J. Appl. Glycosci., **72**, 7201101 (2025)
doi:10.5458/jag.7201101

Regular Paper

Enzymatic Synthesis of a Novel Short Linear Maltodextrin from Starch

(Received September 13, 2024; Accepted November 1, 2024)
(J-STAGE Advance Published Date: January 23, 2025)

Atsushi Kawano,[1] Tomohiro Yamamoto,[1] Yuya Shinagawa,[1] Isao Hanashiro,[2] and Hironori Yoshida[1,†]

[1] *Research & Engineering Department, Showa Sangyo Co., Ltd.*
(*2−20−2 Hinode, Funabashi-shi, Chiba 273−0015, Japan*)
[2] *Department of Food Science and Biotechnology, Kagoshima University*
(*1−21−24 Korimoto, Kagoshima-shi, Kagoshima 890−0065, Japan*)

Abstract: Short linear maltodextrin (SLMD) was synthesized from starch via the combined action of branching and debranching enzymes. The number-average degree of polymerization and number-average chain length of SLMD were 8.49 ± 0.21 and 8.52 ± 0.60, respectively, indicating that it consists of linear chains. In gel permeation chromatography analyses, SLMD showed a single peak at a molecular weight of 1,200. SLMD consisted mainly of linear saccharides with a degree of polymerization of 6−12, without high molecular weight α-glucans or small malto-oligosaccharides. SLMD had a much higher blue value and a longer λmax compared with those of commercial dextrose equivalent (DE) 13 maltodextrin. While the DE 13 maltodextrin solution remained clear, an SLMD solution became turbid upon cooling, with the turbidity reversing upon heating. This interconversion was reproducible. SLMD absorbed moisture only to a limited extent, even under high relative humidity, and remained solid without noticeable viscousness. These results demonstrate the novelty and distinct properties of SLMD compared with those of other maltodextrins available on the market, implying its potential for various applications in the food industry.

Key words: maltodextrin, branching enzyme, debranching enzyme, aggregation

INTRODUCTION

Starch is a major source of polysaccharides in plants. In addition to starch, its hydrolysis products, such as maltodextrin, glucose syrup, and glucose, are widely used in the food industry. Gelatinized starch is treated with carbohydrate-active enzymes, such as α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2), or glucoamylase (EC 3.2.1.3), to produce starch hydrolysis products. Different enzymes with varying specificities have been used, individually or in combination, to obtain products with the desired structures. Starch hydrolysis products are used as sweeteners or energy sources and to control the physical properties of food. To achieve this, various starch hydrolysis products are produced via different enzymatic reactions. Maltotriose, produced by maltotriose-producing amylase (EC 3.2.1.116), prevents the retrogradation of starch in food [1]. Isomalto-oligosaccharides and nigero-oligosaccharides, produced by α-glucosidase (EC 3.2.1.20), are widely used in food processing and manufacture of beverages and confectionery [2]. Thus, the application of enzymatic reactions to create new starch hydrolysis products with unique structures can result in food diversification.

Branching (EC 2.4.1.18) and debranching enzymes (such as isoamylase and EC 3.2.1.68) are widely used in the starch saccharification industry. Branching enzymes catalyze the transglucosylation of α-1,4-glucosyl chains to form branches via an α-1,6-glucosidic linkage. It is used to produce highly branched cyclic dextrins [3] or highly branched glucans [4]. Debranching enzymes catalyze the hydrolysis of α-1,6-glucosidic linkages and are used for producing glucose or maltose along with other amylolytic enzymes such as glucoamylase and β-amylase [5]. As indicated by their names, branching and debranching enzymes exert opposite effects on starch: synthesis and degradation of branch linkages in α-glucans, respectively. Only a few reports on the combined use of these two enzymes for the production of starch-derived products have been published.

In this study, we developed a unique method for producing starch-derived products via the simultaneous action of branching and debranching enzymes. The product was characterized and identified as maltodextrin with a novel structure, designated short linear maltodextrin (SLMD). We report some interesting properties of SLMD compared with other commercially available maltodextrins with similar dextrose equivalent (DE) values.

MATERIALS AND METHODS

Chemicals. Cornstarch and maltodextrin with DE 13 were the commercial products of Showa Sangyo Co., Ltd. (Tokyo, Japan). Unless otherwise specified, all other chemicals were purchased from FujiFilm Wako Pure Chemical Industries Ltd. (Osaka, Japan).

†Corresponding author (Tel. +81−299−92−1463, Fax. +81−299−92−1472, E-mail: hironori_yoshida@showa-sangyo.jp)
Abbreviations: β-AL, β-amylolysis limit; CLn, (number-average) chain length; DE, dextrose equivalent; DPn, (number-average) degree of polymerization; DS, dissolved substances; GPC, gel permeation chromatography; HPLC, high-performance liquid chromatography; SLMD, short linear maltodextrin.

