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Enhanced production of γ-cyclodextrin from corn syrup solids by means of cyclododecanone as selective complexant †

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ABSTRACT

In the presence of cyclododecanone as complexant, corn syrup solids (dextrose equivalent, d.e., 25; $\bar{d}p$ 4.5) were converted into cyclomaltooctaose (γ-cyclodextrin, γ-CD) in 28% yield by incremental addition of cyclodextrin glucanotransferase (CGTase) at 60°C and pH 7.2. Cyclotridecanone was less effective in enhancing γ-CD yield (14%); cyclic complexants with fewer than 12 ring atoms or more than 13 ring atoms were ineffective. In systems containing cyclododecanone, D-glucose strongly inhibited the conversion of both maltodextrin (starch hydrolyzate of $\bar{d}p$ 22) and corn syrup solids ($\bar{d}p$ 5). Maltose, when present in large proportion, also decreased yields of γ-CD from corn syrup solids, but not from maltodextrin. Maltotriose had no inhibiting effect on either substrate. The nature of the high-molecular-weight fraction ($\bar{d}p > 10$) of starch hydrolyzates was found to influence γ-CD production and was the predominant factor causing yields from corn syrup solids to be lower than those from maltodextrin. Maltose itself did not undergo conversion; however, other low-molecular-weight maltooligosaccharides were converted into γ-CD in good yield when treated incrementally with CGTase in the presence of cyclododecanone: 20.1% from maltotriose, 36.5% from maltotetraose, 44.1% from maltopentaose, 41.0% from maltohexaose, and 34.7% from maltoheptaose. Yields from maltooligosaccharides were adversely affected by the presence of both D-glucose and maltose.

INTRODUCTION

Cyclodextrin [cyclomaltooligosaccharides, cyclic (1 → 4)-α-D-glucans, CDs] are produced by the action of cyclodextrin glucanotransferase (CGTase) on starches and long-chain maltooligosaccharides. In the absence of complexants capable of forming insoluble inclusion compounds with CDs, conversion reactions normally favor noncyclic products and produce, in slightly smaller amounts, cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD), and cyclomaltooctaose (γ-CD)[1–3]. Of the three major CDs, γ-CD is favored least. Its yields are generally 4–7%,

† The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

based upon total glucose-unit content of the substrate. Overall yields of CD are usually 35–50%; and the relative proportions of α-CD and β-CD can vary appreciably according to reaction conditions.

Although, in a complexant-free system, maltose (G_2) * is a very poor substrate for conversion into CDs, even after long-term digestion with CGTase, the next two higher homologs, maltotriose (G_3), and maltotetraose (G_4) are convertible at low substrate concentration and provide combined CD yields of 7.6 and 31%, respectively[4]. Such conversions are thought to be preceded by disproportionation reactions that yield maltooligosaccharide chains of length suitable for cyclization. The presence of low-molecular-weight maltooligosaccharides is reported to affect adversely the yields of CDs from starch[5]; starch hydrolyzates of dextrose equivalent (d.e.) 1 and 12 were converted into CDs with combined yields of 45 and 17%, respectively. In the initial stage of CD formation from glycogen, maltooligosaccharides ranging in size from maltose (G_2) to maltoheptaose (G_7) inhibit cyclization[6]. The degree of inhibition is greatest with G_3 and G_4, and becomes progressively less with increasing chain length. Because reactions that led to these findings were conducted in the absence of complexants capable of forming inclusion compounds with CDs, the conclusions concerning effectiveness of inhibition are valid only for complexant-free systems.

Product ratios and CD yields from starch are known to be greatly influenced by complexants that form insoluble inclusion compounds with CDs. For example, 50% yields of α-CD are possible through the use of 1-decanol[7,8], which complexes selectively with the α homolog. β-CD production is enhanced by means of toluene[9,10], trichloroethylene[11], and limonene[12]. Use of a combination of bromobenzene and sodium acetate produces CDs in yields of 8.4 α, 34.3 β, and 18.7% γ (refs 13 and 14). A combination of butanone and 1-naphthol gives a 28% yield of γ-CD[15]. γ-CD yields of 40% are possible through the use of pentacyclic and tetracyclic terpenoids[16], and 34–45% yields are realized with cyclic compounds having 13–24 ring atoms[17,18]. Certain C_{12} cyclic compounds enhance γ-CD production[19] when CGT is added incrementally at 60°C for prolonged periods. Recently, this author reported[19] that, in the presence of cyclododecanone and with CGTase added incrementally at 60°C; starch or maltodextrin with a high average degree of polymerization ($\bar{d}p$ 22) can be converted into γ-CD in yields as high as 50%, with only small amounts of accompanying α-CD (1–2%) and β-CD (3–5%).

