Characterization of Branched Oligosaccharides Produced by *Bacillus licheniformis* Maltogenic Amylase

K.S. Kwon, J.H. Auh, S.K. Choi, G.J. Kang, J.W. Kim, and K.H. Park

ABSTRACT

Highly concentrated branched oligosaccharides (HBOS) were prepared from liquefied corn starch by a continuous process using an immobilized *Bacillus licheniformis* maltogenic amylase (BLMA) and yeast fermentation. Physicochemical properties of HBOS were evaluated and compared with those of sucrose and commercial oligosaccharides. An HBOS solution showed flow behavior nearly Newtonian. At relative humidities of 32% and 90%, HBOS exhibited high moisture retention and absorption properties. HBOS were relatively stable against heating at pH 3. Addition of 10–20% HBOS to starch increased its gelatinization temperature by 5–10°C. The glass transition temperature of freeze-concentrated HBOS was higher than that of sucrose, suggesting the potential of HBOS for use in the storage of frozen foods.

Key Words: branched oligosaccahrides, viscosity, gelatinization, glass transition

INTRODUCTION

INTERESTS IN OLIGOSACCHARIDES HAVE INCREASED DUE TO their useful physicochemical properties in foods and physiological effects on human health. Functional properties of maltooligosaccharides vary with size and linkage. Branched maltooligosaccharides (BOS) are oligomers of α-D-glucopyranoase linked primarily by 1,4-bonds but containing at least one 1,6-glycosidic linkage. They include isomaltose, isomaltotriose, panose and several others composed of four or five glucose residues (Takaku, 1988). The production of BOS has increased due to their diverse applications (Spiegel et al., 1994; Tomomatsu, 1994) in processed foods. They are softer and milder than sugar in taste (Gore et al., 1988) and produce relatively low viscosity and water activity (Yoo et al., 1995) that makes them effective for controlling microbial contamination. Also, BOS have special physiological functions, including lower metabolizable energy than sucrose, favorable production of beneficial intestinal microflora (Park et al., 1992; Tomomatsu, 1994; Hidaka et al., 1986; Oku, 1994), and prevention of dental caries (Gore et al., 1988; Oku, 1994). BOS are used in bakery, confectionery, soft drinks, and other food products to improve physicochemical properties and physiological functions, and to extend shelf life (Nakakuki, 1993; 1995).

In general, BOS mixtures are produced by serial reactions of starch with α-amylase and transglucosidase (Takaku, 1988). Kim et al. (1994) produced a BOS mixture from a 15% starch suspension using a *Bacillus licheniformis* maltogenic amylase (BLMA) which had both hydrolyzing and transglycosylation activities on pullulan and starch. However, the yield of BOS from the process was relatively low, and the BOS mixture contained a high percentage of non-branched sugars such as glucose and maltose. A glucose-free, highly concentrated branched oligosaccharide (HBOS) mixture was prepared in our laboratory from BOS by successive fermentation with immobilized yeast cells (Yoo et al. 1995).

Reliable information is needed on functional properties of HBOS for food applications. Therefore, our objective was to evaluate the physicochemical properties of HBOS produced enzymatically by a novel branching enzyme, BLMA, and to compare it with a commercial isomaltooligosaccharide preparation with different sugar compositions, a maltodextrin, and sucrose.

All authors (except Kwon) are affiliated with the Dept. of Food Science & Technology and Research Center for New Bio-Materials in Agriculture, Seoul National Univ., Suwon 441-744, Korea. Author Kwon is affiliated with the Korea Food & Drug Admistration, Food Packaging Division. Address inquiries to Dr. Kwan-Hwa Park.

MATERIALS & METHODS

Preparation of HBOS

HBOS were prepared according to the method of Yoo et al. (1995). BLMA was purified from *E. coli* HB 101 transformed with the recombinant plasmid containing the BLMA gene as described by Kim et al. (1992). Substrate with BLMA (400 cu/g) was added to 30% (w/v) liquefied corn starch (DE 22, Samyang Genex, Incheon, Korea) dissolved in 50mM maleate-NaOH buffer (pH 6.8) containing 5mM EDTA and allowed to react at 45°C for 15h. The reaction was stopped by boiling for 5 min. One unit of cyclodextrin hydrolyzing activity (CU) of BLMA was defined as the amount of enzyme producing reducing sugar equivalent to one unit change of absorbance at 575 nm (Miller, 1959). The BOS mixture was fermented by immobilized *Saccharomyces cerevisiae* var. *ellipsoideus* cells. Fermentation was carried out in a 1L fluidized bed reactor containing the BOS mixture (400 mL) at 27°C for 2 days. The final HBOS had a dextrose equivalent (DE) ≈33, as measured by the method of Donnelly et al. (1973) and the yield was ≈40% (w/w). A commercial isomaltooligosaccharide preparation (IMO, DE 42) was provided by Doosan Food Co. (Seoul, Korea). A maltodextrin from corn starch (DE 22) was provided by Samyang Genex (Inchen, Korea).

