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ARTICLE

Investigation of Thermal Decomposition as the Kinetic Process That Causes the Loss of Crystalline Structure in Sucrose Using a Chemical Analysis Approach (Part II)

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High performance liquid chromatography (HPLC) on a calcium form cation exchange column with refractive index and photodiode arrary detection was used to investigate thermal decomposition as the cause of the loss of crystalline structure in sucrose. Crystalline sucrose structure was removed using a standard differential scanning calorimetry (SDSC) method (fast heating method) and a quasi-isothermal modulated differential scanning calorimetry (MDSC) method (slow heating method). In the fast heating method, initial decomposition components, glucose (0.365%) and 5-HMF (0.003%), were found in the sucrose sample coincident with the onset temperature of the first endothermic peak. In the slow heating method, glucose (0.411%) and 5-HMF (0.003%) were found in the sucrose sample coincident with the holding time (50 min) at which the reversing heat capacity began to increase. In both methods, even before the crystalline structure in sucrose was completely removed, unidentified thermal decomposition components were formed. These results prove not only that the loss of crystalline structure in sucrose is caused by thermal decomposition, but also that it is achieved via a time−temperature combination process. This knowledge is important for quality assurance purposes and for developing new sugar based food and pharmaceutical products. In addition, this research provides new insights into the caramelization process, showing that caramelization can occur under low temperature (significantly below the literature reported melting temperature), albeit longer time, conditions.

KEYWORDS: Apparent melting; thermal decomposition; caramelization; sucrose; glucose; fructose; 5-HMF; HPLC

INTRODUCTION

Thermodynamic melting of a crystalline material occurs at a single, heating rate independent temperature, where the crystalline solid and corresponding liquid phases are in thermodynamic equilibrium, ($\Delta G = 0$) at a constant pressure (*1*), with no change in chemical composition. However, as detailed in Table 1 in Part I (*2*) (10.1021/jf1042344), a number of investigators have reported a large variation in the melting parameters (the onset melting temperature, $T_{m\,onset}$; the peak melting temperature, $T_{m\,peak}$; and

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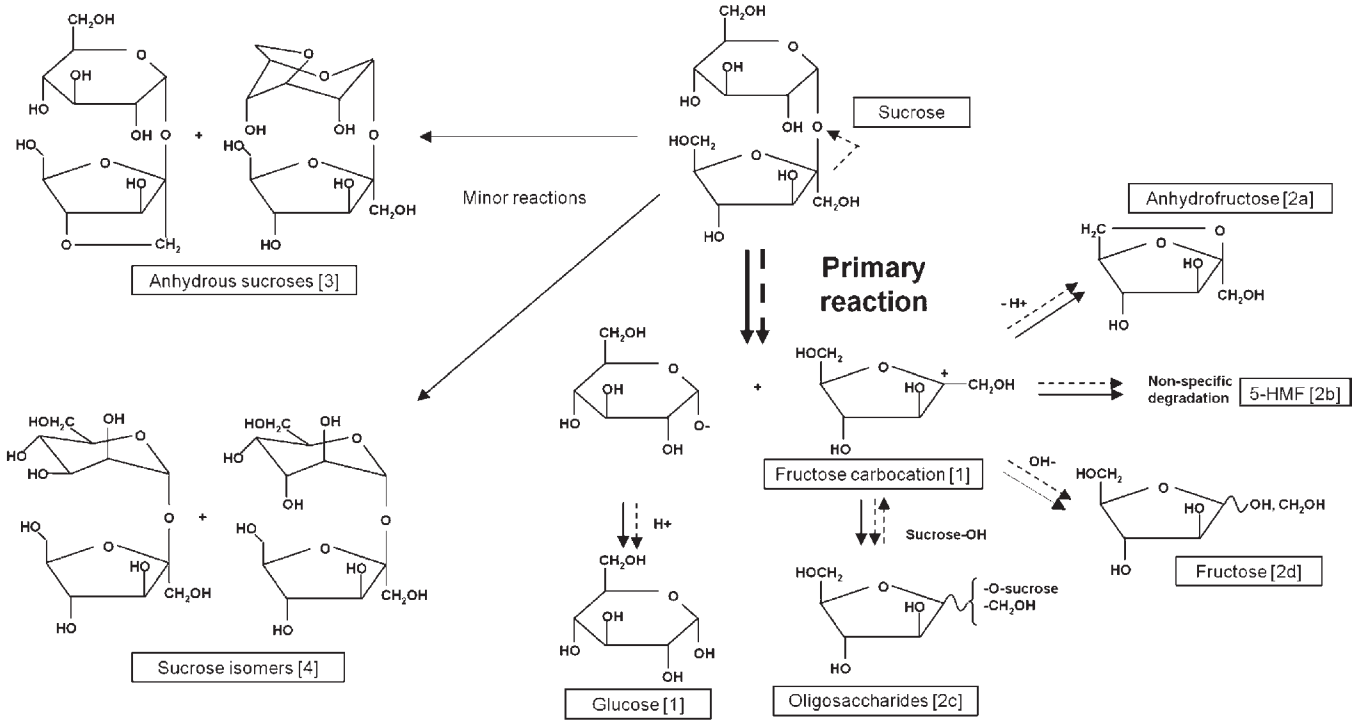


Figure 1. The predominant mechanism of sucrose thermal decomposition in the presence (dashed arrow) and in the absence (solid arrow) of an aqueous solution (synthesized from refs *19*, *22*, *27*, *28*). Numbers identify the different stages and products formed during the decomposition reaction and are referred to in the text.

the enthalpy of melting, ΔH) for sucrose, glucose, and fructose. A critical observation regarding these literature reported melting parameters, which was confirmed using standard differential scanning calorimetry (SDSC) in Part I (*2*) (10.1021/jf1042344), is that they all increase with increasing heating rate. This heating rate dependency led to the hypothesis that a kinetic process was responsible for the loss of crystalline structure (to minimize confusion, the term "loss of crystalline structure" is used instead of melting) in sucrose, glucose, and fructose. In addition, stepwise quasi-isotherm modulated DSC (MDSC) and thermogravimetric analysis (TGA) results suggested that the most plausible kinetic process was thermal decomposition, not as an additional process accompanying thermodynamic melting, but as the kinetic process responsible for the loss of crystalline structure. A number of publications have suggested that thermal decomposition accompanies (along with or right after) sugar melting in sucrose, glucose, and fructose (*3*−*10*). The critical difference between our conclusion regarding the role of thermal decomposition in sugar melting and others is that we propose that thermal decomposition is the kinetic process responsible for the loss of crystalline structure in sucrose, glucose, and fructose; not that thermal decomposition occurs in addition to thermodynamic melting. Additional details are given in Part I (*2*) (10.1021/jf1042344).

However, these thermal analysis experiments alone were not sufficient to unambiguously confirm the specific type of kinetic process. Thus, to identify the specific type of kinetic process responsible for the loss of crystalline structure, a chemical analysis approach, specifically high performance liquid chromatography (HPLC), was proposed. HPLC was selected since it is known to be a successful technique for separating, identifying, and quantifying sugars and their thermal decomposition components (*11*−*16*). Since the presence of tautomers in the case of glucose and fructose would complicate the HPLC analysis, sucrose was chosen for further study because it does not form tautomers and it is also the most commonly used sugar in the food and pharmaceutical industries.