Complexants are not known to have been used by other investigators to enhance γ-CD production from corn syrup solids (low-$\bar{d}p$ starch hydrolyzates). Normally, in the absence of complexant, yields from such hydrolyzates are very low. Recent unreported experiments in this laboratory have shown that, in the presence of cyclododecanone, certain corn syrup solids (d.e. 25; $\bar{d}p$ 4.5) can be converted into γ-CD in moderately high yield (28%). Additional research was, therefore, con-

* The maltooligosaccharides are designated as G_n, where n is the number of α-D-glucopyranose residues.

ducted to determine the factors that limit and promote γ-CD production in these systems. This report shows that, in the presence of cyclododecanone at 60°C, (*1*) only maltooligosaccharides larger than G_2 contribute to γ-CD production, (*2*) in conversions of corn syrup solids, G_1 is a strong inhibitor, whereas the inhibitory ability of G_2 is relatively small, and (*3*) the high-molecular-weight constituents (dp > 10) of corn syrup solids are less convertible into γ-CD than those of maltodextrin ($\bar{d}p$ 22).

EXPERIMENTAL

Materials.—CGTase from *Bacillus macerans* was obtained as an aqueous solution (> 600 units/mL according to the method of Tilden and Hudson)[20] from Amano International Enzyme Co., Inc. In 30 min at pH 6.0 and 60°C, a single 5-μL application of the CGTase to a maltodextrin mixture of $\bar{d}p$ 22.1 (0.3 g in 3 mL of water) produced 16.5 mg of combined CDs (yields: 2.9 α, 1.8 β, and 0.8% γ. Amyloglucosidase (1,4-α-D-glucanglucohydrolase, EC 3.2.1.3, from *Aspergillus niger*, 37 units/mg of solid or 42 units/mg of protein) was obtained from Sigma Chemical Company. The maltodextrin M-050 (7.8% H_2O; d.e. 5; $\bar{d}p$ 22.1) and corn syrup solids M-255 (6.1% H_2O; d.e. 25; $\bar{d}p$ 4.5) were from Grain Processing Corporation (Muscatine, IA). Corn syrup solids Fro-Dex 22 (6.52% H_2O), Fro-Dex 24 (5.96% H_2O) and Fro-Dex 42 (5.8% H_2O) were from American Maize Products Co., Hammond, IN. Maltotriose (6.5% H_2O), maltotetraose (6.1% H_2O), maltopentaose (5.6% H_2O), maltohexaose (5.7% H_2O), and maltoheptaose (5.7% H_2O) were from Aldrich Chemical Co. Saccharides and cyclodextrins were the purest available; the latter contained the following percentages of water: α-CD, 8.75; β-CD, 13.7; and γ-CD, 9.0. Water of hydration was determined by weight loss from heating samples to constant weight at 100°C under vacuum. Values for d.e. and $\bar{d}p$ of maltodextrin and corn syrup solids were provided by the manufacturers. Water was distilled and deionized.

The manufacturer of M-050 and M-255 provided information on the saccharide composition of these two substances. For anhydrous M-050, in wt%: G_1 0.5, G_2 0.5, G_3 0.7, G_4 0.8, G_5 0.8, G_6 0.7, G_7 0.6, G_8 0.5, G_9 0.4, G_{10} 0.1, and dextrins of dp > 10, 94.4. For anhydrous M-255: G_1 2.4, G_2 8.1, G_3 9.5, G_4 6.0, G_5 5.7, G_6 13.7, G_7 9.6, G_8 1.2, G_9 0.8, G_{10} 0.7, and dextrins of dp > 10, 42.3.

The CGTase employed in these studies was described by the manufacturer as being most stable above pH 7, with stability decreasing rapidly below pH 6. Activity decreases rapidly above pH 7 and below pH 5. Thermostability is only moderate at the temperature (60°C) used in the present investigation (~ 93% of the activity remaining after 10 min).

Analytical methods.—CDs and low-molecular-weight saccharides (G_1–G_7) were determined by HPLC, which was performed on a Du Pont Zorbax NH_2 column (4.6 × 250 mm) at 40°C with 13:7 MeCN–water at 1.0 mL/min with refractometric detection. CD reference standard for comparison with elution peaks was a

mixture of pure α-, β-, and γ-CD (0.40 mg of each anhydrous CD per mL of aqueous solution); saccharide reference standard contained G_1–G_7, with each compound at a concentration of 1 mg/mL, on an anhydrous basis. All solutions were filtered through Millipore HV filter units (0.45-μm pore size) prior to injection. Prior to HPLC analysis, cyclodextrin solutions containing maltooligosaccharides were treated with amyloglucosidase to eliminate maltooligosaccharides that would interfere with CD determinations.

Conversion procedure.—Aliquots of substrate solution (usually 10 wt% of carbohydrate in water) were placed in individual screw-capped culture tubes (with or without complexant, as required), the pH was adjusted to 7.2, and a CGTase increment of appropriate size was added to each. Increment size was generally 5 μL per 3 mL of 10% substrate solution. Reactions were conducted in constant-temperature shaker baths at 60°C with periodic adjustment of pH and periodic application of a CGTase increment at a frequency no greater than twice daily. Use of buffers was avoided.

Removal of complexant from reaction mixtures by azeotropic distillation.—Mixtures were heated to ~95°C while N_2 was introduced at a controlled, moderate rate beneath the liquid surface by means of a capillary tube; the length of the operation varied according to the stability of the complex. The complexant-free solutions were then diluted appropriately and filtered through Millipore HV 0.45-μm filters prior to HPLC analysis.