Analyses of sugar composition

The sugar composition of oligosaccharides was determined by a High performance ion chromatography (HPIC) system consisting of a CarboPac PA1 column (Dionex Bio LC 4500I) and a pulsed amperometric detector (PAD, Dionex Co., CA). Samples (20 μL, 0.02%, w/v) were injected, and the column was eluted at 1.0 mL/min with a linear gradient of 150 mM NaOH and a mixture of 150 mM NaOH containing 600 mM sodium acetate (Yoo et al., 1995).

Measurement of rheological properties

Flow properties of the sugar samples were measured using a concentric cylinder viscometer (Haake model RV 12, Fisons Instruments, Valencia, CA) at various temperatures and concentrations. Samples were transferred to the viscometer, and left undisturbed for 5 min to reach a stabilized temperature (25°C). Shear stress was measured while increasing the shear rate from 0 to 2,000 sec^{-1}. Data from the shear test were applied to the power law equation (Evans and Haisman, 1979), to give the relationship between shear stress and shear rate as:

$$\tau = k\gamma^n$$

where τ (Pascal) is shear stress, k ($Pa \cdot s^n$) is the consistency coeffi-

cient, γ (1/sec) is shear rate, and n (dimensionless) is the flow index.

Differential scanning calorimetry (DSC)

DSC analyses were performed using a DSC 120 (Seiko Co., Japan) equipped with an intracooler system. The gelatinization of starch with oligosaccharides was measured as reported by Bello-Perez and Paredes-Lopez, 1995. Corn starch samples were weighed directly into DSC aluminum pans and the sugar solutions (HBOS, IMO, maltodextrin or sucrose; made up with deionized water) were added with a micropipette to make suspensions of 10 and 20% (w/w) oligosaccharides in total samples. After sealing, the pans were equilibrated for 1h at room temperature (≈25°C) and heated from 30°C to 100°C at a rate of 5°C/min. Pans containing deionized water were used as references. Measurement was performed in triplicate for each sample.

The glass transition temperatures of the maximally freeze-concentrated solutions (T_g') were determined by the method of Roos (1993) with modifications. Sugar solutions (10–15mg; 60%, w/w) were weighed into 20 μL aluminum DSC pans, initially cooled at 2.5°C/min to −100°C, and then heated to 25°C to detect their T_m' (the onset temperature of ice melting in a maximally freeze-concentrated solution) as well as their thermal behavior in the non-annealed state. Samples were then cooled to −100°C (2.5°C/min), heated to $T_m'-1$°C (annealing temperature), annealed for 30 min, cooled to -100°C (5°C/min), and scanned from −100°C to 25°°C (2.5°C/min) to determine T_g' and T_m'. The onset temperatures of glass transition and ice-melting were taken as the T_g' and T_m', respectively (Fig. 1). In this experiment, an empty pan was used as reference. Measurement was performed in triplicate for each sample.

Moisture retention and absorption

Moisture retention and absorption properties of HBOS, IMO and sucrose were evaluated at different relative humidities according to the method of Donnelly et al. (1973). Saturated salt solutions of $MgCl_2 \cdot 6H_2O$, and $BaCl_2$ were prepared in separate desiccators to give relative humidities (RH) of 32% and 90%, respectively. Sample syrups of 50% (dry solid) and lyophilized samples in weighing bottles were placed in the desiccator with atmospheres of 32% and 90% RH at 25°C. The weight loss or gain was measured as percentage moisture desorbed or absorbed on the basis of the initial sample weight.

Heat and acid stability

To determine the effect of heat and acidity on sugar composition, 10% HBOS and sucrose solutions were adjusted to pH 3 with 0.1N HCl, heated in a boiling water bath or an oil bath (for 120°C heating) for 30 min, neutralized with 0.1N NaOH, and analyzed by HPIC.

