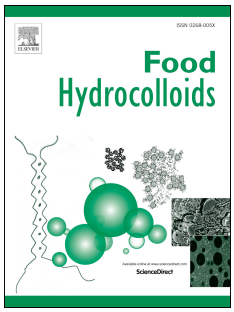

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A facile method for classifying starch fractions rich in long linear dextrin

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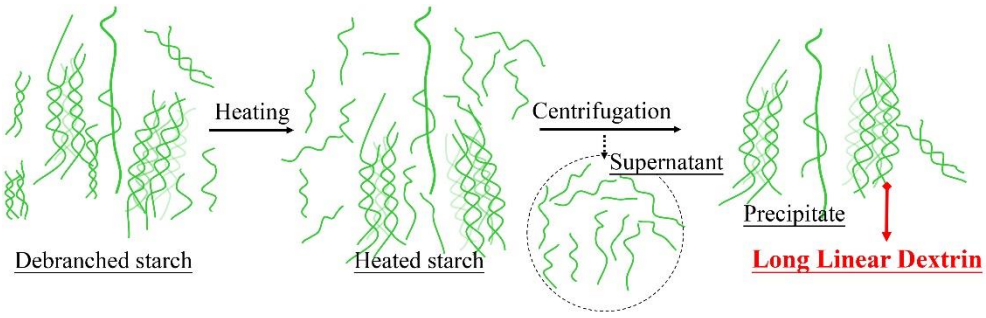

Abstract

Fractionation of linear dextrins with different chain lengths was of great significance for the preparation of functional foods. Traditional gradient non-solvent precipitation method for the fractionation was time-consuming and labor-intensive, making it inconvenient for large-scale dextrin fractionation. The aim of this study was to establish a novel method to fractionate long linear dextrins from debranched waxy maize starch by simply heating (70-80 ℃) and centrifuging the debranched starch solution. The results showed that the linear dextrins produced during the debranching process formed ordered structures with melting temperature of 58.8-95.4 ℃. According to the analysis of starch thermostability and chain length distribution, it was found that thermostability of ordered structure was significantly and positively correlated with the chain length of linear dextrins. During heating at 70 and 80 ℃, less ordered structures with weak thermostability were melted in solution, herein short linear dextrins were removed from debranched starch via centrifugation and thermostable ordered structures could be fractionated to obtain long linear dextrins. By this simple "heating-centrifugation" method, the content of long linear dextrins in starches was increased from 27.08% to 43.40%. This study provided a promising pathway to fractionate long linear dextrins.

Keywords

Starch fractionation, linear dextrins, chain length distribution, molecular size, starch thermostability

1. Introduction

Starch consisted of two groups of glucose polymers: (i) amylose, which was a linear polysaccharide made of α-D-glucose units, and (ii) amylopectin, which was a highly branched polymer of α-D-glucose units. Linear dextrin was a type of linear glucan derived from amylopectin following debranching enzymes such as isoamylase and pullulanase (Liu et al., 2017). It had great applications in many fields such as foods, pharmaceutics, and functional materials (Liu et al., 2017), *e.g.*, it readily formed gel networks and crystalline structures to slow starch enzymatic digestion (Liu et al., 2017), or formed nanoparticles to stabilize emulsion (Ge et al., 2017), or interacted with other ingredients, such as ethylene and curcumin, to form intelligent carriers to protecte these bioactive factors from degradation or rapid escape (Liu et al., 2022; Sun, Tian, Chen, & Jin, 2017). Notably, increasing the length of linear dextrin promoted starch reassembly to slow starch digestion (Chang et al., 2019), and enabled non-starchy ingredients such as ethylene and curcumin to interact with the dextrin, resulting in an ideal delivery system with controllable release behaviors (Liu et al., 2022; Sun et al., 2017; Zhan et al., 2021). Accordingly, fractionation of long-chain linear dextrins was rather important to control functionalities of foods prepared from the linear dextrins.