We hypothesize that, if the loss of crystalline structure in sucrose is caused by thermal decomposition, initial decomposition components, specifically glucose, fructose, and 5-HMF, will be formed and detected by HPLC analysis, at the same temperature (in the SDCS method) or time (in the quasi-isothermal MDSC method) at which apparent melting begins. The term "apparent melting" was proposed in Part I (*2*) (10.1021/jf1042344) to distinguish the loss of crystalline structure due to a kinetic process, such as thermal decomposition, from thermodynamic melting.

Sucrose Thermal Decomposition. Numerous publications have investigated the thermal decomposition of sucrose both in the presence and in the absence of an aqueous solution (*17*−*28*). A schematic overview of the thermal decomposition of sucrose under both conditions is shown in **Figure 1**. In the presence of an aqueous solution, the first step in sucrose thermal decomposition is the splitting of the glycosidic linkage between the glucose and fructose moieties (i.e., sucrose hydrolysis), via protonation of the oxygen atom of the glycosidic linkage. The hydrogen ion (H^+) required for this step is provided by water. In the absence of an aqueous solution, the mechanism and decomposition components of sucrose thermal decomposition are somewhat different. However, Šimkovic et al. (*27*), studying anhydrous sucrose using GC/MS, showed that the splitting of the glycosidic linkage was still the most prominent primary reaction of sucrose thermal decomposition. It was mentioned by Quintas et al. (*28*), based on the work of Richards (*22*) and Lowary and Richards (*23*), that the H^+ required for sucrose hydrolysis could be derived from the dissociation of the sucrose molecule itself at high temperatures. The H^+ required for sucrose hydrolysis also could be derived from acidic products formed via reactions of trace amounts of reducing sugars (i.e., glucose and fructose) and from the effect of salts. Reducing sugars undergo thermal decomposition much faster than sucrose; the acidic products (e.g., acetic, formic, levulinic acids) formed from this source catalyze the sucrose degradation by further protonation of sucrose (termed an "acid-autocatalyzed process") and thus enhance the reaction

Table 1. T_m Parameters ($T_{m\,onset}$, $T_{m\,peak}$, and ΔH) for Sucrose and Mannitol Scanned at a Heating Rate of 10 °C/min up to a Final Temperature of 210.0 °C for Sucrose and 190.0 °C for Mannitol Using SDSC ($N = 6$ and $n = 3$)[a]

	small endothermic peak (first)			large endothermic peak (second)			total
	$T_{m\,onset}$ (°C)	$T_{m\,peak}$ (°C)	enthalpy[b] (ΔH, J/g)	$T_{m\,onset}$ (°C)	$T_{m\,peak}$ (°C)	enthalpy[b] (ΔH, J/g)	enthalpy (ΔH, J/g)
sucrose	150.54 ± 0.34	157.34 ± 0.42	7.03 ± 1.23	185.59 ± 0.72	190.63 ± 0.27	130.20 ± 1.85	137.23 ± 2.37
mannitol				165.43 ± 0.03	166.96 ± 0.55	302.60 ± 3.03	

[a] The average values in the table were obtained by measuring samples in six replicates and by analyzing the T_m parameters for each sample measurement in triplicate. [b] Sigmoidal tangent baseline was used for crystalline sucrose and mannitol using the UA software.

rate of sucrose thermal decomposition by decreasing the pH. Reducing sugars and salts are present as impurities in all grades of sucrose and have been shown to accelerate the initial thermal decomposition of sucrose (*20*, *22*, *26*, *29*). In addition, we hypothesize that another possible source of the H^+ is surface water on the sucrose crystals. Thus, regardless of the presence or absence of an aqueous solution, sucrose thermal decomposition primarily occurs via sucrose hydrolysis as shown in **Figure 1**.

Once sucrose is broken down into glucose [1] and fructose carbocation [1] via sucrose hydrolysis, glucose forms acidic and other decomposition components through further reactions (not shown in **Figure 1**). Fructose carbocation, due to its instability, immediately participates in subsequent, more complex reactions, resulting in the formation of various decomposition components, including anhydrofructose [2a] by cyclization; a wide range of products, such as 5-(hydroxymethyl)furfural (5-HMF) [2b], by nonspecific degradation (e.g., condensation); oligosaccharides (kestoses) [2c] by combining with the hydroxyl oxygen of another saccharide (mostly sucrose); and fructose [2d] by accepting a hydroxyl ion (OH^-) from water. These intermediate products are produced through similar mechanisms in the presence and absence of an aqueous solution. However, in the absence of an aqueous solution, minor products such as anhydrous sucroses [3] and sucrose isomers [4] are also produced through minor reaction pathways.

Though sucrose thermal decomposition continues through a myriad of reaction pathways, in the present study it is important to measure only the initial decomposition components, such as glucose, fructose, and 5-HMF, formed in the early stages of sucrose thermal decomposition, because this study aims to elucidate whether the loss of crystalline structure in sucrose is caused by thermal decomposition, not to detect and quantify all possible sucrose decomposition components. Thus, the specific objective of the present study was to determine if glucose, fructose, and 5-HMF, the three selected thermal decomposition indicator components, are formed at the onset of the loss of crystalline structure in sucrose using HPLC analysis. Sucrose samples for HPLC analysis were prepared using both a SDSC method (fast heating method) and a quasi-isothermal MDSC method (slow heating method).

MATERIALS AND METHODS

Materials. Crystalline sucrose (≥99.5%), D-(−)-fructose (≥99.5%), D-(+)-glucose (99.5%), mannitol (≥99.9%), and 5-(hydroxymethly)furfural (5-HMF,≥99%) were purchased from Sigma-Aldrich Co. (St. Louis, MO) and were used without further purification. HPLC grade water (Ricca Chemical Company, Arlington, TX) was used for the preparation of standard and sample solutions. The water contents (% wet basis, wb) of the three sugars and mannitol were measured by coulometric Karl Fischer titration with Hydranal Coulomat AG as a solvent and were 0.004% wb for sucrose, 0.048% wb for glucose, 0.033% wb for fructose, and 0.060% wb for mannitol. Material information, including trace anions and cations, for sucrose and mannitol obtained from the Sigma-Aldrich Co. and analyzed by the authors of this study is available as Supporting Information.