Solubility of CDs in the presence of complexants at 60°C.—Mixtures of CD and cycloalkanone (mol ratio of 1:1.25) were prepared by mixing 7.00 mL of 8.33 mM CD (0.0583 mmol) and 0.729 mmol of complexant in a capped culture tube. The tube was placed in a 60°C shaker bath for 2 weeks, at the end of which time the contents were rapidly filtered at 60°C by means of a glass syringe equipped with a μStar 0.22-μm filter unit. Syringe and filter were kept at 60°C until ready for use. Filtrates were analyzed by HPLC for unprecipitated CD.

Methanol extraction of maltodextrin and corn syrup solids.—Powdered samples (15.00 g) of hydrated M-050, M-255, Fro-Dex 22, Fro-Dex 24, and Fro-Dex 42 were

TABLE I

Data from methanol extraction of maltodextrin M-050 and corn syrup solids at 25°C

Material [a]	D.e.	Solids removed [b] (%)	Volume of MeOH (mL)	Saccharides removed by extraction [c] (% of M-050 or corn syrup solids)							
				G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
M-050	5	2.6	150	0.45	0.44						
M-255	25	39.0	150 [d]	2.2	9.0	9.3	4.8	4.1	6.6	2.6	0
Fro-Dex 22	22	25.9	152	11.7	13.0						
Fro-Dex 24	24	38.8	204	13.8	8.3						
Fro-Dex 42	42	67.8	412	25.1	14.7						

[a] 15.00 g of hydrated form. [b] Anhyd wt basis. [c] Anhyd wt basis; HPLC analysis. [d] Does not include the approximately 100 mL of washings from filtration operation.

stirred for at least 5 days at 25°C in 150, 150, 150, 200, and 400 mL of anhydrous MeOH, respectively. Aliquots of the extracts were evaporated to dryness under vacuum at 105°C to determine weight losses caused by the extraction and to facilitate saccharide analysis by HPLC. In the case of M-255, the undissolved solids were isolated by filtration, washed rapidly 4 times with 25-mL portions of MeOH, dried at 25°C under vacuum, and equilibrated at 31% relative humidity. Yield, 8.9 g; 9.34% H_2O of hydration; 39.0% loss of weight (anhyd basis) from the extraction. The amounts of G_1, G_2, and G_3 extracted from M-255 were in close agreement with the percentages of these constituents reported by the manufacturer. Extraction data for M-050 and the corn syrup solids are given in Table I.

RESULTS AND DISCUSSION

The basic procedure described earlier[19] for converting potato starch and maltodextrin M-050 into γ-CD in high yield was used. Solutions (10%) of corn syrup solids M-255 treated incrementally with CGTase at 60°C and pH 7.2 in the presence of cyclododecanone resulted in the production of γ-CD in 28% yield. Incremental addition of CGTase was essential because of the relatively low stability of the Amano enzyme at 60°C and the slow rate of formation of γ-CD. The use of various other complexants, including both larger and smaller cycloalkanes or cycloalkanones, bromobenzene, 1,3-diisopropylbenzene, 1-dodecanol, and 1,1,2,2-tetrachloroethane, led to much lower yields of this homolog. These studies, summarized in Table II, suggest that cyclododecanone occupies a possibly unique position among complexants in regard to ability to enhance γ-CD production from corn syrup solids. Yields of γ-CD decrease markedly with increasing size of

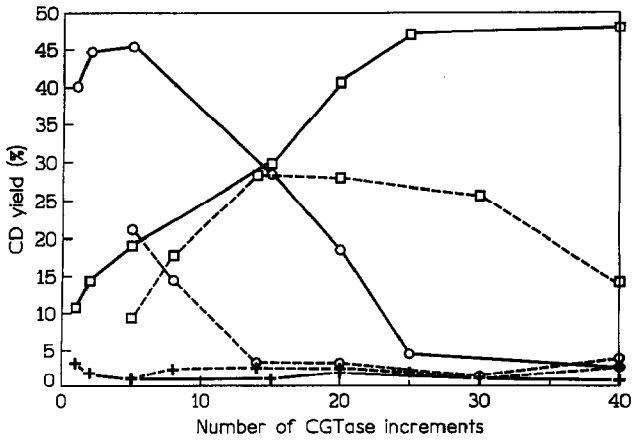

Fig. 1. Conversion of maltodextrin M-050 (———) and corn syrup solids M-255 (— — —) in the presence of cyclododecanone by incremental addition of CGTase at 60°C and pH 7.2. The symbols +, ○, and □ refer to α-CD, β-CD, and γ-CD, respectively. Solutions (3 mL) are 10 wt% in substrate and contain 0.18 mmol of complexant. Increments of CGTase (5 μL each) are added twice daily.