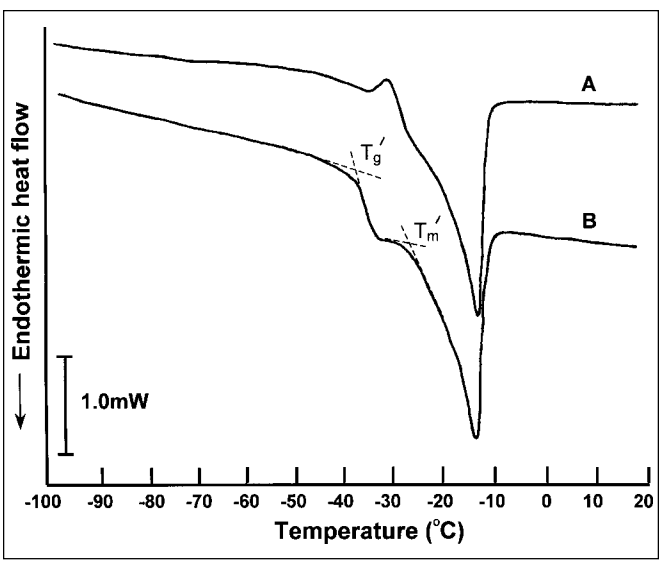

Fig.1—Differential scanning calorimetry (DSC) thermograms of 60% HBOS solution. A, non-annealed solution; B, annealed for 30 min at -25°C.

Statistical analysis

Statistical analyses were performed by non-linear regression, analysis of variance, and pairwise comparisons (Bonferroni's t-test) using *SigmaStat for Windows, version 1.0* (1993, Jandel Corporation, CA). Significance of difference was defined at $p \leq 0.05$.

RESULTS & DISCUSSION

Sugar compositions

The compositions of HBOS and other oligosaccharides determined by HPIC were compared (Table 1). Total amount of branched oligosaccharides in HBOS was 85.7%, which was much higher than that (37.9%) of IMO. HBOS were mainly composed of isomaltose, isopanose, branched tetraose (mainly 6^2-*O*-α-maltosyl-maltose), and branched pentaose (6^2-*O*-α-maltosylmaltotriose and 6^3-*O*-α-maltotriosyl-maltose), it also contained glucose and maltose (Fig. 3A). IMO contained more glucose and maltose, accounting for ≈16% and 27%, respectively. In general, glucose in oligosaccharides syrups may stimulate growth of microorganisms and cause Maillard reactions, producing brown color, therefore the removal of glucose would be desirable for certain applications (Yoo et al., 1995).

Rheological properties

Experimental rheological data were inserted into the empirical power law equation and a series of k and n values were obtained by nonlinear regression (Table 2). The n values of HBOS were close to 1, thus verifying Newtonian flow characteristics. However, IMO showed a behavior close to pseudoplastic flow. The differences of apparent viscosity among samples were little at 10% and 20% concentrations at 25°C, however at 30%, maltodextrin showed the highest viscosity due to a large amount of high molecular weight sugars (DE 22). As the measuring temperature increased, the apparent viscosity decreased, probably due to the increase of molecular mobility.

Water retention and absorption

The ability of many food products to absorb or desorb moisture when exposed to atmospheres of different RH can cause undesirable effects during storage. When 50% syrups were exposed to 32% RH, HBOS and IMO exhibited about 33% weight loss, whereas sucrose desorbed water by more than 56% after 8 days storage. Thus, the viscous syrups of HBOS and IMO remained in a semisolid state, but the appearance of sucrose changed from a liquid syrup to a glassy crystal (Fig. 2A). Lyophilized HBOS and IMO had greater abilities to absorb moisture than sucrose at 90% RH (Fig. 2B). The hygroscopic properties of these oligosaccharides were in order of HBOS > IMO > sucrose. These results had been partly confirmed by the findings that the maltotriose (G_3) and maltotetraose (G_4) constituents of HBOS showed higher moisture absorptive powers than maltose and high-molecular weight oligosaccharides (G_6-G_{11}) (Donnelly et al., 1973). Therefore, HBOS would likely be suitable as a humectant.