Table 2. Target and Sample Temperatures and Corrsponding SDSC Heat Flow Signal Attributes[a] Used in the Preparation of Sucrose and Mannitol SDSC Samples

temp (°C)		
target	sample[b]	corresponding SDSC heat flow signal attributes
		Sucrose
100.0	98.7 ± 0.1	(1) before the appearance of any endothermic peaks
151.0	149.6 ± 0.1	(2) the onset of the first endothermic peak
160.0	158.6 ± 0.1	(3) at the end of the first endothermic peak
186.0	184.1 ± 0.1	(4) the onset of the second endothermic peak
195.0	192.7 ± 0.2	(5) the end of the second endothermic peak
210.0	208.4 ± 0.2	(6) 15 °C above the completion of the second endothermic peak
		Mannitol
158.0	156.7 ± 0.03	(1) before the appearance of an endothermic peak
165.0	163.5 ± 0.02	(2) the onset of the endothermic peak
174.0	172.6 ± 0.03	(3) right after the peak temperature
182.0	180.6 ± 0.02	(4) at the end of the endothermic peak
190.0	188.6 ± 0.03	(5) 8 °C above the completion of the endothermic peak

[a] Observable in **Figures 2** and **4**, respectively. [b] In sample preparation using DSC, the sample temperatures are always slightly lower (approximately 1.6 °C) than the target temperatures, since the sample temperature lags behind the furnace temperature.

Sample Preparation. Sucrose, as an apparent melting material, and mannitol, as a thermodynamic melting comparison material, were individually heated using two methods: a SDSC method and a quasi-isothermal MDSC method. A DSC Q2000 (TA Instruments, New Castle, DE), equipped with a refrigerated cooling system (RCS 90), was utilized for both methods.

***SDSC Method* (*Fast Heating Method*).** Prior to sample preparation, enthalpy (cell constant) and temperature calibrations were done using indium ($T_{m\,onset}$ of 156.6 °C, ΔH of 28.71 J/g, TA Instruments, New Castle, DE) hermetically sealed in a Tzero aluminum DSC pan and lid (TA Instruments, New Castle, DE). The same type of pans and lids was used for the calibrations and sample preparations. An empty pan was used as the reference. Dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas.

Sucrose (approximately 9.75 mg) was hermetically sealed in a pan. Three sample pans of sucrose were loaded in the DSC cell; one pan was placed on the sample platform, and the others were placed on the bottom of the DSC cell. These three pans were heated at a rate of 10 °C/min to six different target temperatures (**Table 2**), corresponding to the SDSC heat flow signal attributes listed in **Table 2** and observable in **Figure 2**, and then immediately cooled at a rate of 50 °C/min to 25 °C. Because the sample temperature lags behind the furnace temperature when heating a sample using DSC, the sample temperatures in **Table 2** are always slightly lower (approximately 1.6 °C) than the target temperatures. In addition, a sample that was not heated at all was prepared and termed the "as is" sample. Images of the "as is" and SDSC samples in DSC pans were taken. Using the same procedure, three more sample pans of sucrose were prepared at each target temperature to make one batch of sucrose samples for HPLC analysis.

For each batch (a total of six sample pans), after heating in the DSC, the DSC lids were removed and each sample plus pan was weighed and then transferred into a small plastic bottle with 5 mL of HPLC water. After the

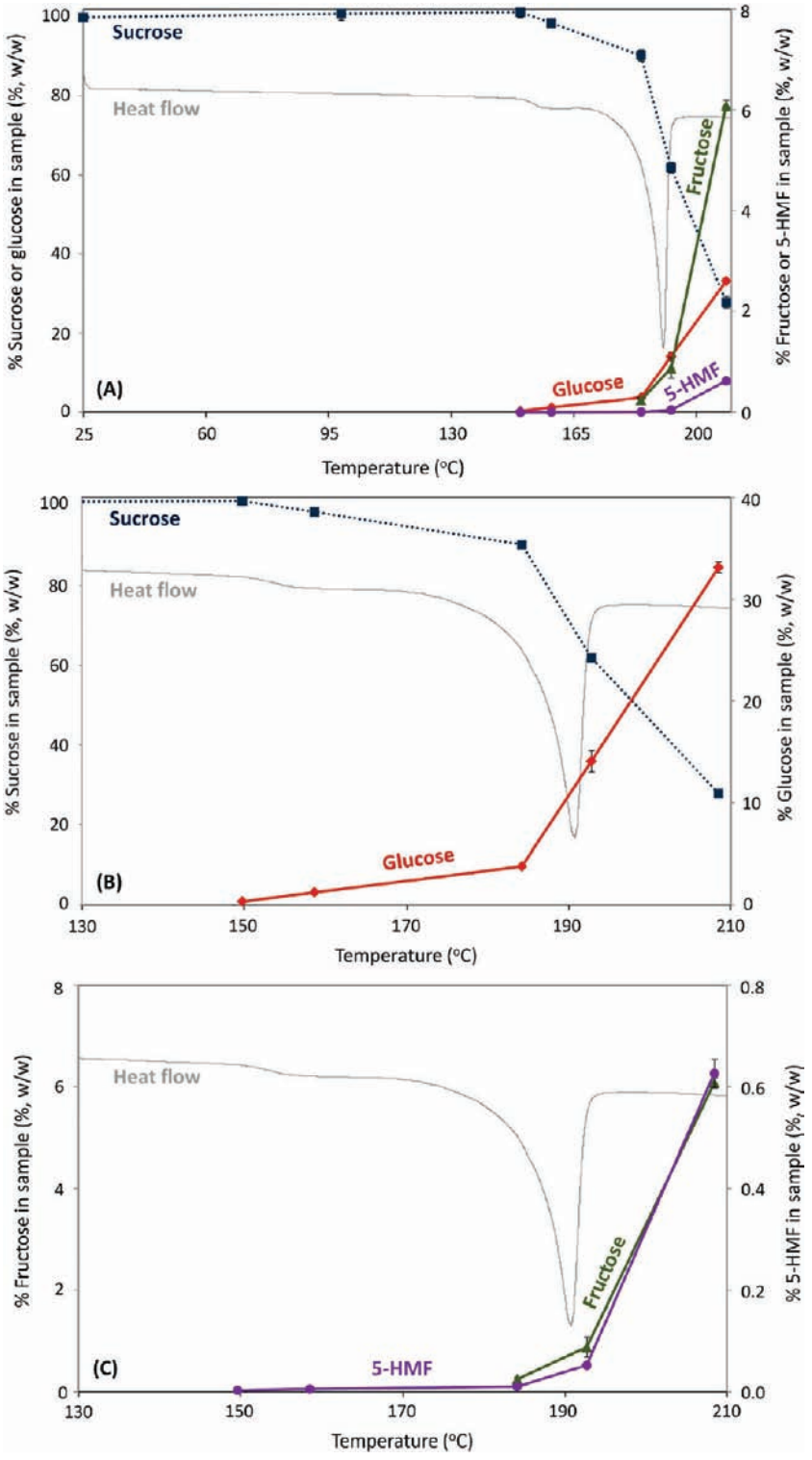


Figure 2. SDSC heat flow scan (at 10 °C/min) and HPLC results for sucrose and indicator thermal decomposition components: (**A**) full temperature scale, (**B**) high temperature region for sucrose and glucose, and (**C**) high temperature region for fructose and 5-HMF.

samples were completely dissolved in the HPLC water, they were filtered through a 0.22 μm Millex syringe filter (Millipore Corp., Temecula, CA) and kept in amber colored HPLC vials (National Scientific Company, Rockwood, TN) in a −70 °C freezer until HPLC analysis. The DSC pans were removed from the plastic bottle, dried off, and reweighed (in case the pan weight had changed due to lid removal). The difference in weight between the dried pan and the sample plus pan before dissolving was used to calculate the sucrose concentration.