Preparation of SLMD. Cornstarch (30 g) was suspended in 20 mM acetate buffer (pH 5.0) containing 2 mM $CaCl_2$ at a solid concentration of 15 % (wt/wt). α-Amylase (Kleistase T10S; Amano Enzyme Inc., Aichi, Japan) was added (2 U/g of starch) and heated to 100 ˚C for 65 min for liquefaction. To terminate liquefaction, the pH was adjusted to 4.0 with 10 % (wt/wt) oxalic acid, and the solution was maintained at 100 ˚C for 30 min. The solution was diluted with water at 10 % concentration, cooled down to 40 ˚C, and its pH was adjusted to 5.5 with 5 % (wt/wt) NaOH. Subsequently, to a 100 g aliquot of liquefied starch, 300 U/g dissolved substances (DS) of branching enzyme (Sensea Form; Novozymes Japan Ltd., Chiba, Japan) and 50 U/g DS of isoamylase (Megazyme Ltd., Wicklow, Ireland) were added and incubated at 40 ˚C for 72 h. The pH was adjusted to 4.0 with 10 % (wt/wt) oxalic acid and heated at 100 ˚C for 30 min. After removing the insoluble precipitate via centrifugation, the solution was desalted using an ion-exchange resin (Amberlite MB-4; ORGANO Corp., Tokyo, Japan) and filtered through filter papers No. 2 and 5C. The filtrate was freeze-dried and 8 g of the SLMD powder was obtained.

DE and iodine absorption properties. DE was determined using the Lane–Eynon method [6]. The iodine absorption spectrum was obtained at 400−800 nm for a solution containing 0.1 % saccharides (wt/wt), iodine (4 mg/100 mL), and potassium iodide (40 mg/100 mL) at 30 ˚C using a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan). The absorbance at 680 nm was recorded as the blue value.

Determination of number-average degree of polymerization (DPn), number-average chain length (CLn), and β-amylolysis limit (β-AL). SLMD (3 mg) was soaked in 55.5 μL of 90 % dimethyl sulfoxide at an ambient temperature overnight. The wetted specimen was heated in a boiling water bath for 5 min, 44.5 μL of deionized H_2O was added, and the mixture was heated continuously until complete dissolution. The dissolved sample was diluted with 1.15 mL of deionized H_2O and used in subsequent experiments. Total carbohydrates and reducing residues were determined using the phenol–sulfuric acid method [7] and the Somogyi–Nelson method [8, 9], respectively. DPn was calculated as the ratio of total carbohydrates to reducing residues. CLn was calculated as the ratio of total carbohydrates to reducing residues after debranching with isoamylase (Nagase Viita Co., Ltd., Okayama, Japan). Debranching was performed under the following conditions: 0.1 U/mg of SLMD in 3 mM acetate buffer (pH 3.5) at 50 ˚C for 2.5 h. β-Amylolysis of SLMD using sweet potato enzyme (Type I-B, Sigma-Aldrich Co., MO, USA) was performed as follows: SLMD (20 U/mg) in 2 mM acetate buffer (pH 4.8) at 37 ˚C for 3 h. After enzymatic hydrolysis, total carbohydrate and reducing sugar contents were determined using maltose as a standard following the method described above, and β-AL (%) was calculated as reducing sugars/total carbohydrates × 100.

Molecular weight distribution analysis using gel-permeation chromatography. The molecular weight distribution of the maltodextrins was analyzed via gel-permeation chromatography (GPC) using connected columns at 35 ˚C, Shodex Ohpak SB-804H and SB802.5HQ (Resonac Corp., Tokyo, Japan). The samples were eluted with distilled water at a flow rate of 0.8 mL/min, and the refractive index was determined using a detector (RID-20A; Shimadzu Corp., Kyoto, Japan). Molecular weight was estimated using a calibration curve prepared using standard pullulans with known molecular weights (Resonac Corp., Tokyo, Japan): P-800, 853,000; P-400, 380,000; P-200, 186,000; P-100, 100,000; P-50, 48,000; P-20, 23,700; P-10, 12,200, and P-5, 5,800.

The size distributions of the maltodextrins were analyzed using cation-exchange chromatography on a high-performance liquid chromatography (HPLC) system equipped with an MCI GEL CK02AS column (Mitsubishi Chemical Corp., Tokyo, Japan) and a refractive index detector. Samples were eluted in distilled water at a flow rate of 1.0 mL/min at 80 ˚C, and the distributions were evaluated based on the DP of the constituent saccharides. DP was determined by comparing the retention time with the reference linear maltosaccharides with known DP.