Fractionation of polysaccharides was mostly performed by chromatography, ultrafiltration and gradient non-solvent precipitation (Hu & Goff, 2018). Chromatography and ultrafiltration required columns and membranes, which limited their applications in polysaccharide fractionation because of their high equipment investment and operating costs (Hu et al., 2018). Fractionation by gradient non-solvent

precipitation corresponded to the gradual addition of a non-solvent (*e.g.*, methanol, ethanol, isopropanol, acetone and 1-butanol) into the polysaccharide solution to gradually precipitate the polysaccharides the solution. Long linear dextrin had a lower solubility in non-solvent solution compared with short linear dextrin, and herein long linear dextrin could be obtained via controlling the non-solvent content in the solution (Hu et al., 2018). As the gradient non-solvent precipitation was independent of special equipment and required simple equipment such as stirred tanks and centrifuges, it was herein widely used in dextrin fractionation (Chang et al., 2018; Hu et al., 2018; Qiu et al., 2016). However, the operation for the gradient non-solvent precipitation was time-consuming and labor-intensive, making this method inconvenient for large-scale dextrin fractionation. The development of a facile and feasible fractionation method will be helpful to isolate long linear dextrins with high efficiency.

It was noted that amylopectin with longer chain length tended to show a higher thermostability in solution (Wang et al., 2021; Zhang, Wang, Chen, & Zhong, 2019; Zhang et al., 2017), indicating ordered structures packed with long amylopectin may endure hot solution while less ordered structures formed with short amylopectin probably dissolved in solution during the heating. Accordingly, we hypothesized that helical structures packed with long linear dextrins had high thermostability and therefore likely to tolerate heated solutions. If helical and crystalline structures formed by linear dextrins were heated with controllable temperature in solution, the structures packed with short linear dextrins were expected to dissolve in solution, and then ordered structures formed by long linear dextrins could be obtained from the solution via

centrifugation (**Figure 1**). Results will offer a promising pathway to fractionate long linear dextrin-rich starch.

2. Materials and methods

2.1 Materials

Waxy maize starch (WMS) was purchased from Suzhou Gaofeng Starch Technologies Co., Ltd. (Suzhou, China). The contents of amylose, protein, lipid, and moisture of WMS were 0.20%, 0.02%, 0.01%, and 10.52%, respectively. Pullulanase (EC 3.2.1.41, enzyme activity 1000 U/mL) was purchased from Novozymes (batch No. ATS20059; Tianjin, China). Isoamylase (EC 3.2.1.68, enzyme activity ≥ 10000000 units/mg protein) and acetate was purchased from Sigma. Sodium azide solution was purchased from Sangon Biotech Co., Ltd (Shanghai, China). Ultra-pure water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Other reagents used were analytical grade.

2.2 Preparation of debranched starch

Starch suspension with a concentration of 10% (w/v) was prepared and cooked at 100 ℃ for 30 min. The temperature of cooked starch was cooled to 58 ℃ and the pH was adjusted to 5.2. Gelatinized starch was then fully debranched by pullulanase at 240 ASPU/g starch for 24 h (Shi, Sweedman, & Shi, 2018). Afterwards, starch suspension was frozen at -80 ℃ for 6 h and freeze-dried using a lyophilizer. The dried starches were added with ethanol to denature the pullulanase and dried at 40 ℃ for 24 h. Finally, samples were ground and sieved for further analysis. Debranched waxy maize starch was abbreviated as DWMS.

2.3 Classification of linear dextrin from debranched starch

Debranched starch was added with distilled water at 25 ℃ until the concentration was 5% (w/v). The suspension was heated at 60-90 ℃ for 20 min and its visual appearance was recorded using a camera. Additionally, the suspensions heated at 70 and 80 ℃ were centrifuged (5000 r/min, 5 min) immediately after the heating. The precipitate and supernatant were cooled to 25 ℃ and frozen at -80 ℃ for 6 h and freeze-dried using a lyophilizer. The precipitates isolated from heated DMS were abbreviated as P70 and P80, respectively, and the corresponding supernatants were abbreviated as S70 and S80, respectively.