Mannitol samples (approximately 9.50 mg per pan) for HPLC analysis were prepared using the same procedures as for sucrose (10 °C/min), except different target temperatures were used. Five different target temperatures (**Table 2**), corresponding to the SDSC heat flow signal attributes listed in **Table 2** and observable in **Figure 4**, were used. In addition, an "as is" mannitol sample with no heat treatment was prepared. Images of the "as is" and SDSC samples in DSC pans were taken. The SDSC mannitol samples for HPLC analysis were prepared using the same procedure as detailed for SDSC sucrose.

Average T_m parameters for sucrose and mannitol were obtained from the complete SDSC thermogram, heated to 210.0 °C for sucrose and 190.0 °C for mannitol.

***Quasi-isothermal MDSC Method* (*Slow Heating Method*).** Prior to sample preparation, DSC and MDSC heat capacity calibrations were done using a 22.93 mg sapphire disk (TA Instruments, New Castle, DE) hermetically sealed in a pan. MDSC heat capacity calibration was carried out using a modulation amplitude of ±1.0 °C and a period of 100 s, which were used for subsequent sucrose sample preparation. An empty pan was used as the reference. Dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas.

As described in the SDSC Method (Fast Heating Method) section, three sample pans of sucrose (approximately 9.75 mg per pan) were loaded in the DSC cell and isothermally held at 120.0 °C for different times (i.e., 0, 10, 50, 100, 200, 250, 300, 350, 400, 450, 500, 1200, 2000, and 3100 min) with a modulation amplitude of ±1.0 °C and a period of 100 s. The 120 °C isothermal temperature was chosen based on the result of stepwise quasi-isothermal MDSC experiments for sucrose performed in Part I (*2*) (10.1021/jf1042344), since at 120.0 °C the C_p began to gradually increase (within the 30 min time step used in the experiment), indicating the onset of the loss of crystalline structure in sucrose. The isothermal time required to remove all crystalline structure in sucrose at 120.0 °C, 3100 min, was determined by adding two standard deviations to the average isothermal time of triplicate measurements obtained via preliminary experiments (data not shown). The sucrose samples held at 120.0 °C at the different times were immediately cooled to 25 °C at a rate of 50 °C/min. Images of the "as is" and quasi-isothermal MDSC sucrose samples in DSC pans were taken. The MDSC sucrose samples for HPLC analysis were prepared using the same procedure as detailed for SDSC sucrose samples.

Since mannitol is a thermodynamic melting material, it melts over a very narrow temperature range. Thus, different quasi-isothermal MDSC experimental conditions were required for mannitol sample preparation: a modulation amplitude of ±0.5 °C and a period of 120 s, a gas flow rate of 1 mL/min, and use of an internal lid (TA Instruments, New Castle, DE) in addition to Tzero hermetic pans and lids. An empty pan with an internal lid was used as the reference for calibration and mannitol sample preparation. Dry nitrogen, at a flow rate of 1 mL/min, was used as the purge gas.

Mannitol (approximately 9.50 mg per pan) was hermetically sealed in a pan with an internal lid. Three sample pans of mannitol were loaded in the DSC cell as described above for sucrose. These pans were isothermally held at 159.9 °C for 5555 min, using a modulation amplitude of ±0.5 °C and a period of 120 s, and were immediately cooled to 25 °C at a rate of 50 °C/min. The isothermal temperature, 159.9 °C, was also selected based on the result of stepwise quasi-isothermal MDSC experiments for mannitol performed in Part I (*2*) (10.1021/jf1042344). The isothermal time required to remove all crystalline structure in mannitol at 159.9 °C, 5555 min, was determined by adding two standard deviations to the average isothermal time of triplicate measurements obtained through preliminary experiments (data not shown). Images of the "as is" and quasi-isothermal MDSC mannitol samples in DSC pans were taken. The MDSC mannitol samples for HPLC analysis were prepared using the same procedure as detailed for SDSC sucrose samples.

HPLC Analysis. Chromatographic analyses were conducted using a Waters 2695 Alliance HPLC system (Waters, Milford, MA), equipped with a Hewlett-Packard interface 35900E A/A converter. The analytical column was an Aminex HPX-87C calcium form cation exchange resin-based column (300 × 7.8 mm) packed with sulfonated divinyl benzene-styrene copolymer with a particle size of 9 μm (Bio-Rad Lab., Richmond, CA). The guard column was a Carbo-C Refill cartridge (30 × 4.6 mm) (Bio-Rad Lab., Richmond, CA). HPLC grade water (Ricca Chemical Company, Arlington, TX) was used for the mobile phase. The analytical column temperature was maintained at 85 °C and the guard column at 30 °C. The flow rate was set to 0.6 mL/min. All samples were injected into the HPLC system using a 20 μL loop injector.

A Waters 410 refractive index (RI) detector (Waters, Milford, MA) was connected to a Hewlett-Packard series 1050 photodiode array (PDA) detector (Hewlett-Packard, Palo Alto, CA) for the sucrose samples. While sucrose, glucose, and fructose were determined using the RI detector, 5-HMF was simultaneously measured using the PDA detector at a wavelength of 284 nm. The same RI detector was used for the mannitol samples. Chromatographic peaks were identified by comparing retention times and spectra to those of known standard solutions. A mixed standard solution, containing sucrose, glucose, fructose, and 5-HMF, was used for HPLC analysis of all sucrose samples. A mannitol standard solution was used for the HPLC analysis of all mannitol samples. All computations were performed using an Agilent ChemStation (ChemStation for LC 3D Rev A. 08. 03, Agilent Technologies, Inc., Santa Clara, CA). HPLC analysis was done in duplicate for each batch of sucrose and mannitol samples.

Sucrose and mannitol results were displayed as the average % ratio of sucrose remaining (grams per liter, g/L) to the total sample concentration (g/L). Glucose, fructose, and 5-HMF results were displayed as the average % ratio of the decomposition component concentration (g/L) formed during the loss of crystalline structure to the total sample concentration (g/L). The limit of quantification (LOQ) of the HPLC analysis was 0.044 g/L for glucose, 0.010 g/L for fructose, and 0.001 g/L for 5-HMF.

