that this product did not contain material >6 kDa, but oligosaccharides with DP 2–6 were clearly visible, indicating the presence of a polymer derived from lactose (Fig. 2B). However, there were considerable proportions of glucose and galactose also present, indicating lactose hydrolysis was occurring under microwave irradiation conditions.

Varying the reaction conditions (microwave power, temperature, time and cycle, citric acid- and water-proportion) did not greatly affect the amount of polymer produced; higher temperatures resulted in a darker product, indicating greater decomposition of the lactose. Further attempts were made to polymerise lactose with microwave irradiation in various organic solvents; toluene, dimethylacetamide, N-methyl-2-pyrrolidone, ethylene carbonate, dimethylformamide, dimethyl sulfoxide and tetramethylene sulfone. Only sulfolane, which had previously been reported for use in the polycondensation of sugars (Mora & Wood, 1958) facilitated any polymerisation, resulting in a greater degree of polymerisation and less monosaccharide than when water was used as the solvent (Fig. 2C).

The presence of glucose and galactose in the reaction mixtures obtained after microwave irradiation of lactose (Fig. 2B) suggested that the oligosaccharides observed were likely the product of melt polymerisation of monomers resulting from lactose hydrolysis, rather than from direct polymerisation of lactose itself. The presence of monomers and small oligomers was also observed under extrusion processing, suggesting a similar mechanism of hydrolysis then polymerisation (Tremaine, Reid, Tyl, & Schoenfuss, 2014). Thus, our attention turned to hydrolysis of lactose with a view to preparing "polylactose" (PL) through a two-step process

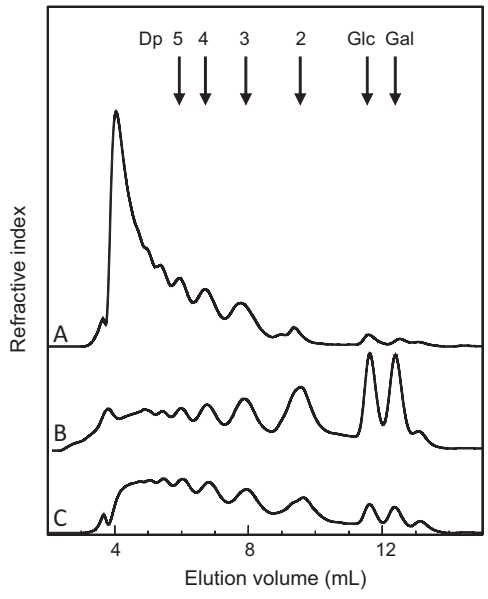

Fig. 2. HPAEC plots for (A) polydextrose, (B) polymer prepared from lactose, (C) polymer prepared from lactose in sulfalone.

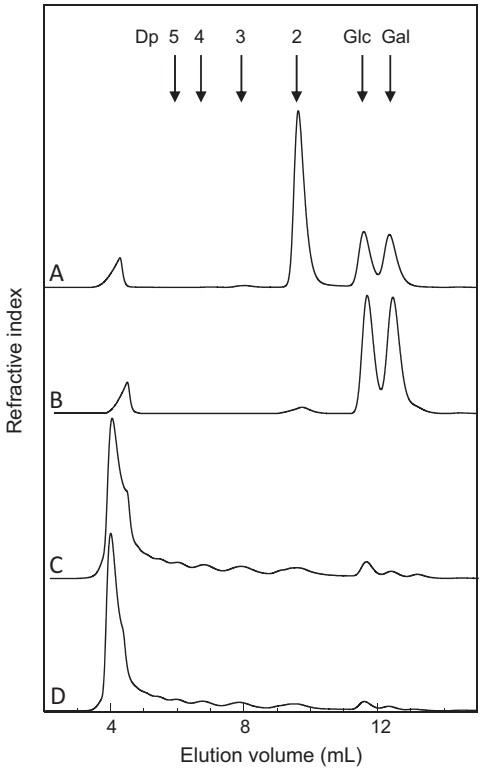

Fig. 3. HPAEC plot of the hydrolysis and melt polymerisation products of lactose. (A) Lactose hydrolysis via conventional heating for 20 h, (B) lactose hydrolysis via microwave irradiation for 12 min, (C) lactose hydrolysis and polymerisation via microwave in sulfolane, (D) melt polymer of 50:50 glucose:galactose.