2.4 Gelatinization properties of starches

Starch in a high-pressure stainless pan was added with distilled water to prepare starch suspension with a concentration of 30% (w/w). The pan was sealed with a gold-plated copper and equilibrated at 25 °C for 12 h before DSC analysis. An empty pan was used as a reference. Sample was heated from 30 to 130 °C at a scanning rate of 10 °C/min. The onset temperature (T_o) and end temperature (T_e) were calculated using DSC software (TA Instruments, New Castle, DE, USA) to evaluate starch thermostability.

In particular, S80 after the heating was cooled from 130 to 30 °C at a cooling rate of 10 °C/min, and then immediately heated from 30 to 130 °C at a scanning rate of 10 °C/min, to unravel how linear dextrin reassembled during the cooling. Rescanned S80 was abbreviated as S80-rescan-X, while the “X” corresponded to rescanning times.

2.5 Fine structures of starches

Molecular weight distributions of starches were characterized by a previous method (Zhao, Henry, & Gilbert, 2021) with slight modifications. Sample (5 mg) was thoroughly mixed with 5 mL of DMSO solution containing lithium bromide (0.5% w/w) (DMSO/LiBr) and heating at 90 °C using a thermomixer for 3 h. The molecular weight of starch sample was analyzed using size exclusion chromatography equipped with a differential refractive index detector (Optilab T-rEX, Wyatt Technology Co., Santa Barbara, CA, USA) (SEC-RI), two tandem columns (300 × 8 mm, Shodex OH-pak SB-805 and 803; Showa Denko K.K., Tokyo, Japan) which was held at 60 ℃ using a model column heater. The flow rate of mobile phase (DMSO/LiBr) was 0.3 mL/min. Standard dextrans of known molecular weights (342, 3650, 21000, 131400, 610500, 821700, 3755000) were used for column calibration. Data were acquired and processed using ASTRA6.1 (Wyatt Technology).

Additionally, starches were further treated with isoamylase and then detected with high-performance anion-exchange chromatography (HPAEC) on a CarboPac PA-200 anion-exchange column (4.0 × 250 mm; Dionex) using a pulsed amperometric detector (PAD; Dionex ICS 5000 system). Starch (10 mg) was dissolved in 5 mL of water in a boiling water bath for 60 min. Sodium azide solution (5 μL, 40 mg/mL), acetate buffer (0.1 mL, 0.1 M, pH 3.5), and isoamylase (2.5 μL) were added to starch solution, and the mixture was incubated in a water bath at 37 ℃ for 24 h. The hydroxyl groups of the debranched glucans were reduced by treatment with sodium borohydride (0.5%, w/v) under alkaline conditions for 20 h. Then, about 600 μL solution was dried in vacuo at

room temperature and redissolved in 30 μL of NaOH solution (1 M) for 60 min. The solution was diluted with 570 μL of distilled water and determined on ICS 5000 system with flow rate of mobile phase of 0.4 mL/min, injection volume of 5 μL, and mobile phase of NaOH solution (0.2 M NaOH, 0.2 M NaAc) with a gradient program: 90:10 (v/v) at 0 min, 90:10 (v/v) at 10 min, 40:60 (v/v) at 30 min, 40:60 (v/v) at 50min, 90:10 (v/v) at 50.1 min, and 90:10 (v/v) at 60 min. Data acquired on the ICS5000 (Thermo Scientific) were processed using chromeleon 7.2 CDS (Thermo Scientific). Technical support is provided by Sanshu Biotech. Co., LTD (Shanghai, China).

2.6 Statistical analysis

All tests were conducted at least in triple and the data were analyzed using IBM SPSS statistics version 21.0 (IBM, Armonk, NY, USA) by means of analysis of variance (ANOVA), followed by the Turkey's HSD test to compare the treatments and the significance level was set at $P < 0.05$.

3. Results and discussion

3.1 Visual appearance of debranched starch solutions at different temperatures

Figure 2 showed the visual appearance of debranched starch solutions at different temperatures. The debranched starch solution was milky and untransparent at 25 ℃, because starch particles with ordered structures did not dissolve in cold solution. The solution gradually became transparent as the temperature increased from 60 to 90 ℃. Notably, the heated solutions at 70 and 80 ℃ also contained starch particles in solution, indicating the heated solutions contained some thermostable starch structures. If starch

thermostability was highly correlated with starch chain length, starch structures packed with long linear dextrins could be fractionated via heating the starch solution and dissolving the less ordered structures in solution at 70 and 80 ℃. Herein, solutions heated at 70 and 80 ℃ were centrifuged to obtain the supernatants and precipitates to verify whether long linear dextrins contributed to the formation of thermostable starch particles and whether long linear dextrins could be obtained by the proposed method as shown in **Figure 1**.

