Food Chemistry 38 (1990) 305–314

Technical Note

Oligosaccharide Formation During the Hydrolysis of Lactose with Hydrochloric Acid and Cation Exchange Resin

ABSTRACT

Oligosaccharides were formed during hydrolysis of 10 and 30% w/w lactose in 2 and 3 M hydrochloric acid by heating to 70 and 100°C, and hydrolysis of 10% w/w lactose using strongly acidic cation exchange resin by heating at 97°C. At least five oligosaccharides were formed in lactose hydrolyzate by hydrochloric acid and by the resin. As the oligosaccharides were not completely hydrolyzed by α-galactosidase, β-galactosidase, α-glucosidase or β-glucosidase, they must have been mixtures of oligosaccharides with different configurations. Twenty-two and eleven per cent of the total sugars were converted to oligosaccharides by hydrochloric acid and the resin, respectively. Six sugars with mobilities greater than that of glucose were formed only in lactose hydrolyzed by hydrochloric acid. These may be anhydrosugars.

INTRODUCTION

Recently, the significance of intestinal flora, especially *Bifidobacterium*, for human health has been suggested by Japanese researchers (Tamura, 1983; Nakaya, 1984; Mutai & Tanaka, 1987). Oral dosage of certain sugars (to volunteers) increased the number of *Bifidobacterium* organisms in the fecal flora (Tanaka *et al.*, 1983; Yazawa *et al.*, 1984; Hidaka *et al.*, 1986). Oligosaccharides containing β-galactosyl residues were among these sugars and are currently used as food additives in Japan (Tanaka *et al.*, 1983). Industrial production of β-galactosyl oligosaccharides is now performed by transgalactosylation reaction from lactose using a costly β-galactosidase enzyme preparation (Tanaka *et al.*, 1983).

Food Chemistry 0308-8146/90/$03·50

Aronson (1952), de Boer and Robbertsen (1981) and Guy and Edmondson (1978) observed oligosaccharide formation during acidic hydrolysis of lactose. It has been reported that the cost of preparing lactose-hydrolyzed syrup by mineral acid or strongly acidic cation-exchange resin was lower than by enzymatic methods (Demaimay *et al.*, 1978; de Boer & Robbertsen, 1981). Preparation of oligosaccharides from lactose by acid or strongly acidic cation-exchange resin must be more economical than by enzyme. So far, there have been many works on acid-catalyzed hydrolysis of lactose (Vujicic *et al.*, 1977; Demaimay *et al.*, 1978; Guy & Edmondson, 1978; de Boer & Robbertsen, 1981), but no works on oligosaccharide production from lactose. The purpose of this paper is to estimate the conditions suitable for oligosaccharide production by hydrochloric acid and strongly acidic cation-exchange resin.

MATERIALS AND METHODS

General

The molar ratios of glucose and galactose were determined as trimethylsilyl (TMS) derivatives by gas chromatography after acid hydrolysis of oligosaccharides with 2 M trifluoroacetic acid for 6 h at 105°C (Toba *et al.*, 1985). Enzymatic digestion of oligosaccharide (1 mg) was performed at 37°C for 24 h using α-galactosidase from green coffee beans (1·8–6·0 units), β-galactosidase from *Escherichia coli* (4·5–6·0 units), α-glucosidase from yeast (10 units) and β-glucosidase from sweet almonds (0·1–0·4 units) which were obtained from Boehringer Mannheim GmbH, Mannheim, FRG.

Sugars

α-Lactose monohydrate, D-galactose and D-glucose were all guaranteed reagents of Japanese industrial standard and obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan) or Nakarai Chemicals Ltd (Kyoto, Japan). Levogalactosan was a gift from Dr T. Urashima *et al.* (1985).