TABLE II

Influence of different complexants and no complexant on conversion of 10% M-255 [a] at pH 7.2 and 60°C

Complexant [b]	No. of 5-μL increments of CGTase	CD yield (%) [c]			
		α	β	γ	Combined
None	2	7.4	6.2	3.1	16.7
Cyclodecane	14	3.7	23.0	2.3	29.0
Cyclodecanone	14	1.4	29.8	0.5	31.7
Cyclododecanone	8	2.3	14.5	17.8	34.6
	14	2.5	3.3	28.3	34.1
	20	2.4	3.2	27.9	33.5
Cyclotridecanone	8	2.8	3.3	13.2	19.2
	14	5.0	5.1	13.8	23.9
	20	5.9	5.8	9.0	20.7
Cyclopentadecanone	8	3.9	4.2	1.0	9.1
	14	7.2	6.1	1.2	14.5
8-Cyclohexacecen-1-one	6	5.0	6.4	1.4	12.8
	10	5.6	7.4	1.1	14.1
Bromobenzene	6	7.1	8.9	2.3	18.3
	10	7.2	9.4	1.8	18.4
1,3-Diisopropylbenzene	6	6.4	12.1	2.0	20.5
	10	3.9	11.2	~0	~15.1
1-Dodecanol	6	7.6	6.7	~0	~14.3
	10	6.4	6.4	1.1	13.9
1,1,2,2-Tetrachloroethane	6	4.3	6.3	2.2	12.8
	10	6.4	6.4	1.1	13.9

[a] Volume of 10% M-255, 3 ml; 1.85 mmol of glucose residues. [b] 0.24 mmol. [c] Based upon total glucose residues in M-255 substrate.

cycloalkanones; no enhancement of this cyclodextrin occurs with cyclic complexants of smaller ring size. With cyclodecane and cyclodecanone, β-CD production is favored, presumably because of the relatively greater stability of the β complex compared with that of the γ complex. In Fig. 1, the ability of maltodextrin M-050 (d.c. 5; $\bar{d}p$ 22.1) to produce CDs under the influence of cyclododecanone is compared with that of corn syrup solids M-255 (d.e. 25; $\bar{d}p$ 4.5). The factors contributing to the substantially lower maximum yield of γ-CD from M-255 were not immediately obvious, although it was initially suspected that the presence of large amounts of low-molecular-weight maltooligosaccharides in M-255 might have an inhibiting effect on CD formation. Such maltooligosaccharides had been suggested by Bender[4] as a probable cause of low CD yields from high-d.e. starch hydrolyzates in complexant-free systems; and Suzuki and coworkers[21] had reported an adverse effect of G_1 and G_2 in both the presence and the absence of the complexants trichloroethylene and 1-decanol.

To determine whether low-molecular-weight saccharides behave as inhibitors in systems containing cyclododecanone and other cycloalkanones known to enhance γ-CD production, an investigation was made of the influence of G_1, G_2, and G_3 on

TABLE III

Influence of added D-glucose (G_1) on conversion of 10% M-050 [a] with and without complexant [b] at pH 7.2 and 60°C

Complexant [b]	Amount of added G_1 in substrate		No. of 5-μL increments of CGTase	CD yield (%) based upon M-050 content [c]			
	wt%	mmol		α	β	γ	Combined
Cyclodo-decanone	0	0	1	3.2	40.0	10.8	54.0
			10	2.0	31.6	23.0	56.6
			20	1.8	18.5	40.6	60.9
			25	1.9	4.5	47.0	53.4
			40	0.8	2.6	47.9	51.3
	2.1	0.036	1	3.8	35.2	12.6	51.6
			5	2.5	41.1	17.7	61.3
			15	2.4	31.1	18.6	52.1
			25	3.0	14.1	34.6	51.7
			35	2.4	3.8	33.1	39.3
	5.4	0.095	1	4.0	31.4	11.0	46.4
			5	2.0	38.3	18.0	58.3
			15	3.3	26.7	24.8	54.8
			25	3.0	4.5	37.2	44.7
			35	3.0	4.0	29.8	36.8
	18.2	0.37	1	3.6	21.6	7.3	32.5
			5	0.5	24.1	12.2	36.8
			10	2.2	6.4	31.6	40.2
			15	1.4	4.4	33.6	39.4
			25	2.0	2.6	30.7	35.3
	26.5	0.60	1	3.0	14.3	5.6	22.9
			5	2.9	16.6	7.9	27.4
			15	3.2	2.5	16.5	22.2
			25	3.4	2.2	15.4	20.9
	42	1.21	25	4.3	1.9	2.7	8.9
Cyclotri-decanone	0	0	1	6.5	22.8	18.1	47.4
			5	2.3	9.2	43.1	54.6
			9	1.4	4.2	51.1	56.7
	18.2	0.37	2	4.7	5.9	14.5	25.1
			6	3.9	4.4	19.4	27.7
			10	2.1	3.6	17.2	22.9
Cyclopenta-decanone	0	0	1	6.2	11.0	25.9	43.1
			5	3.8	11.8	27.4	43.0
			9	3.4	6.7	34.1	44.2
			15	3.6	6.1	29.9	39.6
	18.2	0.37	1	5.5	8.6	2.4	16.5
			15	6.9	8.2	0	15.1
None	0	0	1	9.6	14.3	3.9	27.8
			5	9.7	15.5	2.9	28.1
			10	8.4	13.0	1.6	23.0
			15	8.4	11.0	2.4	21.8
	18.2	0.37	1	7.7	9.0	3.3	20.0
			5	6.2	8.9	1.8	16.9
			10	5.7	7.3	0.7	13.7
			15	6.6	6.8	2.4	15.8

[a] Volume, 3 mL; 1.85 mmol of glucose residues. [b] 0.18 mmol. [c] In the calculation of yields, the presence of added G_1 was ignored.