3.2 Thermal properties of starches

Thermal properties of starches were shown in **Figure 3** and **Table 1**. Both WMS and DWMS exhibited a phase transition peak (G) over DSC curves, corresponding to the melting of helical structures (Liu et al., 2009). Cooked WMS generally did not contain ordered structures but DWMS contained a relatively high content of helical structures. Ordered structures of DWMS must be attributed to the reassembly of linear dextrins during the pullulanase treatment. Notably, the gelatinization of DWMS started at 58.8 ℃ and ended at 95.4 ℃, confirming that the debranched starch partially melted in solution as the temperature increased from 60 ℃ to 90 ℃.

Fractionated starches also showed a peak over DSC curves, indicating that all the starches contained relatively high content of helical structures. P70 and P80 gelatinized at 87.9-116.2 ℃ and 94.0-120.2 ℃, respectively, suggesting that the prepared starches had thermostable structures. P80 exhibited a higher gelatinization temperature than P70, confirming that more less ordered structures were removed from DWMS for preparing P80. Additionally, the gelatinization temperatures of P70 and P80 were higher than 70

and 80 ℃, respectively, which were mostly attributed to the reassembly of starch structures during heating treatment in terms of annealing (Chi et al., 2019). As S70 and S80 were prepared from the supernatant of the solution, S70 and S80 could probably not contain ordered structures because the supernatant only contained some linear dextrins and amorphous structures. However, linear dextrins would form helical and crystalline structures in 30 min at 4 ℃ (Sun, Gong, Li, & Xiong, 2014). This study also found that S80 after gelatinization and cooling still showed a peak over DSC curve during rescanning (**Figure S1** and **Table S1**), confirming that gelatinized S80 would form helical structures rapidly.

3.3 Starch molecular weight distributions

The molecular size distribution of WMS and debranched starches was analyzed using SEC-RI chromatograms. The RI response (**Figure 4A**) and relationships between SEC weight distributions ($w(\log(V_h))$ and molecular size (R_h) of starches (**Figure 4B**) were obtained. According to **Figure 4A**, one elution peak was observed at 35-60 min for WMS, corresponding to the elution of amylopectin. After pullulanase treatment, the peak shifted towards higher elution time, confirming hydrolytic degradation and a decrease in molar mass (Vidal, Bai, Geng, & Martinez, 2022). Notably, DWMS showed three elution peaks at *ca*. 50-65 min, 71-77 min, and 77-90 min, which were probably assigned to amylopectin with low molar mass, long linear dextrins, and linear dextrins, respectively. S70 and S80 only contained an elution peak at 65-90 min, suggesting S70 and S80 mostly contained only linear dextrins. Whilst, P70 and P80 showed three peaks at *ca*. 50-65 min, 71-77 min, and 77-90 min, confirming these two starches contained

small amylopectin, long linear dextrins, and short linear dextrins. Comparing with DWMS, P70 and P80, especially the P80, had higher peak intensity at 50-65 min and 71-77 min, suggesting these starches contained more small amylopectin and long linear dextrins.

According to **Figure 4B**, the molecular size (R_h) of WMS was *ca*. 30-200 nm, while the R_h of all debranched starch was much smaller (< 40 nm), confirming the degradation of starch during the pullulanase treatment (Li et al., 2020). Zhao et al. (2021) reported that starch chains with a R_h of *ca*. 30 nm were the amylopectin with low molar mass. Starch possibly produced small amylopectin with low molar mass during the debranching, and herein the faction with R_h of 5-80 nm mostly assigned to small amylopectin. That further confirmed that DWMS, P70, and P80 contained some small amylopectin. Notably, P70 and P80, especially the P80, showed higher peak intensity at the range of $1.7 < R_h < 5.0$ nm, suggesting these two starches contained more long linear dextrins.