Acid hydrolysis of lactose with hydrochloric acid and strongly acidic cation-exchange resin

Hydrolyzed lactose syrups were prepared by heating 10 and 30% w/w lactose in 2 or 3 M hydrochloric acid to 70°C for 50 h or 100°C for 16 h. The syryps were also prepared by passing 10% w/w lactose solution through a jacketed column (2·0 cm i.d. × 15·5 cm long) packed with a strongly acidic

cation-exchange resin (Bio-Rad AG50W-X8, 100–200 mesh, H^+ form). Before passing through the column, the lactose solution was preheated to 97°C. Then the lactose solution was passed through the column at a constant flow rate of 7·2 or 30 ml/h. The column was heated by circulating steam (97°C) through the jacket.

Samples were removed periodically, cooled in ice water to stop the reaction, and stored at −20°C for analysis.

Paper chromatography

Samples were applied quantitatively on a Whatman No. 3MM paper (40 × 40 cm) with a microsyringe (15 μl for 10% or 5 μl for 30% lactose hydrolyzate) and developed in *n*-butanol–pyridine–water (6:4:3, v/v) four times by the ascending technique. Sugars were stained with silver nitrate reagent (Trevelyan *et al.*, 1950).

Gas chromatography

Sugars were converted to the TMS derivatives by means of hexamethyldisilazane and trifluoroacetic acid in pyridine (Brobst & Lott, 1966). The TMS derivatives were analyzed on a Hitachi 163 gas chromatograph equipped with a stainless steel column (3 mm i.d. × 500 mm long) packed with 2% Dexsil 300GC on Uniport HP (60–80 mesh). Carrier gas: N_2, 40 ml/min; injection and detector temperature: 350°C; column temperature: 150–350°C, 10°C/min.

Degree of polymerization of oligosaccharides was estimated by comparing their retention times with those of standards. β-Galactosyl oligosaccharides, structures of which were previously elucidated, were used as standards (Toba & Adachi, 1978; Toba *et al.*, 1985).

RESULTS AND DISCUSSION

Oligosaccharide formation with hydrochloric acid

Oligosaccharide formation during hydrolysis of lactose with hydrochloric acid under eight conditions was observed by paper chromatography. The conditions and time course of oligosaccharide formation are summarized in Table 1. On paper chromatograms, oligosaccharides were detected as spots having lower mobility than that of galactose, while degradation products of monosaccharides were detected as spots having higher mobility than that of glucose. Glucose and galactose were already liberated within 5 min of heating in all eight conditions (data not shown).

TABLE 1

Oligosaccharide Formation[a] During Hydrolysis of Lactose with Hydrochloric Acid under Various Conditions

Temperature (°C)	*Acid concentration* (M)	*Initial lactose concentration* (%)	*Time of heating*							
			5 min	*30 min*	*1 h*	*3 h*	*6 h*	*16 h*	*32 h*	*50 h*
70	2	10	− (−)	+ (−)	+ (−)	NT[b]	+ + + (+)	+ + + (+)	+ + + (+ +)	+ + + (+ +)
70	2	30	− (−)	− (−)	+ (−)	NT	+ + + (+)	+ + + (+)	+ + + (+ +)	+ + + (+ + +)
70	3	10	− (−)	+ (−)	+ (−)	NT	+ + + (+)	+ + + (+ +)	+ + + (+ +)	+ + + (+ + +)
70	3	30	− (−)	− (−)	+ (−)	NT	+ + + (+)	+ + + (+)	+ + + (+)	+ + + (+ +)
100	2	10	+ (−)	+ (−)	+ + (+)	+ + (+)	+ (+)	− (+)	NT	NT
100	2	30	− (−)	+ (−)	+ + + (+ +)	+ + + (+ +)	+ + + (+ +)	+ + + (+ + +)	NT	NT
100	3	10	+ (−)	+ + (−)	+ + + (+ +)	+ + (+ + +)	+ + (+ + +)	+ (+ +)	NT	NT
100	3	30	+ (−)	+ (−)	+ + (+)	+ + (+ + +)	+ + (+ +)	+ (+ +)	NT	NT

[a] Intensity of oligosaccharide spot on paper chromatogram: −, not detected; +, weak; + +, middle; + + +, strong.
[b] NT: not tested.
Compounds having mobilities faster than that of glucose in parentheses.