Heat and acid stability

The changes in oligosaccharide composition by boiling at pH 3 were determined (Fig. 3). Heating at 120°C in acid caused complete degradation of sucrose (data not shown), but degraded only about 17% of HBOS oligosaccharides (Fig. 3). The concentration of glucose and isomaltose in HBOS hydrolysates increased with increasing temperature. However the amount of isopanose and branched tetraose decreased, indicating that these oligosaccharides were degraded to small molecules. These data showed that the exposure of samples to high temperature alone did not cause the breakdown of oligosaccharides. Branched oligosaccharides of HBOS were relatively stable in an acidic environment as had been reported (Nakakuki, 1995; Kim et al., 1995).

Table 1—Sugar compositions of HBOS, IMO and maltodextrin

Saccharide	HBOS (%)	IMO (%)	Maltodextrin (%)
Glucose	—	15.94	3.92
Maltose	0.9	26.51	12.84
Isomaltose	7.3	9.82	
Maltotriose	7.0	13.70	15.48
Panose	21.0	16.79	
Isopanose	7.5	3.64	
Maltotetraose	2.4	4.20	>67.74
Branched tetraose	28.6	6.13	
Maltopetaose	2.4	3.43	
Branched pentaose	14.9	1.21	
Maltohexaose	1.6	1.10	
Branched hexaose	6.4	—	
> Branched hexaose	Trace	—	
Total branched Oligosaccharides	85.7	37.88	
DE value	33	42	22

Table 2—Rheological parameters of HBOS, IMO, maltodextrin and sucrose solutions calculated using the power law equation

Sample/ concentration (%)	n	[a]k (Pa·s^n)[b]	r^2
HBOS			
10	0.999 (±0.037)	1.384e-3 (±3.726e-4)	0.995
20	1.179 (±0.014)	6.657e-4 (±6.919e-5)	0.999
30	1.099 (±0.007142)	2.134e-3 (±1.112e-4)	0.999
IMO			
10	0.950 (±0.033)	1.976e-3 (±4.668e-4)	0.993
20	0.955 (±0.018)	2.942e-3 (±3.738e-4)	0.998
30	0.970 (±0.006099)	4.706e-3 (±2.085e-4)	0.999
Maltodextrin			
10	1.137 (±0.024)	4.708e-4 (±8.343e-5)	0.997
20	1.018 (±0.027)	2.603e-3 (±5.152e-4)	0.996
30	0.988 (±0.005929)	6.576e-3 (±2.835e-4)	0.999
Sucrose			
10	1.492 (±0.047)	2.098e-5 (±7.270e-6)	0.995
20	1.028 (±0.031)	1.722e-3 (±3.881e-4)	0.995
30	1.306 (±0.020)	2.934e-4 (±4.404e-5)	0.999

[a]The flow index.
[b]The consistency coefficient.

Gelatinization of starch

The effects of HBOS and other oligosaccharides on gelatinization of starch were followed (Table 3). Corn starch powder containing oligosaccharides showed endotherms characterized as the gelatinization phase transition from DSC thermograms (data not shown). The onset temperature (T_o) at which the DSC curve began to deviate from the base line was considered as the gelatinization temperature. The gelatinization temperatures of starch shifted ($p<0.05$) to higher temperatures in the presence of oligosaccharides (Table 3), and the extent of shift increased with increasing concentration ($p<0.05$). There are several possible reasons for the increase in T_o on addition of sugars: (1) Sugar limits the availability of water in the starch solutions (Hoseney et al., 1977). (2) Stereoconformation due to the α-1,6 linkage of glucose units stabilizes the amorphous region of starch (Spies and Hoseney, 1982). Maltose and gentiobiose (maltose, α-1,4; gentiobiose, β-1,6) were shown to exhibit different gelatinization-delaying characteristics. Sugars were hypothesized to increase the gelatinization temperature through a combination of two independent mechanisms. When sugar is added to a starch-water system, it lowers the water activity of the solution, and then interacts with starch chains to stabilize the amorphous regions of the starch granule. (3) Slade and Levine (1991) explained the re-