Molecular weight distribution of starch was also shown in **Table 2**. All fractions of WMS showed molar mass higher than 800k g/mol. After pullulanase treatment, starches did not contain fraction larger than 800k g/mol, confirming starch degradation. Comparing with DWMS, S70 and S80 did not contain the fractions of $80k < M_w < 800k$ g/mol (DP 500-5000) but contained less fractions of $8k < M_w < 80k$ g/mol (DP 50-500) and more fractions of M_w < 8k g/mol (DP <50), suggesting S70 and S80 mostly contained more short linear dextrins. P70 and P80 contained more fractions of $80k < M_w < 800k$ g/mol comparing with DWMS, S70, and S80, corresponding to higher

content of small amylopectin. Additionally, P70 and P80, especially the P80, showed a lower proportion of fraction of M_w < 5k g/mol but higher proportions of fractions of 5k < M_w < 8k g/mol (DP 31-50) and 8k < M_w < 80k g/mol (DP 50-500), indicating these two starches probably contained more long linear dextrins.

3.4 Chain length distribution of starches

High-performance anion-exchange chromatograms of debranched starches were shown in **Figure S2**, and the chain length distribution of amylopectin were summarized in **Table 3**. The average chain length of different starches showed in the orders of S70 < S80 < DWMS < P70 = P80, indicating our proposed fractionation method showed high efficiency in fractionating long chain length. Comparing with DWMS, S70 and S80 showed more fractions of DP 6-24. S80 contained more fractions of DP > 25 in comparison with S70. Ordered structures packed with longer amylopectin showed higher thermostability (Wang et al., 2021; Zhang et al., 2019; Zhang et al., 2017). Accordingly, the supernatant of heated debranched starch solution contained more long chains at 80 ℃ than that at 70 ℃. P70 and P80 displayed less fractions of DP 6-24 and more fractions of DP > 25 comparing with DWMS. Additionally, P80 contained more fractions of DP ≥ 37 than P70 did, suggesting the heating at 80 ℃ favored the fractionation of long chains. According to **Figure 4**, P70 and P80 showed high peak intensity at the range of 1.7 < R_h < 5.0 nm (long linear dextrins). Herein, the long chains of P70 and P80 were assigned to long linear dextrins rather amylopectin with long chain length. That confirmed that our proposed method could be used for isolating long linear dextrins from debranched waxy maize starch via simply heating the solution containing

242 debranched starch.

243 **3.5 Underlying mechanism for the fraction of long linear dextrins**

244 Structural changes of starch structures during the fraction of long linear dextrins
245 were shown in **Figure 5**. Debranched starch produced during the pullulanase treatment
246 formed ordered structures with melting temperature of 58.8-95.4 ℃ (**Table 1**).
247 Thermostability of ordered structure was positively correlated with chain length of
248 linear dextrins (**Table 1** and **Table 3**). Accordingly, debranched starch partially
249 gelatinized and contributed to the melting of less ordered structures in solution at 70
250 and 80 ℃, and then released the short linear dextrins (**Table 2** and **Table 3**). The
251 supernatant of the centrifuged solution herein contained a relatively high content of
252 short linear dextrins. Notably, the short linear dextrins probably with DP 13-24 (**Table**
253 **3**) formed helical and crystalline structures rapidly during the cooling. Accordingly, the
254 short linear dextrins separated from the supernatant could be used to prepare functional
255 starch rich in helical and crystalline structures. On the other hand, the precipitates were
256 rich in long linear dextrins because of the removal of short linear dextrins. It seemed
257 that the thermostability of ordered structures determined the fractionation of long linear
258 dextrin according to our proposed "heating-centrifugation" method. Ordered structures
259 packed with long linear dextrins could tolerate hydrothermal treatment, and in turn,
260 thermostable ordered structures could be obtained via removing the short linear dextrins
261 simply by "heating-centrifugation" method to obtain starch rich in long linear dextrins.