Evaluation of aggregation properties. To evaluate aggregation, 10 % (wt/wt) solutions of dextrins were heated at boiling temperature for one minute for complete dissolution of the dextrins, and the solutions were stored at 4 ˚C for 18 h. Turbidity was visually observed. The stored solution was boiled again for 1 min, and changes in turbidity were observed. Additionally, the SLMD solution was subjected to three repetitions of a similar treatment protocol (storage at 4 ˚C for 24 h followed by re-heating at boiling temperature for 2 min), and the changes in turbidity were monitored by visual observation and measuring absorbance at 720 nm. Before observation, the test tube was vortexed and the precipitate was dispersed.

Moisture absorption under high humidity. Powders of maltodextrins were stored at 25 ˚C and a relative humidity of 94 % (under the saturated potassium nitrate aqueous solution atmosphere). The time course of the weight change was recorded, and after 99 h, the moisture-absorbed samples were evaluated visually.

RESULTS AND DISCUSSION

Structural characterization of SLMD via terminal-residue determination. DPn of the SLMD was 8.49 ± 0.21 (n = 4), indicating that the product's major components were maltooligosaccharides. This was smaller than the reported CLn values for maize amylopectins, which generally fall within 20−24 [10−12]. Based on their length, amylopectin unit chains can be divided into two groups: short cluster-composing chains (A and B1) and long cluster-connecting chains (B2 and longer) [13]. The CLn value of amylopectin is related to the ratio and average length of the two groups of unit chains. The short-chain group constitutes approximately 90 % by mole of the total unit chains and has a CLn of approximately 13 for A and 24 for B1 chains [12]. The long-chain group has a larger CLn of approximately 50−60 [12]. Therefore, the smaller DPn of SLMD compared to the CLn of the groups of unit chains indicated that the branching enzyme used to prepare SLMD acted on all B chains and some A chains as donor chains for the transfer reaction. The CLn (13.2) of the A chains of maize amylopectin [12] was overestimated because of the overlapping elution peaks of the A and B1 chains, and thus, the actual CLn of the A chains should be shorter. For the branching properties of bacterial branching enzymes, Guan et al. reported that the minimum

CL required for branching of both amylose and amylopectin substrate by *Escherichia coli* glycogen branching enzyme is 12 [14]. The *Rhodothermus* and *E. coli* enzymes used amylose as the preferred substrate over amylopectin [15], suggesting similar branching properties between the two enzymes. Takata et al. reported that the *Bacillus stearothermophilus* enzyme acted mainly on long B chains (B2 and longer) [16]. Therefore, only a small portion of the A chains fulfilling the criteria for minimum CL may act as donor chains in the branching reaction catalyzed by bacterial enzymes.

CLn, determined after debranching of SLMD with bacterial isoamylase, was 8.52 ± 0.60 (n = 4), consistent with the DPn value. The agreement between the DPn and CLn values indicated that the SLMD possessed no branched side chains hydrolyzable by isoamylase; thus, SLMD comprised linear chains. The linear nature of SLMD was further evidenced by complete digestion with β-amylase; the β-AL value was 106.8 ± 4.86 (n = 4).

A possible mechanism via which SLMD is formed from liquefied starch is shown in Fig. 1. Branching enzymes act on the A and B chains of amylopectin or amylose in liquefied starch and transfer an external part of a donor chain to form a new unit chain (shown as filled circles in Fig. 1 (b) and (c)). Thus, the chain lengths of the newly formed chains and the left part on the used donor chains inevitably become shorter than that of the original chain (Fig. 1 (b)). Debranching enzymes release these chains (Fig. 1 (c)). Although branching and debranching actions are separately depicted in the figure, both reactions are supposed to occur simultaneously. The enzymatic branching and debranching actions continue until the chain length becomes lower than the minimum requirement of the branching enzyme for a donor chain. Finally, a mixture of short linear malto-oligosaccharides, called SLMD, remains with no further reduction in chain length.

Analysis of the molecular weight of SLMD using GPC. In the GPC analysis, SLMD showed only one peak at a molecular weight of approximately 1,200 (Fig. 2 (a)). This result indicated that the major components of SLMD were saccharides with approximately DP 8, consistent with the DPn value of SLMD mentioned above. We analyzed commercially available DE 13 maltodextrin with a DE similar to that of the SLMD (13.7). DE 13 maltodextrin showed two major peaks at molecular weights of 21,000 and 660 and two minor peaks at 350 and 170 (Fig. 2 (b)). Similar results have been reported for commercial maltodextrins with a DE of approximately 10 [17]. Despite similar DE values, DE 13 maltodextrin and SLMD exhibited completely different compositions, and SLMD was unique as it was predominantly composed of saccharides of intermediate molecular weights. SLMD contained negligible amounts of polysaccharides of molecular weights exceeding 10^4 and a small amount of oligosaccharides of low DP. The unique saccharide composition of SLMD may be indicative of novel physicochemical properties.