TABLE IV

Influence of added G_2 and G_3 on conversion of 10% M-050 [a] with and without complexant [b] at pH 7.2 and 60°

Complexant	No. of 5-μL increments of CGTase	CD yield (%) based upon M-050 content [c]							
		With added G_2 (0.37 mmol) [d]				With added G_3 (0.37 mmol) [e]			
		α	β	γ	Combined	α	β	γ	Combined
None	1					9.5	13.5	6.7	29.7
	5	9.6	7.0	0	16.6				
	10	13.7	3.5	0	17.2	11.2	15.6	2.8	29.6
	15					12.0	14.9	2.6	29.5
	25	10.3	7.2	0	17.5	11.2	13.3	2.1	26.6
	35	10.9	0	0	10.9				
Cyclododecanone	1	8.6	10.8	1.4	20.8	6.1	31.5	10.3	47.9
	5	1.8	36.0	14.7	52.5				
	10	2.2	26.1	23.8	52.1	3.7	37.6	41.8	83.1
	15	2.9	7.2	38.1	48.2	3.1	16.1	51.8	71.0
	25	2.9	1.1	46.3	50.3	3.1	5.0	71.8	79.9
	35	1.9	1.6	44.2	47.7	2.4	2.9	72.2	77.5
Cyclotridecanone	6	4.4	5.9	33.6	39.9				
	8	4.2	5.9	34.9	45.0				
	10	3.7	4.0	40.4	48.1				
Cyclopentadecanone	1	6.5	3.1	4.9	14.5	11.7	15.9	4.6	32.2
	5	6.3	7.0	9.7	23.0	6.4	10.1	38.2	54.7
	10	6.3	4.3	24.3	34.9	5.2	8.0	44.8	58.0
	15	7.8	4.7	15.1	27.6	6.6	8.5	35.2	50.3
	25	11.2	5.4	8.1	24.7	6.4	7.4	28.4	42.2
	35	11.8	4.1	3.7	19.6				

[a] Volume of 10% M-050, 3 mL; 1.85 mmol of glucose residues. [b] 0.18 mmol. [c] In the calculation of yields, the presence of added maltooligosaccharide was ignored in order to facilitate recognition of influence or lack of influence of the maltooligosaccharide on yield. [d] 30 wt% of total substrate. [e] 38 wt% of total substrate.

the conversion of M-050, a maltodextrin of low-saccharide content (0.4% G_1, 0.4% G_2, and ~4.6% combined G_3–G_{10}). The results (Tables III and IV) showed that, with cyclododecanone as complexant, only G_1 inhibited the formation of maximum yield. Inhibition was particularly strong at high levels of G_1; a γ-CD yield of only 17% resulted from using a substrate containing 26.5% (by wt) of G_1. G_2 had no apparent influence on yield. (It should be noted that, in a complexant-free system, both G_1 and G_2 are strong inhibitors.) In the presence of cyclododecanone, although both G_2 and G_3 fail to inhibit, G_3 differs from G_2 by being able to undergo conversion to γ-CD and, thereby, contribute greatly to overall γ-CD yield from mixtures of M-050 and G_3. For example, where the weight of added G_3 was 36.7% of the weight of total substrate (M-050 + G_3), a 72.2% yield of γ-CD, based upon M-050 content only, was obtained (Table IV). Based upon combined M-050 and G_3, the yield was 60.8%, which is substantially higher than the 45–50% yield

TABLE V

The influence of complexants and added G_1 and G_2 on the conversion of maltooligosaccharides (10% solutions) at pH 7.2 and 60°C