At 70°C oligosaccharides were first detected in the samples after 30 min or 1 h of heating. Steady accumulation of oligosaccharides was observed in the samples between 6 and 50 h of heating. Compounds having mobilities greater than that of glucose were first observed in the samples after 6 h of heating, and increased as the time progressed. These observations were independent of lactose concentration and acid concentration. Maximum accumulations of oligosaccharides and compounds having greater mobility than that of glucose were observed when 30% lactose was hydrolyzed in 2 M hydrochloric acid for 50 h (Fig. 1) or 10% lactose in 3 M hydrochloric acid for 50 h.

At 100°C the reaction rates were accelerated compared to those at 70°C. At 100°C oligosaccharides were formed within 5 to 30 min of heating, and steady accumulation of them was observed between 1 and 16 h, when 30% lactose was hydrolyzed in 2 M hydrochloric acid. In the other acid concentration and lactose concentration, oligosaccharides were accumulated between 1 and 6 h, and hydrolyzed again as the time progressed to 16 h. Compounds having mobilities greater than that of glucose were observed first in the samples after 1 h of heating, and accumulated between 3 and 16 h of heating. Maximum accumulation of oligosaccharides and compounds having greater mobility than that of glucose was observed when 30% lactose was hydrolyzed in 2 M hydrochloric acid for 16 h (Fig. 2). There are no qualititative differences between Figs 1 and 2. Gas chromatographic analysis of the hydrolyzate (30% lactose in 2 M hydrochloric acid at 100°C for 16 h) showed that it contained di-, tri-, tetra-, penta- and hexasaccharides (Fig. 3). Twenty-two per cent of total sugar was converted to oligosaccharides (calculated from peak area ratio, without statistical treatment).

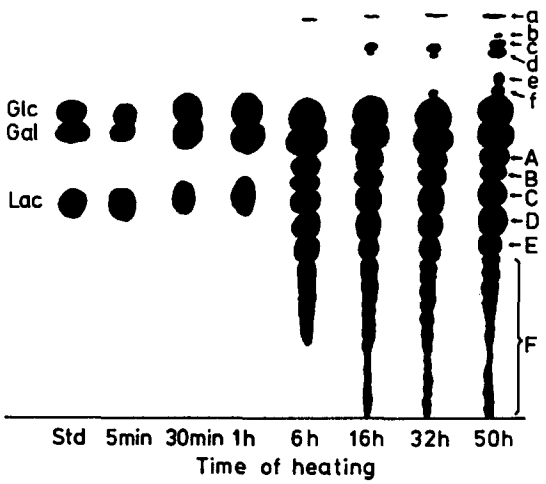

Fig. 1. Paper chromatogram of lactose (30%) hydrolyzate with 2 M hydrochloric acid at 70°C. Std = standard mixture of Glc (glucose), Gal (galactose) and Lac (lactose).

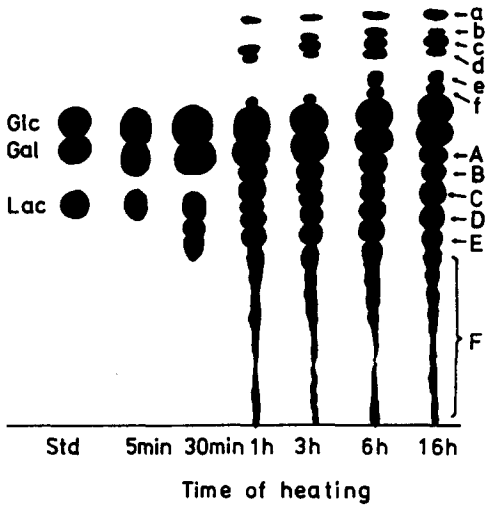

Fig. 2. Paper chromatogram of lactose (30%) hydrolyzate with 2 M hydrochloric acid at 100°C. Std = standard mixture of Glc (glucose), Gal (galactose) and Lac (lactose).