Analysis of the DP distribution of SLMD using HPLC. The composition of SLMD was examined in detail. The DP distribution of SLMD is shown in Fig. 3 and Table 1. Medium and large oligosaccharides (DP 6−9) and small megalosaccharides (DP 10−12) were the most abundant components of SLMD. The DP distribution of the constituent saccharides of SLMD differed from that of other commercially available maltodextrins, for which a bimodal molecular weight distribution was observed, and the weight ratios of small oligosaccharides increased as the DE values of the maltodextrins increased [18, 19]. The novel DP distribution of SLMD was likely due to the combined action of a branching enzyme and isoamylase.

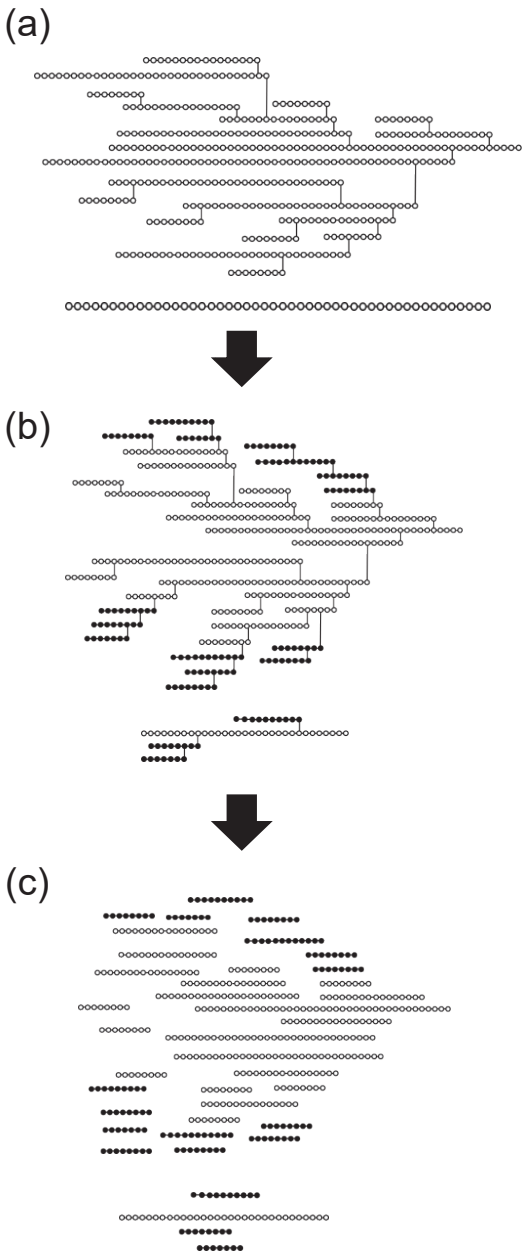

Fig. 1. A possible mechanism of SLMD formation.
(a) Model diagrams of amylopectin and amylose in liquefied starch, (b) after branching enzyme treatment of (a), (c) after debranching enzyme treatment of (b). Open circle, glucosyl residue in the unit chains; filled circle, glucosyl residues in the chains newly formed by branching enzyme action; vertical line, α-1,6-linkage.

Iodine absorption properties. The blue value and wavelength at maximum absorption (λmax) were determined from the saccharide–iodine spectra (Fig. 4). Iodine absorption of SLMD and DE 13 maltodextrin is presumably because the lengths of the component chains were sufficient to form a glucan–iodine complex. As shown in Table 1, linear chains of DP >20 constitute approximately 30 % of the weight of the

SLMD preparation. Similarly, DE 13 maltodextrin may contain unit chains capable of complex formation. The blue value of SLMD was 0.75, which was 4.7-fold higher than that of DE 13 maltodextrin (0.16). The λmax of SLMD was approximately 530 nm (Fig. 4), longer than that of DE 13 maltodextrin (500 nm). The DP distributions and degree of branching of the respective dextrins could explain these differences in the iodine absorption properties between the two maltodextrins. SLMD is rich in linear chains of DP 6−12 (Fig. 3), whereas these oligosaccharides are minor components of the DE 13 maltodextrin (Fig. 2). For linear malto-oligosaccharides, iodine absorption increases logarithmically with DP within the DP range of 6−13 and linearly for DP over 14 [20]. These short linear chains of SLMD exert negligible effect on the λmax values [20]; thus, the higher linearity of SLMD could be attributed to the longer λmax. The DPn of SLMD is presumed to result from the specificities of the branching and debranching enzymes used in the preparation, especially those for the lengths of the donor and transferred chains of the branching enzyme and for the cleavable lengths and positions of the branched side chains of the debranching enzyme. Therefore, the DP distribution, and consequently DPn, can be controlled by choosing the two enzymes used for SLMD production. The complex formation properties of the products can be altered by controlling the DP distribution.