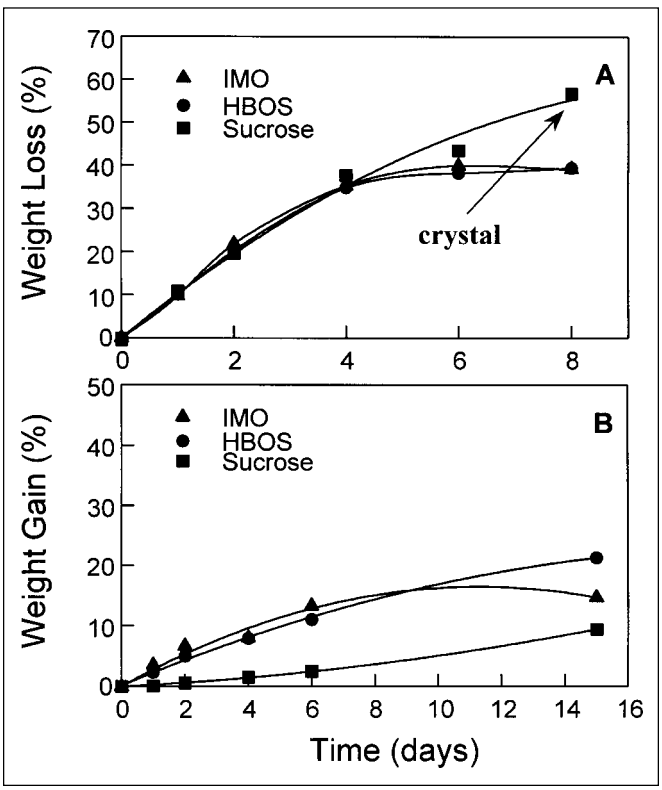

Fig. 2—Moisture retention and absorption properties of HBOS, IMO, and sucrose at 25°C. (A) moisture retention of 50% syrups at 32% relative humidity; (B) moisture absorption of lyophilized powders at 90% relative humidity.

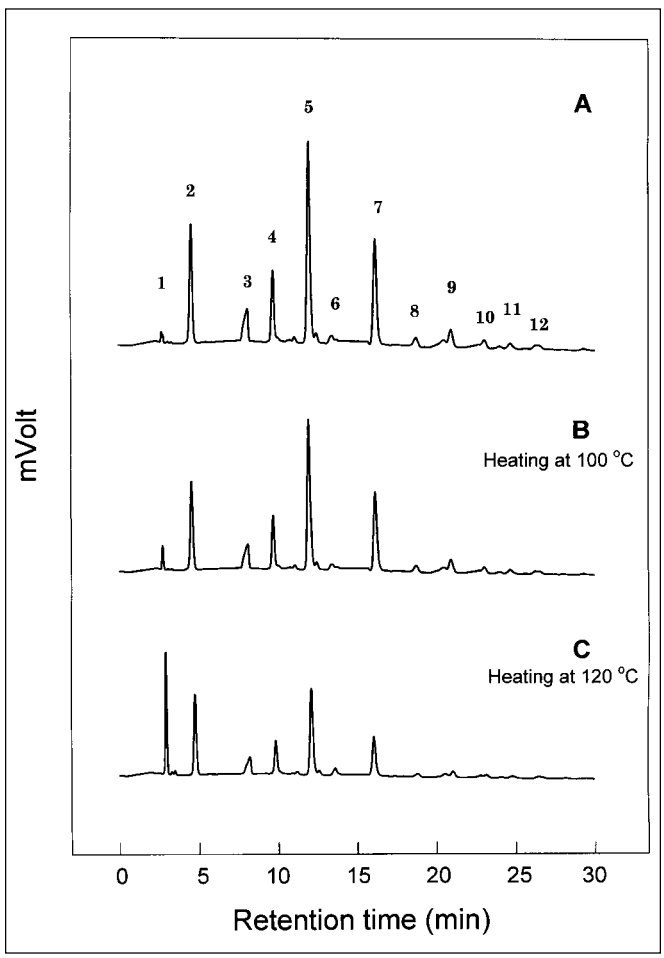

Fig. 3—Effect of heating 30 min at pH 3 on the sugar composition of HBOS. Sugar composition was analyzed by HPIC after no heating (A), heating at 100°C (B), or at 120°C (C). 1–glucose; 2–isomaltose; 3–maltose; 4–isopanose; 5–panose; 6–maltotriose; 7–branched maltotetraose (6²-*O*-α-maltosyl maltose); 8–maltotetraose; 9–branched pentaose (6²-*O*-α-maltosyl maltotriose and 6³-*O*-α-maltotriosyl maltose; 10–pentaose; 11–branched hexaose; and 12–hexaose.