In Figs 1 and 2, six spots having higher R_{Gal} values than glucose were detected: a (R_{Gal}(mobility relative to that of D-galactose) = 1·39), b (R_{Gal} = 1·34), c (R_{Gal} = 1·30), d (R_{Gal} = 1·27), e (R_{Gal} = 1·17) and f (R_{Gal} = 1·13). Five distinct spots and other inseparable spots of oligosaccharide were also detected: A (R_{Gal} = 0·87), B (R_{Gal} = 0·80), C (R_{Gal} = 0·73), D (R_{Gal} = 0·66), E (R_{Gal} = 0·58) and F (origin to R_{Gal} = 0·54). Although the spot C has the same R_{Gal} values as authentic lactose, paper electrophoresis (Toba & Adachi,

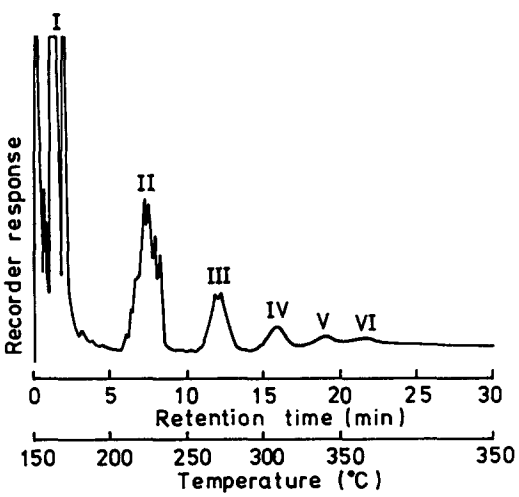

Fig. 3. Gas chromatogram of lactose (30%) hydrolyzate with 2 M hydrochloric acid at 100°C for 16 h. I = glucose and galactose, II = disaccharides, III = trisaccharides, IV = tetrasaccharides, V = pentasaccharides and VI = hexasaccharides.

TABLE 2
Sugar Composition of the Oligosaccharides

Oligosaccharide	R_{Gal} *values on paper chromatogram*	*Molar ratio*	
		Galactose	*Glucose*
A	0·87	1·0	2·1
B	0·80	1·0	1·2
C	0·73	1·0	1·6
D	0·66	1·0	0·8
E	0·58	+	−

+, Detected; −, not detected.

1978) showed that it contained at least two sugars besides lactose (data not shown). Thus we counted this spot as an oligosaccharide. As shown in Table 2, oligosaccharides A, B, C and D contained glucose and galactose, while E contained only galactose. When these oligosaccharides were subjected to hydrolysis by α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase, in all cases there was incomplete hydrolysis of oligosaccharide by a single glycosidase.

Oligosaccharide formation with strongly acidic cation-exchange resin

As shown in Fig. 4, oligosaccharides were formed when 10% lactose solution was passed through strongly acidic cation-exchange resin at flow rates of 30 and 7·5 ml/h. Five oligosaccharides having the same R_{Gal} values as A, B, C, D and E in Figs 1 and 2 were detected in both hydrolyzed lactose samples on paper chromatograms (data not shown). Spots having R_{Gal} values greater than that of glucose and less than $R_{Gal} = 0{\cdot}58$ were, however, not detected on paper chromatograms. Di- and trisaccharide peaks were detected but tetra- and higher oligosaccharide peaks were not detected on the gas chromatogram as shown in Fig. 4. A small amount of unhydrolyzed lactose was detected in the hydrolyzed lactose at a flow rate of 30 ml/h (Fig. 4a), while no detectable amount of lactose remained in that at a flow rate of 7·5 ml/h (Fig. 4b). In the former case 11% of the total sugars was converted to oligosaccharides (calculated from peak area ratio, without statistical treatment).