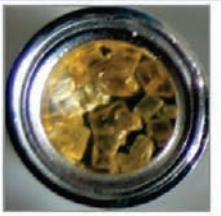
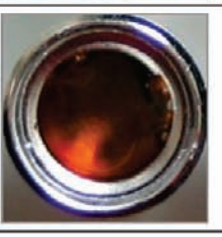
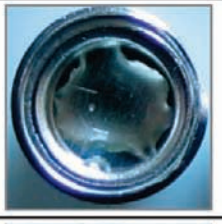
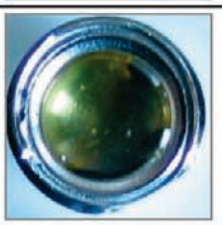
RESULTS AND DISCUSSION

SDSC Method (Fast Heating Method). Figure 2 shows the SDSC heat flow signal for sucrose, the percent glucose, fructose, and 5-HMF (the three selected thermal decomposition indicator components) formed in the sample, and the percent sucrose remaining in the sample during the loss of crystalline structure when heating sucrose at 10 °C/min. As observed in the heat flow signal of **Figure 2**, sucrose exhibited two endothermic peaks during the loss of crystalline structure. The number of endothermic peaks obtained for sucrose varies in the literature from one to three, with one peak often being obtained when analyzing commercial sources (e.g., Domino, C&H, and United Sugar), two peaks for analytical grade sucrose (e.g., Sigma and Fischer), and three peaks for laboratory-recrystallized sucrose (e.g., using methanol or ethanol). The difference in the number of peaks between different sucrose sources is currently under study in our laboratory. **Table 1** contains the corresponding sucrose melting parameters for both the small and large endothermic peaks. These T_m parameters are very similar to those reported in Table 3 in Part I (*2*) (10.1021/jf1042344), for smaller sample sizes (2.75 mg for sucrose and 2.00 mg for mannitol) compared to the larger sample sizes (9.75 mg for sucrose and 9.5 mg for mannitol) needed herein for HPLC analysis. In addition, images of the "as is" and heated sucrose samples in DSC pans are shown in **Table 3**.

As can be observed in **Figure 2**, no thermal decomposition indicator compounds were detected via HPLC analysis for either the "as is" or the 98.7 °C (target temperature 100 °C) samples. However, concomitant with the $T_{m\,onset}$ of the first endothermic peak (sample temperature 149.6 °C ± 0.1 °C; target temperature 151.0 °C), a small amount of glucose (0.365%) and 5-HMF (0.003%) were detected in the sample via HPLC analysis. However, no obvious changes in crystal appearance were noted until the 158.6 °C sample temperature (target temperature 160.0 °C), where very slight yellowing was observed (**Table 3**). The amount of glucose and 5-HMF increased gradually, until the $T_{m\,onset}$ of the second endothermic peak (sample temperature 184.1 ± 0.1 °C; target temperature 186.0 °C), where the amount of these indicator compounds began to increase more substantially and the change in crystal appearance and color became more obvious (**Table 3**).

Also at $T_{m\,onset}$ of the second endothermic peak (sample temperature 184.1 ± 0.1 °C; target temperature 186.0 °C), fructose (0.218%) was first detected (**Figure 2**). The later detection of fructose compared to glucose and 5-HMF was discussed by Mauch (*19*) and attributed to the lack of free water, which is required to form fructose from fructose carbocation, until it is produced by further decomposition reactions, such as condensation reactions. The minute quantity of surface water may also be a source of free water for fructose formation, but, more than likely, it is used primarily for the initial sucrose hydrolysis reaction. In addition, if surface water were available for fructose

Table 3. Images of "As Is" and Thermally Treated Sucrose Samples

SDSC sucrose samples			Quasi-isothermal MDSC sucrose samples		
sample temp. (°C)	target temp. (°C)	images	holding time (min)	target temp. (°C)	images
"as is"			"as is"		
98.7	100.0		500	120.0	
149.6	151.0		1200	120.0	
158.6	160.0		2000	120.0	
184.1	186.0		3100	120.0	
192.7	195.0				
208.4	210.0				

formation, the fructose would probably have been detected earlier. In addition to the formation of these indicator components, unidentified HPLC peaks began to be observed in the sucrose chromatogram (**Figure 3**) at a sample temperature of 158.6 °C (target temperature 160.0 °C). These unidentified peaks indicate the formation of diverse decomposition components, most likely produced from fructose carbocation via further reactions, as shown in **Figure 1**. At a sample temperature of 184.1 °C (target temperature 186.0 °C) and beyond, the amount and diversity of these additional decomposition components

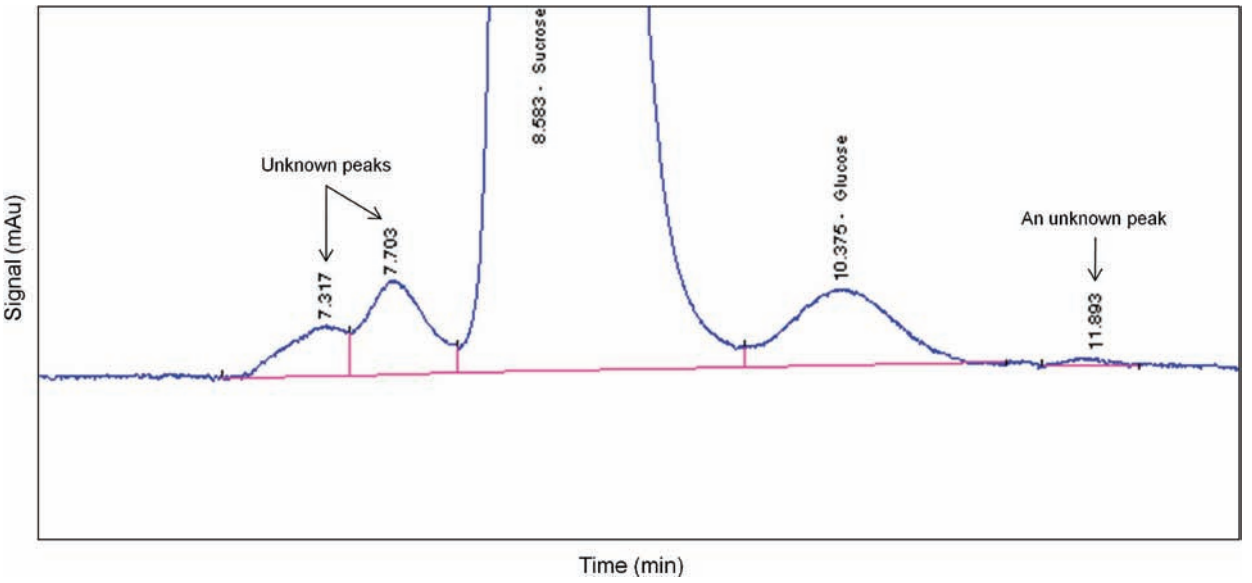


Figure 3. A sample chromatogram (refractive index detector) showing three unidentified HPLC peaks, in addition to sucrose and glucose, at a sample temperature of 158.6 °C (target temperature 160.0 °C).

increased as evidenced by an increase in the number and size of the unidentified HPLC peaks and the corresponding large decrease in % sucrose (**Figure 2**). It is interesting to note that, despite the considerable decrease in sucrose content at sample temperatures of 192.7 °C (target temperature 195.0 °C) (61.87%) and 208.4 °C (target temperature 210.0 °C) (27.82%), the samples were only light and medium yellow in color, respectively (**Table 3**).

These results indicate that thermal decomposition causes the loss of crystalline structure in sucrose, and the observed endothermic peaks measured by SDSC are due to the energy of amorphization (the energy difference between the crystalline and amorphous phases, at a specific temperature) plus the energy associated with thermal decomposition. As proposed in Part I (*2*) (10.1021/jf1042344), the loss of crystalline structure caused by a kinetic process (thermal decomposition herein) is termed "apparent melting" in order to distinguish it from thermodynamic melting.