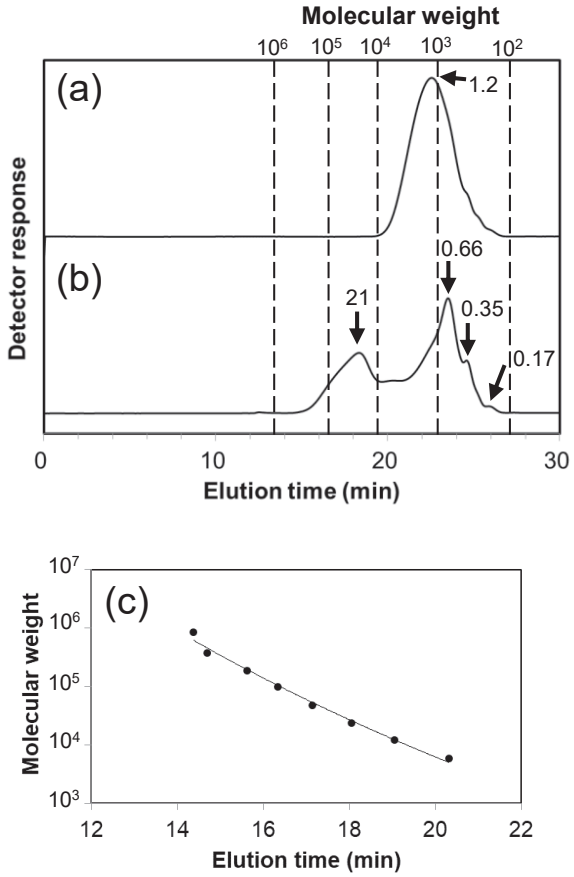

Fig. 2. Molecular weight distributions of maltodextrins.
(a) SLMD, (b) DE 13 maltodextrin, and (c) calibration curve. The arrows and numbers indicate molecular weight (×10³) at the indicated elution positions.

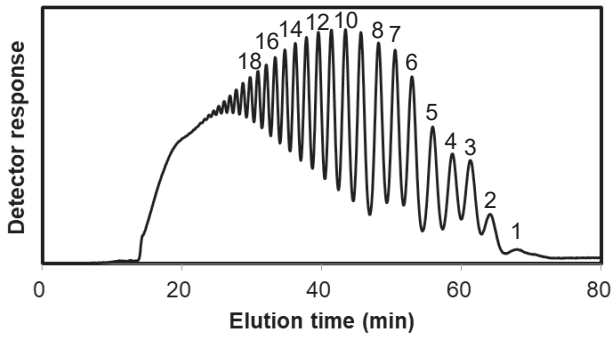

Fig. 3. Size (DP) distribution of SLMD.
The numbers indicate the degree of polymerization of each peak.

Aggregation of SLMD. The aggregation properties of SLMD were evaluated and compared with those of DE 13 maltodextrin. Clear solutions of saccharides were prepared by heating and stored at 4 ˚C for 18 h. The cooling phase promoted turbidity only in SLMD, and a portion of the turbid materials showed precipitation (Fig. 5). DE 13 maltodextrin was not turbid under the same conditions. Turbidity development and precipitate formation are attributed to the aggregation of the dispersed SLMD. Notably, the turbidity and precipitates observed in the SLMD solution completely disappeared after heating to a boiling temperature. Moreover, this interconversion of the turbid (by cooling) and clear (by reheating) states was reproducible (Table 2). In this study, up to three interconversion trials were successfully achieved and more rounds of conversion were feasible. These results indicated that the formation of turbid materials by SLMD can be reversed by heating, which is generally not the case for the retrogradation of starch or

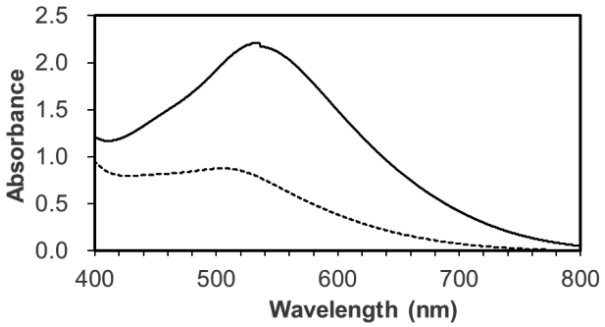

Fig. 4. Iodine absorption spectrum of a 0.1 % (wt/wt) solution of maltodextrins.
Solid line, SLMD; broken line, DE 13 maltodextrin.