Complexant (0.18 mmol/3 mL)	No. of CGTase increments [a]	% Yield of CDs [b]			
		α	β	γ	Combined
Maltotriose					
None	1	0	0.5	0	0.5
	2	5.0	1.4	0	6.4
	5	6.1	3.2	0	9.3
	10	7.3	1.9	0	9.2
Cyclooctane (1.2 mmol/3 mL)	10	4.0	17.6	0	21.6
Cyclodecanone	25	2.0	20.9	0	22.9
Cycloundecanone	25	6.2	16.2	0	22.4
Cyclododecanone	1	3.8	2.8	0	6.6
	5	1.3	18.9	5.0	25.2
	15	1.9	2.5	20.1	24.5
	25	2.1	2.1	17.0	21.2
Cyclotridecanone	8	0	3.5	3.2	6.7
	25	5.5	5.1	0	10.6
Cyclopentadecanone	25	5.3	3.5	0	8.8
Maltotriose + D-glucose (26 wt%)					
Cyclododecanone [c]	15	3.2	1.1	0	4.3
Maltotriose + maltose (25 wt%)					
Cyclododecanone	15	0	1.1	14.8	15.9
Maltotetraose					
None	1	7.2	5.5	2.8	15.5
	15	8.2	7.0	0	15.2
Cyclododecanone	1	2.9	19.7	9.9	32.4
	15	1.5	3.1	36.5	41.2
	25	1.7	2.3	34.0	38.0
Maltopentaose					
None	1	9.1	9.6	4.4	23.1
	15	8.9	5.8	0	14.6
Cyclododecanone	1	2.9	27.2	11.4	41.5
	15	2.1	5.5	39.5	47.1
	25	2.1	3.0	44.1	49.2
Maltopentaose + maltose (8 wt%)					
Cyclododecanone	15	2.9	9.3	25.7	37.9
Maltohexaose					
Cyclododecanone	1	1.3	41.2	17.2	59.7
	15	1.8	8.2	40.3	50.3
	25	1.5	7.5	41.0	50.0
Cyclotridecanone	10	1.9	3.4	40.5	45.8
Cyclopentadecanone	15	3.5	4.0	25.2	32.7
Maltohexaose + D-glucose (26 wt%)					
Cyclododecanone	25	1.8	0.9	16.2	18.9

TABLE V (continued)

Complexant (0.18 mmol/3 mL)	No. of CGTase increments [a]	% Yield of CDs [b]			
		α	β	γ	Combined
Maltoheptaose					
None	1	10.4	10.3	5.8	26.5
	15	10.3	14.0	3.8	28.1
Cyclododecanone	1	4.9	30.7	13.1	48.7
	15	1.6	29.5	26.1	57.2
	25	5.3	24.2	34.7	64.2

[a] Increment size equivalent to 5 μL per 3 mL of 10% substrate solution. [b] Based exclusively upon millimoles of glucose residues in G_3, G_4, G_5, G_6, or G_7; does not include glucose residues from added G_1 or added G_2. [c] No precipitation of complex occurred during the course of reaction.

normally obtained from M-050 in the absence of added G_3.

With cyclopentadecanone as complexant, both G_1 and G_2 are strong inhibitors, and G_3 contributes only moderately to the yield of γ-CD (Tables III and IV). The influence of cyclotridecanone is intermediate between that of cyclododecanone and that of cyclopentadecanone: G_1 inhibits strongly and G_2 inhibits weakly. In the absence of added saccharide, maximum γ-CD yields from M-050 at pH 7 and 60°C are 35 and 51% in the presence of cyclopentadecanone and cyclotridecanone, respectively.

Studies with individual maltooligosaccharides as sole substrates showed that cyclododecanone at 60°C enhances γ-CD production from all maltooligosaccharides but G_2 (Table V). G_2 resisted conversion into CDs in both the presence and the absence of complexants. Of the larger oligosaccharides, G_3 gave the lowest CD yields. In contrast with the behavior of cyclododecanone, larger cyclic complexants (cyclotridecanone and cyclopentadecanone) were unable to enhance γ-CD yields from G_3; however, these same complexants did enhance yields from G_6.

Only limited data were obtained that pertained to the influence of G_1 and G_2 on convertibility of maltooligosaccharides in the presence of cyclododecanone. Both G_1 and G_2 were found to retard the conversion of maltotriose and certain larger oligomers (Table V). On a weight basis, G_1 was a stronger inhibitor of maltotriose conversion than was G_2. More research in this area is needed in order to clarify what appears to be a significant difference between the behavior of G_2 in a low-dp maltooligosaccharide system and its behavior in a high-dp maltodextrin system.

Relationship of D-*glucose content of corn syrup solids to yield of γ-CD.*—Because of their widely differing G_1 contents, M-255 (2.2% G_1; 9.0% G_2), Fro-Dex 22 (11.7% G_1; 13.0% G_2), Fro-Dex 24 (13.8% G_1; 8.3% G_2), and Fro-Dex 42 (25.1% G_1; 14.7% G_2) were chosen for the purpose of determining whether any relationship exists between γ-CD yield and G_1 content of corn syrup solids. Table VI summarizes data from conversions conducted in the presence of cyclododecanone, cyclotridecanone, and cyclopentadecanone. The yields are maximum or near-maxi-

TABLE VI

Conversion of different corn syrup solids in the presence of various cyclic complexants at pH 7.2 and 60°C [a]

Solids	D.e.	G_1 (%) [c]	G_2 (%) [c]	% Yield of CDs [b]								
				With cyclododecanone			With cyclotridecanone			With cyclopentadecanone		
				α	β	γ	α	β	γ	α	β	γ
M-255	25	2.2	9.0	2.4	3.2	27.9	2.8	3.3	13.2	7.2	6.1	1.2
Fro-Dex 22	22	11.7	13.0	1.5	3.3	25.3	2.8	3.9	12.8	2.6	5.0	1.0
Fro-Dex 24	24	13.8	8.3	1.7	1.5	14.2	2.8	3.6	0.9	1.4	1.0	0
Fro-Dex 42	42	25.1	14.7	1.4	1.4	1.4	1.1	1.2	0	0	2.0	0