Unlike sucrose, no decomposition components were observed during the loss of crystalline structure in mannitol by heating at 10 °C/min using the SDSC method (**Figure 4**). Although the mannitol used was of very high purity (99.9%), an unknown peak in the HPLC chromatographs ("as is" and all heat treated samples) was observed at a retention time of 22 min (data not shown), which was identified as sorbitol. The percent sorbitol peak area, based on the total area, was the same for all samples (0.95 ± 0.01), including the "as is" mannitol sample. Hence, sorbitol was found to be a minor impurity in the mannitol sample but, in the case of SDSC, was not a thermal decomposition component.

Even at a sample temperature of 188.6 °C (target temperature 190.0 °C), which is much higher than the temperature at which crystalline structure is completely removed in mannitol, no decomposition components were detected. This result shows that the loss of crystalline structure in mannitol is accomplished via thermodynamic melting. Furthermore, it confirms that, as suggested in Part I (*2*) (10.1021/jf1042344), the very small weight loss for mannitol observed in the proximity of its melting temperature is due to sublimation, not thermal decomposition. No color changes (remained white) were observed for any of the SDSC mannitol samples (**Table 4**). Only the "as is" and 188.6 °C (target temperature 190.0 °C) mannitol images are shown in **Table 4** since the 188.6 °C sample was representative of all SDSC thermally treated mannitol samples. All SDSC mannitol samples were in the crystalline phase, because, as documented in the literature (*30*, *31*), molten mannitol tends to recrystallize during cooling regardless of the preparation methods (e.g., melt-quenching, freeze-drying, and partially or completely melted).

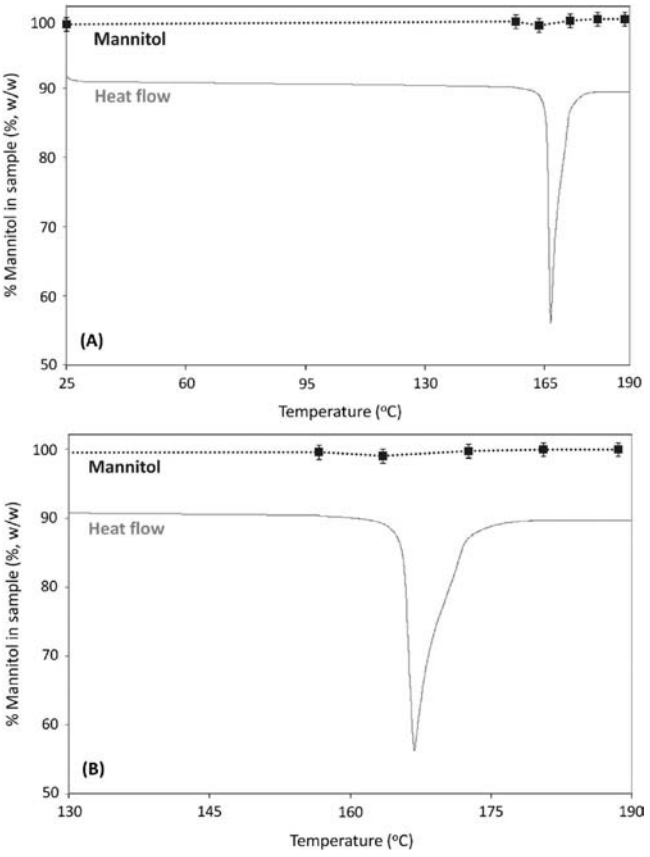


Figure 4. SDSC heat flow scan (at 10 °C/min) and HPLC results for mannitol: (**A**) full temperature scale and (**B**) high temperature region.

Table 4. Images of "As Is" and Thermally Treated Mannitol Samples

SDSC mannitol samples			Quasi-isothermal MDSC mannitol samples		
sample temp. (°C)	target temp. (°C)	images	holding time (min)	target temp. (°C)	images
"as is"			"as is"		
188.6	190.0		5555	159.9	

Quasi-isothermal MDSC Method (Slow Heating Method). **Figure 5** shows the C_p signal for sucrose, the percent glucose, fructose, and 5-HMF (the three selected thermal decomposition indicator components) formed in the sample, and the percent sucrose remaining in the sample during the loss of crystalline structure when holding the sucrose at 120.0 °C using the quasi-isothermal MDSC method. A holding time of 3100 min was required to completely remove the crystalline structure in sucrose at 120.0 °C.

As can be observed in **Figure 5**, no thermal decomposition indicator compounds were detected via HPLC analysis for either the "as is" or the 10 min samples. However, at a holding time of 50 min, where the C_p began to increase (indicating the onset of the loss of crystalline structure), small amounts of glucose (0.411%) and 5-HMF (0.003%) were detected. These results confirm that the loss of crystalline structures in sucrose is due to thermal decomposition. Since thermal decomposition is a kinetic process, the loss of crystalline structure in sucrose can take place even at 120.0 °C, which is far below the reported melting temperature for sucrose (approximately 169 to 192 °C as shown in Table 1 in Part I (*2*) (10.1021/jf1042344)), if the holding time is sufficiently long.

Others have mentioned the possibility of sucrose decomposition at temperatures below its melting temperature. Hirschmüller (*17*) stated, "At temperatures below the melting point, the decomposition of sucrose is slow." Sakamoto et al. (*9*) reported, using HPLC, that a commercial granulated sucrose turned to a light brown color along with the formation of glucose and fructose after holding for 48 h at 100 °C. However, no complete theoretical framework was available to explain how this low temperature decomposition was possible, until the studies herein. The temperature at which sucrose decomposition begins may be related to the difference in the presence of trace amounts of water, salts, reducing sugars (i.e., glucose and fructose), and organic acids in the sucrose.

Similar to the SDSC results discussed above, fructose was not detected until a holding time of 200 min, which was later than the detection of glucose and 5-HMF (**Figure 5**). Peaks corresponding to unidentified decomposition components began to appear at a holding time of 200 min. Up to the holding time of 1200 min, the C_p increased relatively slowly. Sucrose samples at holding times of 500 and 1200 min were visually intact (i.e., retained their outer crystalline shape), but had slightly yellowed (**Table 3**). After a holding time of 1200 min, a considerable increase in C_p was observed and glucose, fructose, and 5-HMF production increased. At a holding time of 2000 min, sucrose crystals had turned a deep yellow color and had partially adhered to each other (**Table 3**). At a holding time of 3100 min, the time at which all crystalline structure was removed, a number of unknown peaks of varying size were observed in the chromatograph, the sucrose sample had turned a dark brown color, and only 24.043% sucrose content remained in the sample.