Although Vujicic *et al.* (1977) and Demaimay *et al.* (1978) did not detect oligosaccharides during acidic hydrolysis of lactose, they were detected in this study. Aronson (1952), Guy and Edmondson (1978) and de Boer and Robbertsen (1981) also observed oligosaccharide formation. De Boer and Robbertsen (1981) showed that only trace amounts of oligosaccharides (about 0·1%) were formed by cation-exchange resin catalyst while there were

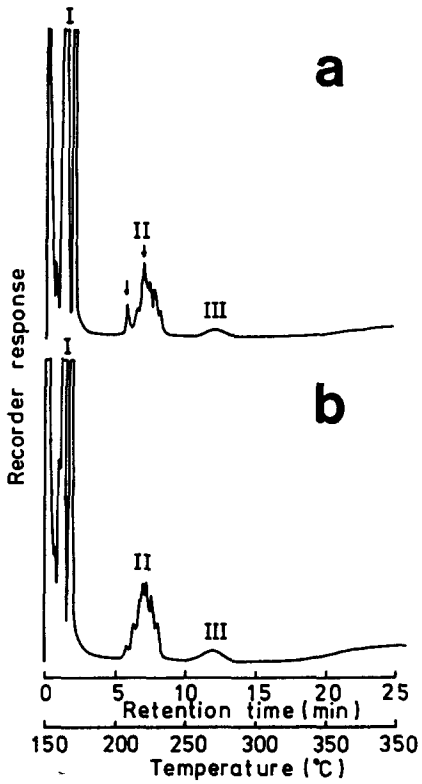

Fig. 4. Gas chromatogram of the eluent passed through the column (2·0 i.d. × 15·5 cm) packed with strongly acidic cation-exchange resin (Bio-Rad AG50W-X8) at 97°C. 10% lactose solution was passed through the column at a flow rate of (a) 30 ml/h or (b) 7·5 ml/h. I = glucose and galactose, II = disaccharides, III = trisaccharides, ↓ = α- and β-lactose.

no quantitative data for mineral acid. We showed that about 10% of the total sugars were converted to oligosaccharides by strongly acidic cation-exchange resin. The extent of conversion by us was considerably higher than that reported by de Boer and Robbertsen (1981). The absence of tri- and higher oligosaccharides in the lactose hydrolyzed by the resin offered a possible explanation for lower oligosaccharide yield than that obtained by hydrochloric acid (Fig. 4). As no higher oligosaccharide formation was observed in the lactose hydrolyzed at a flow rate of 7·5 ml/h and 30 ml/h by the resin (Fig. 4), time of contact on the resin might not be important for this reaction. High cross-linkages in the resin might be rather an important factor for higher oligosaccharide formation.

Hydrochloric acid gave a higher oligosaccharide yield (about 20%) than the resin. Results in this experiment showed that heating 30% lactose in 2 M hydrochloric acid to 100°C for 16 h was optimum for oligosaccharide production by acid-catalyzed hydrolysis of lactose. Although the ratio of

oligosaccharide formation by hydrochloric acid was lower than that of enzymatic methods (10–50% conversion, Toba, 1985), hydrochloric acid-catalyzed oligosaccharide production is of great interest from an economical point of view.

Oligosaccharide structure is an important factor for choice of hydrochloric acid-catalyzed hydrolysis instead of an enzymatic method for its production. Aronson (1952) detected three oligosaccharides and Guy and Edmondson (1978) observed inseparable oligosaccharides, which have R_F values below that of lactose. We detected not only the spots with lower mobility (D and E in Figs 1 and 2) but also those with higher mobility than that of lactose (A, B and C in Figs 1 and 2). There is no report on the structural elucidation of oligosaccharides formed during acidic hydrolysis of lactose. As the oligosaccharides A, B, C, D or E were not completely hydrolyzed by α-galactosidase, β-galactosidase, α-glucosidase or β-glucosidase, each of them must be a mixture of oligosaccharides with different configurations. Enzymatic hydrolysis of lactose gives oligosaccharides with β-linkages only, while acidic hydrolysis of lactose will give both α- and β-linkages. It is reported that 4 and 11 disaccharides containing both α- and β-linkages are formed by acid reversion from galactose and glucose, respectively (Staněk *et al.*, 1965).

Furthermore, we detected spots with R_{Gal} values higher than that of glucose (Figs 1 and 2). One of those spots, c, had the same R_{Gal} as authentic levogalactosan. Thus these spots seem to be anhydrosugars. Reducing the formation of anhydrosugars seems to increase oligosaccharide production. The structural elucidation of oligosaccharides and anhydrosugars will be reported elsewhere.