262 **Conclusions**

263 In this study, we opened a promising pathway to fractionate long linear dextrin-

264 rich starch from DWMS by simply heating and centrifuging the debranched starch
265 solution. Linear dextrins produced during the debranching process formed ordered
266 structures with melting temperature of 58.8-95.4 ℃. Whilst, thermostability of ordered
267 structures was significantly and positively correlated with chain length of linear
268 dextrins. During heating at 70 or 80 ℃, less ordered structures with weak
269 thermostability were melted in solution and herein the short linear dextrins could be
270 removed by centrifuging the solution to obtain long linear dextrin-rich starch. It seemed
271 that the heating at high temperature (below the end temperature of starch gelatinization)
272 will be helpful to fractionate long linear dextrins. Although this study verified the
273 "heating-centrifugation" method could be used for fractionating long linear dextrin-rich
274 starch, how temperature and centrifugal force affected the fractionation efficiency of
275 long linear dextrins should be further investigated in the future. Additionally,
276 physicochemical properties and functionalities of these fractionated starch were also
277 parts of our future works.

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340 **Tables**

341 **Table 1** Gelatinization parameters of starches

Sample	T_o (°C)	T_e (°C)
WMS	71.4 ± 0.1^{c}	88.08 ± 0.1^{e}
DWMS	58.8 ± 0.5^{e}	95.35 ± 0.2^{c}
S70	63.4 ± 0.6^{d}	91.08 ± 0.5^{d}
P70	87.9 ± 0.8^{b}	116.23 ± 0.1^{a}
S80	55.7 ± 0.1^{f}	85.57 ± 0.1^{f}
P80	94.0 ± 0.6^{a}	120.19 ± 0.0^{b}

342 Values are the means of three replicates ± SD. Values with different letters in the same

343 column are significantly different ($P < 0.05$).

344 **Table 2** Molecular weight distribution of starches

Sample	< 1.6 k (g/mol) (%)	1.6k-5k (g/mol) (%)	5k-8k (g/mol) (%)	8k-80k (g/mol) (%)	80k-800k (g/mol) (%)	> 800k (g/mol) (%)
WMS	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}	100 ± 0.00^{a}
DWMS	23.67 ± 0.16^{b}	49.44 ± 0.06^{c}	11.88 ± 0.03^{d}	11.49 ± 0.06^{c}	1.53 ± 0.00^{c}	0.00 ± 0.00^{b}
S70	26.63 ± 0.19^{a}	56.82 ± 0.11^{b}	10.34 ± 0.08^{e}	6.20 ± 0.15^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
P70	9.87 ± 0.09^{c}	44.59 ± 0.04^{d}	18.13 ± 0.02^{b}	21.69 ± 0.01^{a}	2.81 ± 0.00^{b}	0.00 ± 0.00^{b}
S80	23.79 ± 0.18^{b}	57.20 ± 0.08^{a}	12.09 ± 0.04^{c}	6.92 ± 0.06^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}
P80	9.47 ± 0.08^{d}	39.72 ± 0.04^{e}	26.64 ± 0.07^{a}	14.86 ± 0.07^{b}	5.07 ± 0.00^{a}	0.00 ± 0.00^{b}

345 Values are the means of two replicates ± SD. Values with different letters in the same

346 column are significantly different ($P < 0.05$).

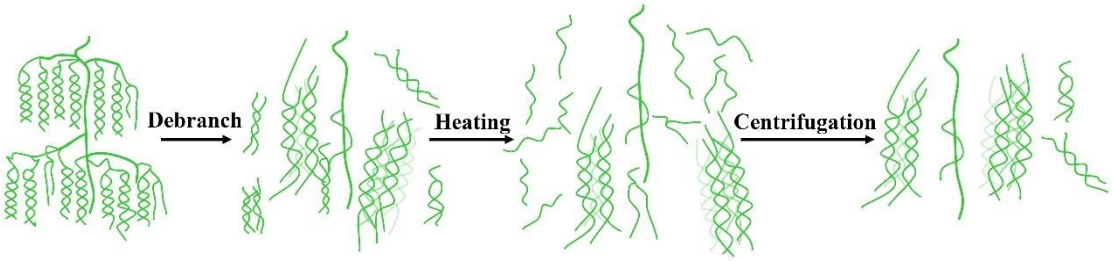
347 **Table 3** Chain length distribution of amylopectin of debranched starches