Table 1. Saccharide composition of SLMD

DP	1	2	3	4	5	6	7	8	9	10
Area %	0.5	1.5	3.0	3.0	3.6	4.6	5.0	5.1	5.0	4.9

(Continued)

DP	11	12	13	14	15	16	17	18	19	20+
Area %	4.7	4.5	4.3	4.0	3.8	3.5	3.4	3.0	2.9	29.7

Table 2. Changes in the turbidity of the SLMD solution subjected to repeated cooling and heating.

Trial	A_{720}			
	SLMD		DE 13 Maltodextrin	
	Cooling	Heating	Cooling	Heating
Dextrin solution*	–	0.02	–	0.00
First	3.01	0.02	0.00	0.00
Second	3.12	0.02	0.00	0.00
Third	3.17	0.04	0.00	0.00

*Dextrins were dissolved via heating to obtain a 10 % (wt/wt) solution.

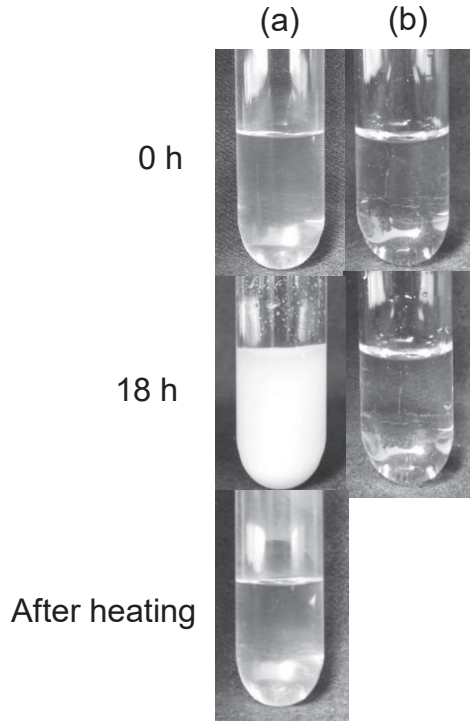

Fig. 5. Changes in the solubility of maltodextrins following storage at a low temperature.
(a) SLMD and (b) DE 13 maltodextrin.

amylose [21] owing to the formation of amylose networks with crystalline junction zones by six-fold amylose double helices [22]. Some commercial maltodextrins have been reported to produce thermally reversible gels, although their DE values are very low (DE 2−3) [23] or they contain large branched molecules as the major components (50 %) [24]. Thus, understanding the mechanism underlying the reversible aggregation of SLMD is of interest, considering their linear nature and relatively low molecular weight.

Recently, the self-assembly of linear malto-oligosaccharides and their resulting products have attracted research attention. Linear saccharides are prepared via the enzymatic debranching of gelatinized starches, and self-assembled spherulites display different susceptibilities to amylolytic enzymes [25, 26], along with the ability to encapsulate hydrophobic compounds, such as polyphenols, with various health-promoting effects [27]. The mechanism underlying self-assembly is essentially the same as that underlying retrogradation/aggregation. Considering the narrow DP distribution and moderate DPn of the saccharide components, SLMD is expected to exhibit distinct behaviors in self-assembly phenomena and complex formation; thus, the resulting assembly or complex could display properties different from those reported previously. In principle, the methods for preparing linear chains to be self-assembled in these previous studies inevitably resulted in a CL distribution of the products being similar in some ways to those of the starches used for preparing linear chains. Amylopectins are converted into a mixture of unit chains with bi- or trimodal CL distribution by enzymatic debranching. The steps involved in removing insoluble materials and/or self-assembly are thought to influence the CL distribution of glucans in the assembled spherulites, reportedly rich in B_1 chains (DP 13−24) [28].

Kim et al. [29] reported that the self-assembly conditions significantly affected the yields and physicochemical characteristics of the products. In this study, SLMD was produced without self-assembly. Therefore, together with the unique method of linear-chain preparation, incorporation of strategies for the self-assembly of debranched starch into SLMD production is expected to further expand the variations in the structural and physicochemical properties of the SLMDs.

The property of solidifying of SLMD under high humidity. When the powder of SLMD was stored at 25 ˚C, 94 % relative humidity, its weight increased due to water absorption up to 18 h. However, no more increase in weight was observed between 18−99 h (Fig. 6). This result differs from that of DE 13 maltodextrin, which showed a continuous weight increase during the time-course experiment. After 99 h, the appearance of the samples was visually evaluated. While the DE 13 maltodextrin powder changed to a syrup-like viscous fluid, the SLMD powder remained in a solid state without any sensible stickiness, binding to each other, forming a solid body (Fig. 7). This suggests that some structural changes occurred in SLMD upon moisture absorption, although the details of these changes are not fully understood.