REFERENCES

Aronson, M. (1952). Transgalactosidation during lactose hydrolysis. *Archs Biochem. Biophys.*, **39**, 370–8.

Boer, R. de & Robbertsen, T. (1981). A purified, hydrolysed lactose syrup made from ultrafiltration permeate. *Neth. Milk Dairy J.*, **35**, 95–111.

Brobst, K. M. & Lott, C. E., Jr (1966). Determination of some compounds in corn syrup by gas-liquid chromatography of the trimethylsilyl derivatives. *Cereal Chem.*, **43**, 35–43.

Demaimay, M., Henaff, Y. Le & Printemps, P. (1978). Hydrolysis of lactose on catalytic resin. *Process Biochem.*, **13**, 3–5.

Guy, E. J. & Edmondson, L. F. (1978). Preparation and properties of sirups made by hydrolysis of lactose. *J. Dairy Sci.*, **61**, 542–9.

Hidaka, H., Eida, T., Takagi, T., Tokunaga, T. & Tashiro, Y. (1986). Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora*, **5**, 37–50.

Mutai, M. & Tanaka, R. (1987). Ecology of *Bifidobacterium* in human intestinal flora. *Bifidobacteria Microflora*, **6**, 33–41.

Nakaya, R. (1984). Role of *Bifidobacterium* in enteric infection. *Bifidobacteria Microflora*, **3**, 3–9.

Staněk, J., Černý, M. & Pacák, J. (1965). Synthesis of oligosaccharides by the action of mineral acids; reversion. In *The Oligosaccharides*. Academic Press, New York, pp. 108–15.

Tamura, Z. (1983). Nutriology of bifidobacteria. *Bifidobacteria Microflora*, **2**, 3–16.

Tanaka, R., Takayama, H., Morotomi, M., Kuroshima, T., Ueyama, S., Matsumoto, K., Kuroda, A. & Mutai, M. (1983). Effects of administration of TOS and *Bifidobacterium breve* 4006 on human fecal flora. *Bifidobacteria Microflora*, **2**, 17–24.

Toba, T. (1985). β-Galactosidase. Its application to lactose hydrolysis and galactooligosaccharide production. *Jap. J. Dairy Food Sci.*, **34**, A169–A182.

Toba, T. & Adachi, S. (1978). Hydrolysis of lactose by microbial β-galactosidases. Formation of oligosaccharides with special reference to 2-*O*-β-D-galactopyranosyl-D-glucose. *J. Dairy Sci.*, **61**, 33–8.

Toba, T., Yokota, A. & Adachi, S. (1985). Oligosaccharide structures formed during the hydrolysis of lactose by *Aspergillus oryzae* β-galactosidase. *Food Chemistry*, **16**, 147–62.

Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). Detection of sugars on paper chromatograms. *Nature, Lond.*, **166**, 444–5.

Urashima, T., Suyama, K. & Adachi, S. (1985). The formation of 1,6-anhydro-3,4-*O*-[5-(hydroxymethyl)-2-furfurylidene]-β-D-galactopyranose from lactose during pyrolysis. *Carbohydr. Res.*, **135**, 324–9.

Vujicic, I. F., Lin, A. Y. & Nickerson, T. A. (1977). Changes during acid hydrolysis of lactose. *J. Dairy Sci.*, **60**, 29–33.

Yazawa, K., Nakajima, A. & Tamura, Z. (1984). Growth of bifidobacteria in adults' intestines on oral administration of sugar source, pantethine and riboflavin. *Bifidobacteria Microflora*, **3**, 43–9.

K. T. Huh,* T. Toba‡ & S. Adachi
Laboratory of Animal Products Technology, Faculty of Agriculture, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aobaku, Sendai 981, Japan

(Received 30 August 1989; revised version received and accepted 20 December 1989)

* Present address: Department of Animal Science, Sang-Ji Junior College attached to Sang-Ji University, Usan-Dong 41, Won-Ji, Kangwon-Do, Korea.
‡ To whom correspondence should be addressed.