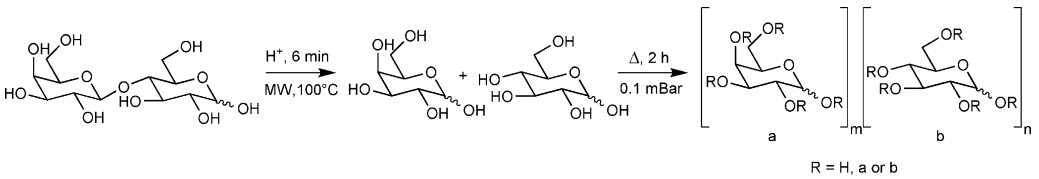

Fig. 4. Reaction scheme for the preparation of a polysaccharide mixture from lactose via a two-step process of microwave hydrolysis and melt polymerisation.

of microwave hydrolysis and subsequent conventional melt polymerisation.

3.3. Two-step microwave hydrolysis of lactose and subsequent melt polymerisation

There have been many methods reported for the hydrolysis of lactose with the most common being enzymatic methods or acidic hydrolysis, either with a mineral acid or ion-exchange resin (Harju, Kallioinen, & Tossavainen, 2012). To optimise the conditions required for lactose hydrolysis a comprehensive study was undertaken, investigating water dilutions, acid concentrations, acid type, temperature, microwave irradiation (power and time), and conventional heating. Ultimately, acid hydrolysis of lactose proved to be far more efficient in the microwave reactor. Lactose hydrolysis by conventional heating (cat. phosphoric acid, 100 °C, 20 h) gave only ~25% hydrolysis (Fig. 3A), with full hydrolysis still not observed after 4 days reaction (data not shown), whereas hydrolysis was almost 100% complete after just 12 min of microwave irradiation (cat. phosphoric acid, 100 °C; Fig. 3B). However, an undesired side effect of lactose microwave hydrolysis was the production of specks of dark decomposition products. It was found that a 10% (w/v) aqueous solution of lactose, with 5% phosphoric acid present as a catalyst, processed in the microwave for 2 × 6 min at 100 °C and 100 W gave almost complete hydrolysis (~98%), with minimal colour generation. Decreasing any of these parameters lead to incomplete hydrolysis, while increasing them either had no effect on the overall outcome, or gave rise to an increase in the amount of coloured by-products produced.

Treatment of the lactose hydrosylate under melt polymerisation conditions readily produced a "polylactose" material that was comparable in both size and composition to that derived from the monomers glucose and galactose. Comparison of Fig. 3C and D demonstrates the conversion of the monomers to a much larger polymer distribution and the composition as determined by linkage analysis was very similar (Table 1). This provided an efficient mechanism for the rapid hydrolysis and conversion of lactose into material with a high proportion of polymeric content, comparable in gross structural-composition to commercial PD (see Fig. 4).

3.4. Utilisation of polysugars as growth substrates for bifidobacteria

The growth of 19 bifidobacterial strains in media incorporating PL and polygalactose was tested and compared with media charged with commercially available, enzymatically prepared, GOS and to carbohydrate-free media (Fig. 5). The GOS medium enhanced the growth of all of the strains relative to basal medium, and was marked in the case of *B. longum* subsp. *infantis* strains that displayed two to three times the growth profile compared to both other bacterial species or carbohydrate sources.