Sample	DP 6-12 (%)	DP 13-24 (%)	DP 25-36 (%)	DP ≥ 37 (%)	Average DP (%)
DWMS	22.84 ± 0.18^{c}	50.08 ± 0.15^{c}	14.85 ± 0.25^{c}	12.23 ± 0.28^{c}	21.37 ± 0.29^{b}
S70	25.68 ± 0.21^{a}	52.77 ± 0.24^{a}	13.63 ± 0.16^{d}	7.92 ± 0.26^{e}	19.47 ± 0.22^{d}
P70	12.90 ± 0.19^{e}	44.05 ± 0.22^{d}	21.04 ± 0.23^{a}	22.01 ± 0.25^{b}	26.22 ± 0.26^{a}
S80	24.00 ± 0.22^{b}	50.93 ± 0.28^{b}	14.72 ± 0.17^{c}	10.35 ± 0.29^{d}	20.58 ± 0.30^{c}
P80	13.63 ± 0.27^{d}	42.97 ± 0.19^{e}	20.74 ± 0.16^{b}	22.66 ± 0.31^{a}	26.37 ± 0.32^{a}

348 Values are the means of two replicates ± SD. Values with different letters in the same

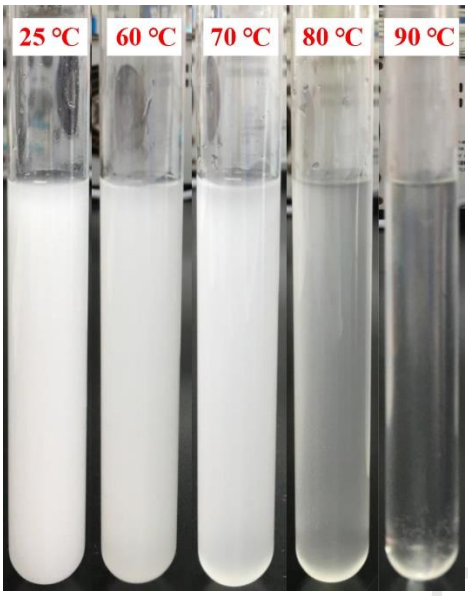
349 column are significantly different ($P < 0.05$).

350 **Figures**



351

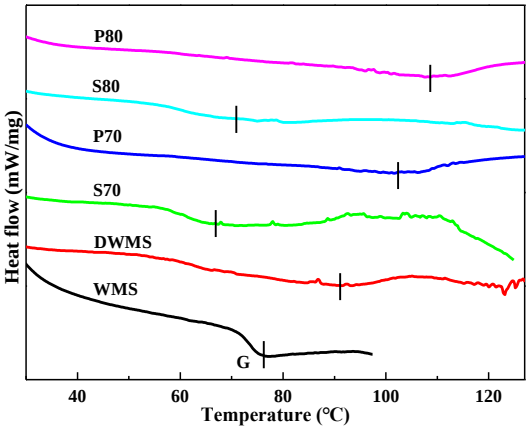
352 **Figure 1** Schematic illustration of long linear dextrin fractions



353

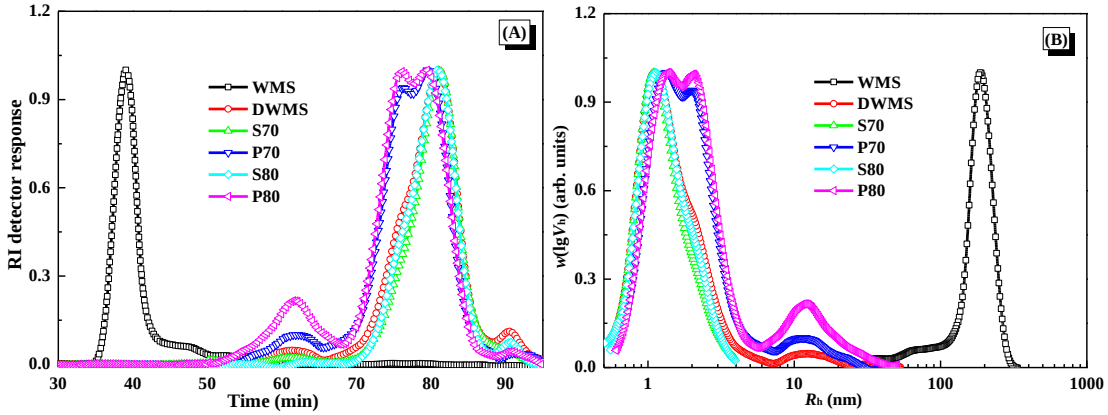
354 **Figure 2** Visual appearance of debranched starch solutions at different temperatures.