Conclusions. We developed a production method for novel maltodextrins with short linear chains (SLMD) using a combination of branching and debranching enzymes. SLMD is distinct from other commercially available maltodextrins with similar DE values in several respects. SLMD mostly comprised saccharides with a DP of 6−12 and no high molecular weight glucans or small malto-oligosaccharides. SLMD showed a high tendency to aggregate and form inclusion complexes with iodine.

Remarkably, the interconversion of dissolution and aggregation occurred multiple times. SLMD absorbed moisture only to a limited extent, even under high relative humidity RH conditions, and did not become viscous but remained solid. These findings hint at the several potential applications of SLMD in the food industry. For example, in the confectionery industry, the solids of SLMD could be

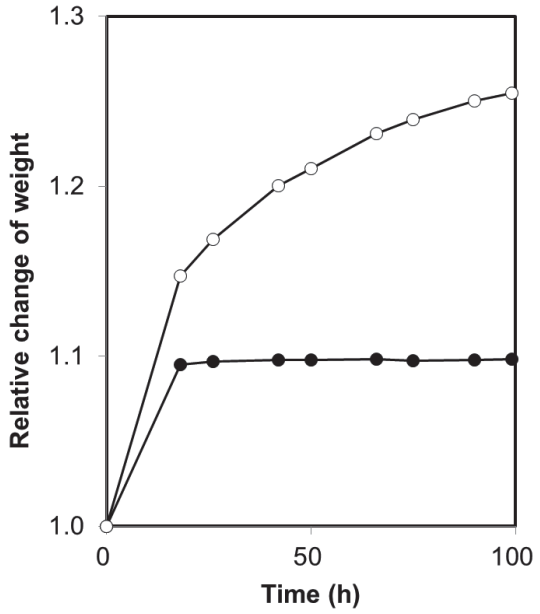

Fig. 6. Changes in the weight of maltodextrins over time under humid conditions.

The dextrins were placed under conditions of 94 % relative humidity at 25 ˚C. Closed circle, SLMD; open circle, DE 13 maltodextrin.

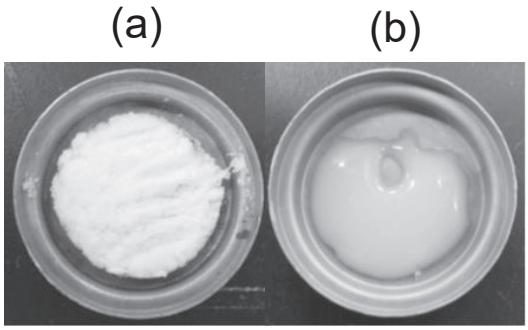

Fig. 7. Appearance of maltodextrins stored under high humidity conditions.

(a) SLMD and (b) DE 13 maltodextrin.

used as decorations or non-sticky sugar coatings, taking advantage of the fact that the solids do not absorb moisture. In addition, SLMD may be useful for imparting white color or changing the physical properties of various processed food items because of its ability to become cloudy or solidify in the presence of water. By replacing the enzymes used and incorporating a controlled self-assembly process, SLMD variants with different structures and properties can be expanded. Further research is needed to explore the variations and applications of SLMD.

CONFLICTS OF INTEREST

Atsushi Kawano, Tomohiro Yamamoto, Yuya Shinagawa, and Hironori Yoshida are employees of Showa Sangyo Co., Ltd.

ACKNOWLEDGEMENT

The authors thank Ms. S. Kimura for technical assistance.

REFERENCES

[1] Takasaki Y, Kitajima M, Tsuruta T, Nonoguchi M, Hayashi S, Imada K. Maltotriose-producing amylase from *Microbacterium imperial*. Agric Biol Chem. 1991; 55: 687−92.

[2] Nakakuki T. Present status and future prospects of functional oligosaccharide development in Japan. J Appl Glycosci. 2005; 52: 267−71.

[3] Takata H, Takaha T, Nakamura H, Fujii K, Okada S, Takagi M, et al. Production and some properties of a dextrin with a narrow size distribution by the cyclization reaction of branching enzyme. J Ferment Bioeng. 1997; 84: 119−23.

[4] Takata H, Kato T, Takagi M, Imanaka T. Cyclization reaction catalyzed by *Bacillus cereus* branching enzyme, and the structure of cyclic glucan produced by the enzyme from amylose. J Appl Glycosci. 2005; 52: 359−65.