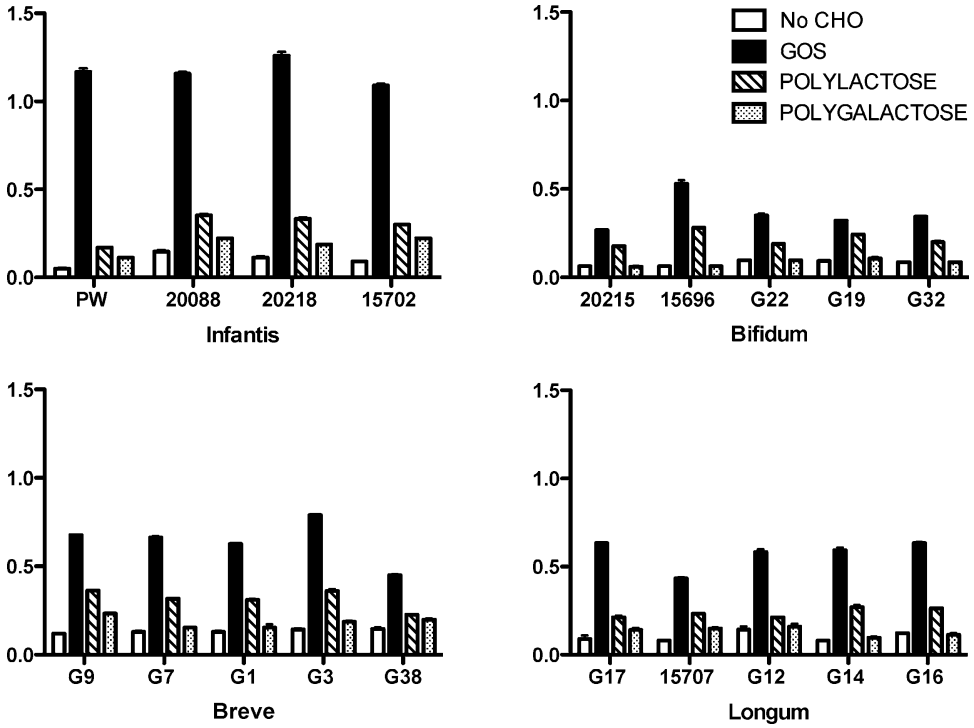

Fig. 5. Optical density ($A_{600\,nm}$) measurements of growth of bifidobacterial strains in basal medium (no carbohydrate added, No CHO) or test media (basal medium containing 0.2%, w/v) polylactose, polygalactose, or galacto-oligosaccharide (GOS). Means and standard error of the mean are shown ($n = 3$).

It is interesting to note that the polygalactose medium differentiated between bifidobacterial species, in that *B. bifidum* did not grow better in this medium compared to basal medium, whereas the other species showed improved growth.

There are two significant differences between the carbohydrate sources assessed for microbial growth. GOS is a series of linear sugars of mostly tri- to pentasaccahrides, whilst the melt polymerised materials demonstrate both significant branching and larger molecular weight. Secondly, the melt polymerised materials also indicate a large proportion of monosaccharide present after the reaction. The microbial utilisation of the various substrates would indicate that whilst there is still monosaccharide present in the melt-derived materials, the smaller linear polymers of GOS are a far superior substrate. Analysis of substrate consumption by high performance anion exchange chromatography indicated that there were differences in the consumption of individual oligosaccharides in PL, polygalactose and GOS among the various bifidobacterial species and strains tested. The isolation and identification of the individual species utilised by the microorganisms is the subject of ongoing research.

4. Conclusions

Polysugars are readily prepared from the melt polymerisation of individual or mixtures of monosaccharide's generating polydextrose-like materials and these have been characterised. Experiments to polymerise lactose by microwave irradiation in the presence of sorbitol, citric acid and a small amount of water suggest that the oligosaccharides observed were the result of melt polymerisation of monomers produced from lactose hydrolysis, rather than from direct polymerisation of lactose itself. Such microwave assisted lactose hydrolysis was far more efficient than conventional acid hydrolysis and gave almost complete hydrolysis in only 12 min. Polymerisation of these lactose hydrolysates by conventional melt-polymerisation gave polymers with similar chemical properties to those made from prepared from mixtures of monomeric galactose and glucose. The ability of bifidobacteria to utilise these polylactoses as substrates for growth, particularly in a semi-selective fashion, suggested that they may have utility as novel prebiotics.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.09.061.

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