355 The concentration of debranched starch was 5% (w/v)



356

357 **Figure 3** DSC curves of starches



358

359 **Figure 4** RI response of SEC-RI chromatogram (A) and relationships between SEC

360 weight distributions ($w(\log(V_h))$ and molecular size (R_h) of starches

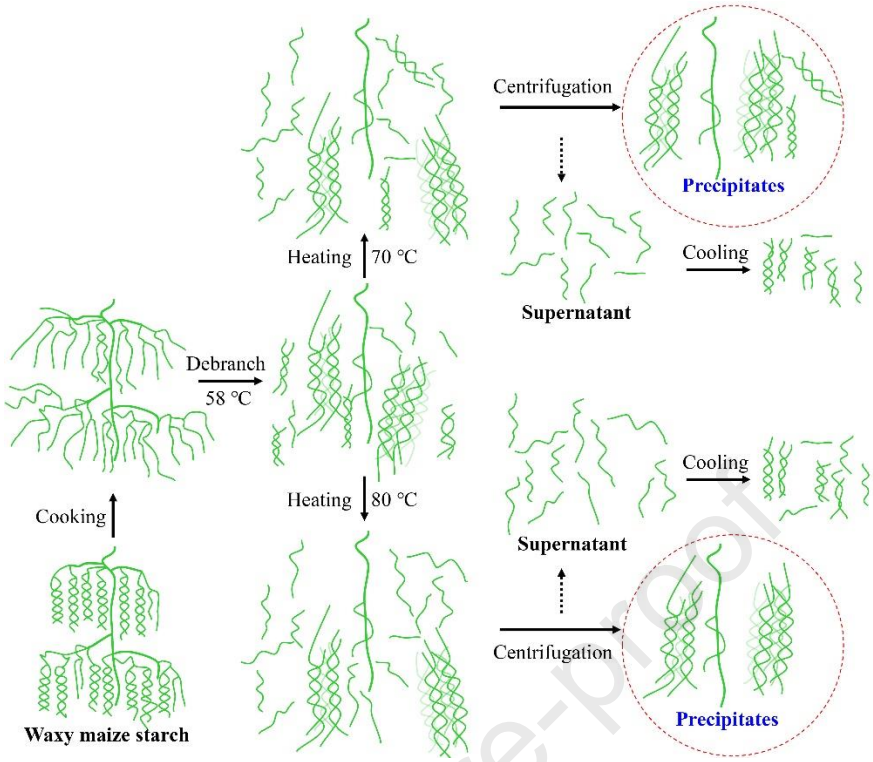


Figure 5 Schematic illustration of starch structure changes during the fractionation of long linear dextrins

- A method for isolating long linear dextrin from debranched starch was established.
- Ordered structure packed with long linear dextrin (LD) had high thermostability.
- Ordered structure was fractionated to obtain long LD by heating and centrifugation.
- Long LD content in starch was increased from 27.08% to 43.40% after the treatment.
- Short LD with polymerization units of 13-24 formed helical structure rapidly.

Conflict of Interest

The authors declare no competing financial interest.

Chengdeng Chi: conceptualization, literature analysis, investigation, writing, reviewing, and editing; Youcai Zhou: investigation and reviewing; Bilian Chen and Yongjin He: reviewing and editing; Yingting Zhao: writing, reviewing, and editing.