[a] 5-μL increments of CGTase were added to 10% solutions (3 mL), each containing 0.18 mmol of complexant. The number of increments varied according to the complexant employed: 20–25, 6–10, and 12–15 in systems with cyclododecanone, cyclotridecanone, and cyclopentadecanone, respectively. These numbers correspond to the optimum number of CGTase increments required to reach maximum γ-CD yield with starch, M-050, and M-255. [b] Based upon millimoles of glucose residues in substrate. [c] Wt% of saccharide constituent in corn syrup solids.

mum. The ability to enhance γ-CD production decreases rapidly with increasing ring size: cyclododecanone > cyclotridecanone > cyclopentadecanone; and γ-CD production decreases with increasing G_1 content of the substrate. With cyclododecanone as complexant, however, the extremely low γ-CD yield (1.4%) from Fro-Dex 42 cannot be explained solely on the basis of G_1 content (25.1% by wt), in view of the fact that, under similar conditions, a higher yield (15%) can be produced from a mixture of M-050 and G_1 where total G_1 content is 26.9%. Although maltose, if present in large amount, probably contributes to low yields from corn syrup solids, existing evidence does not indicate that the contribution is major. For example, with cyclododecanone as complexant, the addition of 0.1 g of G_2 to 0.3 g of M-255 merely reduces the maximum convertibility of the latter from 28 to 21%. Similar additions of G_1 and G_3 give γ-CD yields of 0 and 34%, respectively, based upon M-255 content of the substrate. Evidence that the inherent maltose content (9%) of M-255 has little, if any, adverse effect on γ-CD yields will be provided later in this report.

Examination of the high-dp fraction of M-255 as substrate for CD production.—In the absence of any strong evidence implicating low-molecular-weight maltooligosaccharides as major factors in the lowering of γ-CD yields from corn syrup solids in the presence of cyclododecanone, the possibility was examined that the high-dp fraction of these solids was related to low yield. Experiments were designed to determine whether high-dp components of corn syrup solids M-255 are less easily converted than those of maltodextrin M-050. For this purpose, M-255 was subjected to extraction with methanol to give a mixture of solids that was either free or almost free of G_1–G_5, but which still contained important amounts of G_6 and G_7. Although residual, unextracted G_8–G_{10} were not measured, they

TABLE VII

Conversion of MeOH-extracted M-255 [a], unreconstituted and fully reconstituted with G_3–G_7, at pH 7.2 and 60°C

Complexant [b]	No. of 3.5-μL increments of CGTase	% Yields of CDs [c]			
		α	β	γ	Combined
Unreconstituted [d]					
None	2	4.9	9.5	3.0	17.4
Cyclododecanone	5	1.8	25.6	9.7	37.1
	10	2.1	15.7	15.1	32.9
	15	1.4	11.4	19.8	32.6
	20	2.2	2.9	23.1	28.2
	25	1.1	2.8	23.3	27.2
Cyclopentadecanone	5	7.6	10.3	1.8	19.7
	10	7.6	9.6	2.0	19.2
	15	7.8	10.0	1.8	19.6
	25	6.1	7.0	0.6	13.7
Reconstituted [e]					
None	8	14.2	17.6	3.8	35.6
Cyclododecanone	15	2.5	3.8	29.7	36.0
	25	1.4	2.5	29.3	33.2
	30	3.2	2.4	29.2	34.8

[a] Volume, 2 mL. [b] 0.18 mmol. [c] Based upon total glucose residues (1.09 mmol) in substrate. [d] Wt% of saccharide components in substrate: $G_1+G_2+G_3=0$, $G_4=2.0$, $G_5=2.7$, $G_6=12$, $G_7=12$, $G_8\leq 2$, $G_9\leq 1$, $G_{10}\leq 1$. [e] Wt% of saccharide components in substrate: $G_1+G_2=0$, $G_3=10.4$, $G_4=6.6$, $G_5=6.2$, $G_6=14.5$, $G_7=9.9$, $G_8\leq 2$, $G_9\leq 1$, $G_{10}\leq 1$.

were not expected to contribute importantly to the composition of the extracted M-255. The extraction process removed 39.0% (by wt) of the M-255 constituents; saccharides G_1–G_7 comprised 99% of the extracted solids. Although several repeated extractions probably would have removed almost all of the remaining G_4–G_7, complete removal of these oligomers was not deemed essential for the proposed investigation.