Unlike mannitol samples prepared by the SDSC method (fast heating method), mannitol samples prepared by the quasi-isothermal MDSC method (slow heating method) (**Figure 6**) exhibited a small, but measurable, decrease in % mannitol content, a 2.47% decrease after 5555 min at a holding temperature of 159.9 °C. The recrystallized mannitol turned light brown from its original white color, as shown in **Table 4**. In addition, after 5555 min at 159.9 °C, trace amounts of two unidentified decomposition components were also observed in mannitol samples. The amount of these two unidentified decomposition components was only 1.49%, calculated based on the % ratio of unidentified components peak area to total peak area. The formation of unidentified decomposition components in the mannitol samples is not surprising since it was held for 5555 min at 159.9 °C, which is only 5.5 °C lower than its $T_{\mathrm{m\,onset}}$ of 165.4 °C (**Table 1**). In comparison, in the case of the slow heating method for sucrose only 24.043% sucrose remained after being held for 3100 min at 120.0 °C, which was 30.5 °C lower than its $T_{\mathrm{m\,onset}}$ for the first endothermic peak of 150.5 °C (**Table 1**). Therefore, based on this comparison, it is very likely that the loss of crystalline structure in mannitol is not caused by thermal decomposition but rather by thermodynamic melting followed by thermal decompostion.

Comparison of SDSC Method (Fast Heating Method) and Quasi-isothermal MDSC Method (Slow Heating Method). Compared to the SDSC method (fast heating method), complete loss of crystalline structure occurred more slowly in the quasi-isothermal MDSC method (slow heating method). Since thermal decomposition is a kinetic (time-dependent) process, we hypothesize that the loss of crystalline structure in sucrose is a time−temperature combination process. That is, the loss of crystalline structure in sucrose occurs more quickly at higher temperatures (shorter times) compared to lower temperatures (longer times); however, the degree of thermal decomposition (measured as the amount of sucrose remaining as well as the amount of thermal

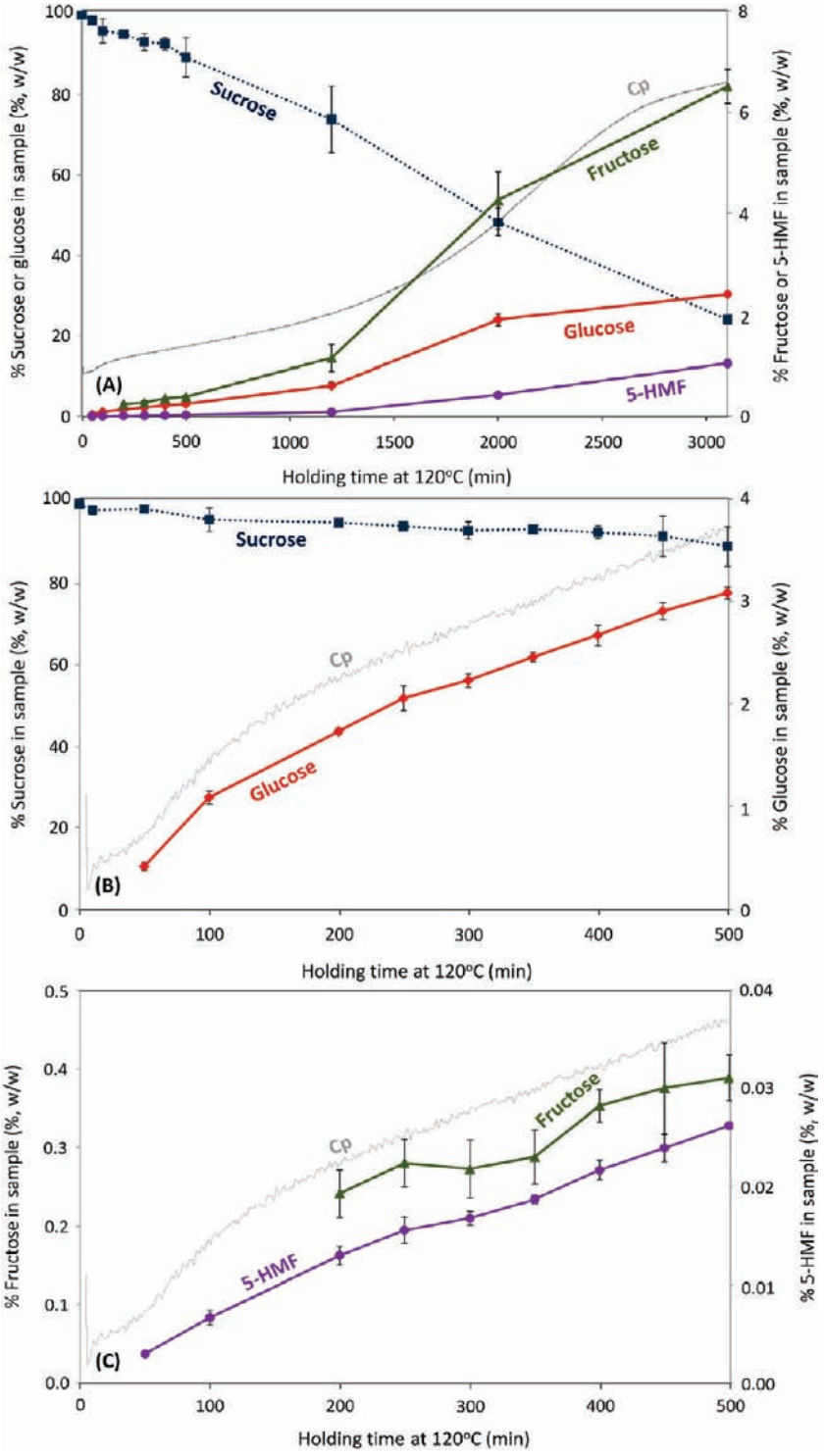


Figure 5. C_p obtained via quasi-isothermal MDSC (at 120.0 °C for 3100 min) and HPLC results for sucrose and indicator thermal decomposition components: (**A**) full time scale, (**B**) short time region for sucrose and glucose, and (**C**) short time region for fructose and 5-HMF.

decomposition components produced) is more extensive at lower temperatures. As observed in the present study, upon complete loss of crystalline structure in the SDSC method (fast heating method) (sample temperature of 192.7 °C), the sucrose content decreased to 61.871% and the glucose, fructose, and 5-HMF formed were 14.088%, 0.882%, and 0.053%, respectively, whereas, in the quasi-isothermal MDSC method (slow heating method) (120.0 °C for 3100 min), the sucrose content decreased to 24.043% and the glucose, fructose, and 5-HMF formed were 30.322%, 6.499%, and 1.043%, respectively. In addition, the amount and diversity of unidentified decomposition components were greater in the slow heating method, compared to the fast heating method.