[5] Harada T. Isoamylase and its industrial significance in the production of sugars from starch. Biotechnol Genet Eng. 1984; 1: 39−64.

[6] Lane JH, Eynon L. Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. J Chem Soc Ind Trans. 1923; 42: 32−6.

[7] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956; 28: 350−6.

[8] Somogyi M. Notes on sugar determination. J Biol Chem. 1952; 195: 19−23.

[9] Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem. 1944; 153: 375−80.

[10] Takeda Y, Preiss J. Structures of B90 (*sugary*) and W64A (normal) maize starches. Carbohydr Res. 1993; 240: 265−75.

[11] Jane JL, Chen YY, Lee LF, McPherson AE, Wong KS, Radosavljevic M, et al. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. Cereal Chem. 1999; 76: 629−37.

[12] Hanashiro I, Tagawa M, Shibahara S, Iwata K, Takeda Y. Examination of molar-based distribution of A, B and C chains of amylopectin by fluorescent labeling with 2-aminopyridine. Carbohydr Res. 2002; 337: 1211−5.

[13] Hizukuri S. Polymodal distribution of the chain lengths of amylopectins, and its significance. Carbohydr Res. 1986; 147: 342−7.

[14] Guan H, Li P, Imparl-Radosevich J, Preiss J, Keeling P. Comparing the properties of *Escherichia coli* branching enzyme and maize branching enzyme. Arch Biochem Biophys. 1997; 342: 92−8.

[15] Shinohara ML, Ihara M, Abo M, Hashida M, Takagi S, Beck TC. A novel thermostable branching enzyme from an extremely thermophilic bacterial species, *Rhodothermus obamensis*. Appl Microbiol Biotechnol. 2001; 57: 653−9.

[16] Takata H, Takaha T, Okada S, Hizukuri S, Takagi M, Imanaka T. Structure of the cyclic glucan produced from amylopectin by *Bacillus stearothermophilus* branching enzyme. Carbohydr Res. 1996; 295: 91−101.

[17] Wang YJ, Wang L. Structures and properties of commercial maltodextrins from corn, potato, and rice starches. Starch/Stärke. 2000; 52: 296−304.

[18] Avaltroni F, Bouquerand PE, Normand V. Maltodextrin

molecular weight distribution influence on the glass transition temperature and viscosity in aqueous solutions. Carbohydr Polym. 2004; 58: 323−34.

[19] Castro N, Durrieu V, Raynaud C, Rouilly A. Influence of DE-value on the physicochemical properties of maltodextrin for melt extrusion processes. Carbohydr Polym. 2016; 144: 464−73.

[20] Manners DJ, Stark JR. α-(1→4)-D-Glucans Part XXII. The iodine staining properties of linear maltosaccharides. Starch/Stärke. 1974; 26: 78−81.

[21] Miles MJ, Morris VJ, Orford PD, Ring SG. The roles of amylose and amylopectin in the gelation and retrogradation of starch. Carbohydr Res. 1985; 135: 271−81.

[22] Imberty A, Perez S. A revisit to the three-dimensional structure of B-type starch. Biopolymers. 1988; 27: 1205−21.

[23] McPherson AE, Seib PA. Preparation and properties of wheat and corn starch maltodextrins with a low dextrose equivalent. Cereal Chem. 1997; 74: 424−30.

[24] Schierbaum F, Radosta S, Vorwerg W, Yuriev VP, Braudo EE, German ML. Formation of thermally reversible maltodextrin gels as revealed by low resolution H-NMR. Carbohydr Polym. 1992; 18: 155−63.

[25] Kiatponglarp W, Rugmai S, Rolland-Sabaté A, Buléon A, Tongta S. Spherulitic self-assembly of debranched starch from aqueous solution and its effect on enzyme digestibility. Food Hydrocoll. 2016; 55: 235−43.

[26] Cai L, Shi YC. Self-assembly of short linear chains to A- and B-type starch spherulites and their enzymatic digestibility. J Agric Food Chem. 2013; 61: 10787−97.

[27] Remanan MK, Zhu F. Encapsulation of chrysin and rutin using self-assembled nanoparticles of debranched quinoa, maize, and waxy maize starches. Carbohydr Polym. 2024; 337: 122118.

[28] Oh SM, Park CS, Kim YR, Baik MY. Preparation and characterization of self-assembled short-chain glucan aggregates (SCGAs) derived from various starches. Food Hydrocoll. 2021; 114: 106517.

[29] Kim J, Oh SM, Kim HY, Choi JH, Shin JS, Bae JE, et al. Self-assembly kinetics of short-chain glucan aggregates (SCGA). Food Chem. 2023; 403: 134361.