The approximate composition of the methanol-extracted M-255 (the methanol-insoluble fraction) is given in Table VII, which presents the results of conversion reactions with extracted M-255 and with extracted M-255 reconstituted with G_3–G_7. Reconstitution resulted in a substrate essentially identical with M-255, but free of G_1 and G_2. M-255 with G_1 and G_2 removed underwent conversion in the presence of cyclododecanone to give a maximum γ-CD yield of 32%, which was only slightly greater than the 28% yield obtained with M-255 (Table II). CD yields from unreconstituted, methanol-extracted M-255 were exceptionally low in the absence of complexant: 4.9 α, 9.5 β, and 3.0% γ. Under similar, complexant-free conditions, yields from maltodextrin M-050 are higher: 10.9 α, 17.8 β, and 4.1% γ. In the presence of cyclododecanone, maximum conversion of the same unreconstituted substrate into γ-CD (23.3% yield) was also low, relative to maximum γ-CD

TABLE VIII

Conversion of MeOH-extracted M-255 [a], partially reconstituted with G_2–G_7 and G_3–G_7, at pH 7.2 and 60°C

Complexant [b]	No. of 3.5-μL increments of CGTase	% Yields of CDs [c]			
		α	β	γ	Combined
Reconstituted with G_2–G_7 [d]					
Cyclododecanone	5	1.2	24.7	10.1	36.0
	10	1.0	16.2	14.3	31.5
	15	1.1	12.5	16.5	30.1
	20	2.1	4.3	23.6	30.0
	25	1.1	2.4	27.7	31.2
Cyclopentadecanone	4	6.2	7.7	2.3	16.2
	8	5.2	6.5	2.7	14.4
	12	5.3	5.8	2.7	13.8
	15	5.3	5.6	3.3	14.2
	20	5.3	5.6	1.5	12.4
Reconstituted with G_3–G_7 [e]					
Cyclododecanone	5	1.2	28.0	14.0	43.2
	10	3.0	26.0	21.3	50.3
	15	1.6	14.6	20.7	36.9
	20	2.3	3.1	27.1	32.5
	25	2.1	2.8	27.6	32.5
Cyclopentadecanone	4	6.9	9.3	3.9	20.1
	8	5.7	6.7	5.6	18.0
	12	4.8	4.2	6.2	15.2
	15	5.1	5.9	6.7	17.7
	20	5.1	5.1	5.4	15.6

[a] Volume, 2 mL. [b] 0.18 mmol. [c] Based upon total glucose residues in substrate (1.54 and 1.43 mmol, respectively, for mixtures reconstituted with G_2–G_7 and G_3–G_7). [d] Wt% of saccharide components in substrate: $G_1 + G_2 = 0$, $G_3 = 8.0$, $G_4 = 5.1$, $G_5 = 4.7$, $G_6 = 11.1$, $G_7 = 7.6$, $G_8 \leq 2$, $G_9 \leq 1$, $G_{10} \leq 1$. [e] Wt% of saccharide components in substrate: $G_1 = 0$, $G_2 = 7.2$, $G_3 = 7.4$, $G_4 = 4.7$, $G_5 = 4.4$, $G_6 = 10.3$, $G_7 = 7.0$, $G_8 \leq 2$, $G_9 \leq 1$, $G_{10} \leq 1$.

yields (45–50%) from M-050 under similar conditions. This striking difference between M-050 and methanol-extracted M-255 indicates that an important difference, perhaps structural, exists between the high-dp fraction of M-050 and that of M-255 and suggests the possibility that high-dp components of M-255 are, on the average, more highly branched and/or contain shorter branches than those of M-050. Regardless of the precise reason for the difference, it may be concluded that the nature of the high-dp fraction of corn syrup solids is a major factor determining the extent of CD production, and that this nature might possibly vary according to the manufacturing process.

Table VIII contains data on conversions of extracted M-255 reconstituted with G_2–G_7 and with G_3–G_7. Here the level of reconstitution was somewhat less than that used for the studies in Table VII. With cyclododecanone as complexant, maximum γ-CD yield (27.7%) achieved in the presence of G_2 was approximately

the same as that (27.6%) reached in its absence. Consequently, in the presence of this complexant, G_2 has little effect on CD production. With cyclopentadecanone, however, CD production was severely retarded, even in the absence of G_2, which indicates that cyclododecanone is the preferred complexant for converting corn syrup solids. The reason for the low combined yields and the inability of cyclopentadecanone to enhance γ-CD production is not clear. In regard to enhancement, the difference between the solubility of γ-CD in the presence of cyclopentadecanone and the solubility of γ-CD in the presence of cyclododecanone does not seem to be involved, since the two complexants affect solubility to similar extents at 60°C [from 7 mL of 8.33 mM γ-CD (0.0583 mmol) to which was added 0.0729 mmol of guest compound, 93% of the γ-CD precipitated as cyclododecanone complex and 97% precipitated as cyclopentadecanone complex]. Perhaps the difference in enhancement ability is related in some way to the great difference in the influence of these two complexants on the solubility of β-CD [from 7 mL of 8.33 mM β-CD (0.0583 mmol) to which was added 0.0729 mmol of complexant, no β-CD precipitated in the presence of cyclopentadecanone, whereas 85% of the β-CD precipitated in the presence of cyclododecanone]. An alternative, but unsupported, explanation for the difference in enhancement is that cyclododecanone, but not cyclopentadecanone, is intimately involved in the cyclization mechanism as a guest–substrate–enzyme intermediate that favors γ-CD formation. It is conceivable that the formation of such an intermediate might be hindered competitively by G_1 and, depending upon the molecular size of the substrate, by maltose.

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