An interesting observation is the dramatic decrease in sucrose content between the sample heated to 192.7 °C (the temperature for the complete loss of crystalline structure) and the sample heated to 208.4 °C using the SDSC method (fast heating method) (**Figure 2** and **Table 3**). During this change in temperature, which took only 1.6 min, the sucrose content decreased from 61.871% to 27.819%, nearly equal to the sucrose content (24. 043%) in the

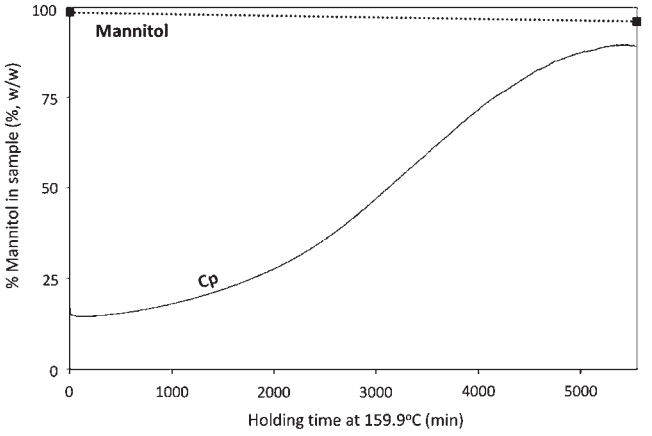


Figure 6. C_p obtained via quasi-isothermal MDSC (at 159.9 °C for 5555 min) and HPLC results for mannitol.

quasi-isothermal MDSC method (slow heating method) (3100 min, the holding time for the complete loss of crystalline structure at 120.0 °C). In addition, the amount of indicator components in the sample heated to 208.4 °C (33.167% glucose, 6.082% fructose, and 0.627% 5-HMF) also became similar to the quasi-isothermal MDSC method (slow heating method) (values given previously). Despite these component content similarities, the 208.4 °C sample was only medium yellow in color, while the 120.0 °C for 3100 min sample was deep brown in color (**Table 3**).

This dissimilarity in color was attributed to the difference in the diversity of thermal decomposition components between the two heating conditions. Based on the HPLC data, more unidentified peaks with larger peak areas, which indicate the formation of a large amount and wide variety of decomposition components, were observed in the 120.0 °C for 3100 min sample than in the 208.4 °C sample. The more diverse thermal decomposition components formed in the 120.0 °C for 3100 min sample were themselves subjected to further, more advanced stages of the thermal decomposition reaction scheme, yielding complex color producing components, whereas the 208.4 °C sample, even though it contained a similar amount of indicator components (detailed previously), had fewer and smaller unidentified peaks compared to the 120.0 °C for 3100 min sample, indicating that less advanced thermal decomposition reactions had occurred. These results further support that the slow heating method (longer heating time at a lower temperature) causes more severe thermal decomposition (measured as the amount of thermal decomposition components produced, not as the amount of sucrose remaining) than the fast heating method (faster heating rate to a higher temperature).

These results provide new insights into the caramelization process. Caramelization has generally been thought of as a complex series of reactions *occurring under higher temperature conditions* and involving degradation of sugar molecules and polymerization of the reaction intermediates and reactants (*32*, *33*). However, based on the research reported herein, since the loss of crystalline structure in sucrose occurs via a time−temperature combination process, caramelization does not have to be carried out under higher temperature conditions, but can also be carried out under lower temperature, albeit longer time, conditions. Thus, the desired degree of caramelization required for producing a specific final product can be "controlled" by selecting appropriate time−temperature conditions. Controlled is in quotes, since by its very nature, caramelization (decomposition) is a highly variable scheme of reactions, so control depends not only on time and temperature but also on other factors that affect sucrose decomposition. For example, decomposition of sucrose has been shown to be catalyzed by trace amounts of water, salts, reducing sugars (i.e., glucose and fructose), and organic acids in the sucrose (*26*, *29*). Therefore, from a practical viewpoint caramelization of sucrose can simply be defined as browning of sucrose (or other apparent melting sugar) by applying heat for a length of time. In turn, the conversion of crystalline sucrose to amorphous sucrose by applying heat for a length of time (i.e., apparent melting) can be thought of as "controlled caramelization".

These results further suggest that, since the applied heating conditions determine the amounts and types of thermal decomposition components, apparent melting of sucrose influences several important final product characteristics, such as sweet taste, flavor, color, texture, and shelf life. For example, in regard to flavor, the applied heating conditions could be optimized so as to maximize the key caramel flavor compounds. Another example, in regard to shelf life, is the stability of amorphous sucrose prepared by melt-quenching (heating followed by quick cooling), where the decomposition components formed during heating will have a significant impact on the resultant glass transition temperature, a critical factor determining the physicochemical stability of amorphous materials. It is important to point out that in the case of preparing amorphous sucrose by melt-quenching it is probably not necessary to quench cool the sample to keep the sucrose from crystallizing, since the formation of thermal decomposition components formed during melting serves to retard crystallizing. Therefore, the elucidation of the cause of the loss of crystalline structure in sucrose is very important from both a theoretical and practical viewpoint.

Conclusion. Regardless of the heating method employed, the formation of initial decomposition components was coincident with the loss of crystalline structure in sucrose. In sucrose samples prepared using the SDSC method (fast heating method), glucose and 5-HMF were observed immediately after the $T_{\mathrm{m\,onset}}$ of the first endothermic peak, at a sample temperature of 149.6 ± 0.1 °C (target temperature 151.0 °C). In sucrose samples prepared using the quasi-isothermal MDSC method (slow heating method), glucose and 5-HMF were detected at the holding time of 50 min (at 120.0 °C), where the C_p began to increase, signaling the onset of the loss of crystalline structure. In addition to glucose and 5-HMF, a variety of unidentified thermal decomposition components were continuously formed even before all crystalline structure was removed in sucrose. These results confirm our hypothesis that the loss of crystalline structure in sucrose is caused by thermal decomposition; thus, sucrose does not go through thermodynamic melting, but rather apparent melting. Further research on the kinetics of sucrose appearent melting is currently being carried out in the Schmidt laboratory.

In addition, a higher amount and more diverse thermal decomposition components were observed in the quasi-isothermal MDSC method (slow heating method), compared to the SDSC method (fast heating method). This result suggests that the loss of crystalline structure in sucrose is a time−temperature combination process. That is, longer times are required for the loss of crystalline structure in sucrose at lower temperatures, whereas shorter times are required at higher temperatures. This knowledge that the loss of crystalline structure in sucrose is caused by the time−temperature thermal decomposition process is significant because melting is not only a common property used in sugar characterization, but also a general method used to prepare amorphous sugar, which is widely used as a key ingredient in the food and pharmaceutical industries. Consequently, heating conditions employed can affect the final properties of sugar-based

products, such as the product's flavor and glass transition temperature.

In addition, this research provides new insights into the caramelization process. Caramelization has generally been thought of as a complex series of reactions *occurring under higher temperature conditions*. However, based on the research reported herein, caramelization can occur under lower temperature, albeit longer time, conditions. Therefore, the caramelization reaction can be "controlled" via selection of appropriate heating conditions from a wide range of time−temperature combinations. Lastly, besides sucrose, the melting parameters of other materials, both organic (e.g., glucose, fructose, lactose, xylose, and acetylsalicylic acid) and inorganic (e.g., silicon), have been shown to exhibit a heating rate dependency. Thus, additional fundamental studies are needed to investigate the possibility of apparent melting in these materials, as well as the resultant consequences to the properties of the finished products.

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Supporting Information Available: Material information for sucrose and mannitol obtained from the Sigma-Aldrich Co. and analyzed by the authors of this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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