Table 3—Gelatinization characteristics of starch in the presence of HBOS, IMO, maltodextrin, or sucrose from DSC thermograms

Samples	T_o (°C)[a,c]	T_p(°C)[b,c]	H (J/g)[c]
Starch (control)	61.2 (±0.08291)	66.7 (±0.08660)	14.1 (±0.3419)
Starch + HBOS 10%	65.3 (±0.6982)	70.8 (±0.7950)	13.1 (±0.4968)
Starch + HBOS 20%	71.0 (±0.3999)	76.8 (±0.1700)	14.1 (±0.1886)
Starch + IMO 10%	64.9 (±0.5166)	70.2 (±0.5025)	13.8 (±0.3897)
Starch + IMO 20%	69.0 (±0.3700)	74.6 (±0.2915)	13.2 (±0.1225)
Starch + maltodextrin 10%	63.9 (±0.5895)	69.5 (±0.6457)	13.1 ±1.082)
Starch + maltodextrin 20%	67.3 (±0.6139)	73.6 (±0.5362)	13.1 (±0.9960)
Starch + sucrose 10%	64.4 (±0.2160)	70.0 (±0.2867)	12.0 (±0.2055)
Starch + sucrose 20%	69.9 (±0.1920)	75.4 (±0.1785)	13.5 (±0.6379)

[a]The onset temperature of gelatinization.
[b]The peak temperature of gelatinization.
[c]Mean values of three replicates

tardation effect of sugar solutions as a result from antiplasticization by sugar-water cosolvant, relative to the extent of plasticization by water alone.

Bello-Perez and Paredes-Lopez (1995) reported that addition of sucrose or glucose in normal corn starches decreased the gelatinization enthalpy (ΔH). However, there were no differences among samples ($p>0.05$) in that study. This implies that oligosaccharides delayed gelatinization by interacting with starch chains without affecting the melting enthalpies.

Glass transition temperature of maximally freeze concentrated solutions (T_g')

Glass transition is the phase transition of polymers, including many food materials from a glassy state to a rubbery state, and this occurs at a specific temperature, T_g. The main consequence of glass transition is an increase of molecular mobility and free volume above T_g, which may result in physicochemical deteriorative changes (Slade and Levine, 1991). The T_g' and T_m' of oligosaccharides at maximum freeze concentration were compared (Table 4). Isothermal holding (annealing) was necessary to obtain maximum freeze-concentration and to get accurate T_g' and T_m' values. Results for sucrose and maltodextrin agreed in general with values reported by Roos and Karel (1991a, b). Results showed that T_g' and T_m' of the samples increased as DE values of oligosaccharides decreased (that is, as average molecular weight of the molecules increased). These results also confirmed the findings by Levine and Slade (1988) that low molecular weight molecules decreased the T_g' values of sugar mixtures more than high molecular weight molecules. The relation between glass transition temperature and solute is in general a colligative property. However, T_g' also depends on the molecular configuration of the solute molecules as influenced by the nature of the glycosidic linkages. Comparisons of the T_g' between linear and branched glucose oligomers have supported that 1→4 linked (linear amylose-like) glucose oligomers manifest larger hydrodynamic volumes than oligomers containing 1→6 (branched amylopectin-like) links (Slade and Levine, 1991). This has suggested that the compositional effects of the sugar mixtures may be due to the principal sugar (Slade and Levine, 1991, Roos, 1995). T_g' values of IMO (Table 4) indicated that the major components, glucose and maltose, were responsible for the physical state of IMO solution. T_g' and T_m' values are important for cryostabilization of frozen foods because they control the diffusion of water within a frozen food to maintain integrity of the product. Levine and Slade (1986) proposed that the stability of frozen foods was controlled by the temperature difference ($\Delta T = T_f - T_g'$) between the freezer temperature (T_f) and the T_g' of the specific solutes. T_g' of HBOS was about 10°C higher than that of sucrose. Therefore, HBOS show good potential as a cryostabilizer.

Table 4—Glass transition temperature (T_g'), ice melting temperature (T_m') of maximally freeze-concentrated HBOS, IMO, maltodextrin, and sucrose

Sugars	T_g' (°C)[a]			T_m' [a]
	Onset	Midpoint	End-point	
HBOS	−37.1 (±0.2046)	−35.6 (±0.3766)	−34.7 (±0.0707)	−24.1 (±0.6722)
IMO	−59.8 (±0.1414)	−55.9 (±0.2624)	−53.9 (±0.2160)	−42.5 (±0.1247)
Maltodextrin	−22.5 (±0.4272)	−21.6 (±0.2121)	−20.7 (±0.1225)	−14.5 (±0.2598)
Sucrose[b]	−46	−41	−36	−34

[a]Mean values of three replicates.
[b]Data from Roos and Karel (1991).

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