Enzymatic modification of corn starch with 4-α-glucanotransferase results in increasing slow digestible and resistant starch

Huan Jiang, Ming Miao*, Fan Ye, Bo Jiang*, Tao Zhang

State Key Laboratory of Food Science & Technology, Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, 1800 Liha Avenue, Wuxi, Jiangsu 214122, PR China

A R T I C L E   I N F O

Article history:
Received 9 November 2013
Received in revised form 9 January 2014
Accepted 17 January 2014
Available online 24 January 2014

Keywords:
Slowly digestible starch
Resistant starch
4-α-Glucanotransferase
Amylose
Molecular weight
Chain length

A B S T R A C T

In this study, partial 4-α-glucanotransferase (4αGT) treatment was used to modulate the fine structure responsible for the slow digestion and resistant property of starch. Normal corn starch modified using 4αGT for 4 h showed an increase of slowly digestible starch from 9.40% to 20.92%, and resistant starch from 10.52% to 17.63%, respectively. The 4αGT treatment decreased the content of amylose from 32.6% to 26.8%. The molecular weight distribution and chain length distribution of 4αGT-treated starch showed a reduction of molecular weight and a great number of short (DP < 13) and long (DP > 30) chains through cleaving and reorganization of starch molecules. Both the short and long chain fractions of modified amyllopectin were attributed to the low in vitro digestibility. The viscosity was inversely related to the digestibility of the 4αGT-treated starch. These results suggested that the 4αGT modified starch synthesized the novel amyllopectin clusters with slow digestible and resistant character.

Crown Copyright © 2014 Published by Elsevier B.V. All rights reserved.

1. Introduction

In human nutrition, starch plays a major role in supplying metabolic energy, which enables the body to perform a multitude of functions. Based on the rate and extent of digestibility, starch can be quantified into three consecutive fractions using the in vitro Englyst assay: the starch fraction digested within 20 min of incubation is classified as rapidly digestible starch (RDS), the starch fraction digested between 20 and 120 min corresponds to slowly digestible starch (SDS), and the remaining fraction that is not further digested is resistant starch (RS) [1,2]. RDS induces a fast increase in blood glucose and insulin levels, which can induce a series of health complications, such as diabetes and cardiovascular diseases. SDS is slowly digested throughout the small intestine, resulting in a slow and prolonged release of glucose into the blood stream, namely low glycaemic response. This starch type may be helpful in controlling and preventing hyperglycemia-related diseases. Moreover, RS is the starch portion that cannot be digested in the small intestine, but instead is fermented in the colon as dietary fiber, which may prevent disease and lead to better colonic health [1–5]. Therefore, improving food quality with higher amounts of SDS is becoming an area of interest for researchers from industry and academia, due to fewer amounts of SDS in thermal processing carbohydrate products. Meanwhile, there are limit reports on SDS preparation by using enzymatic method [2,6].

4-α-Glucanotransferase (4αGT, EC 2.4.1.25) is a member of the α-amylase super-family, and also known as amylomalase or D-enzyme involved in starch metabolism with multiple action modes (disproportionation, cyclization, coupling and hydrolysis) [7–10]. This enzyme catalyzes a chemical reaction that transfers a segment of a 1,4-α-D-glucan to a new position in an acceptor carbohydrate, which may be glucose or a 1,4-α-D-glucan [9]. In the earlier studies, 4αGT was widely used in starch processing, such as cycloamylose [11–13], isomalto-oligosaccharide [14], thermo-reversible starch gel [15–18] and fat substitute [19,20]. Also, 4αGT has the ability to retard starch retrogradation and improve the shelf life of rice cake and bread [21]. However, little work has been reported 4αGT using as a tool to sculpt the structure of starch for in vitro digestion modulation. In the present study, corn starch was subjected to 4αGT treatment and the changes of structure and digestibility were investigated. The impact of fine structure on the low digestibility of corn starch was also characterized.

2. Materials and methods

2.1. Materials

Normal corn starch was purchased from Shanghai Yuanju Biological Technology Co., Ltd. (Shanghai, China). Alpha-amylase (Cat. No. A3176, type VI-B, ≥ 10 units/mg solid) from porcine pancreas
and amyloligosidase (Dextrzyme® GA, 51,000 units/g) from Aspergillus niger were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO) and Novozymes (Tianjin, China), respectively. Isoamylase and the glucose oxidase-peroxidase assay kits were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). The pET-22b(+) expression vector was obtained from Novagen (Darmstadt, Germany). Oligonucleotides were synthesized by Generay Biotech Co., Ltd. (Shanghai, China). The resin for protein purification and the Chelating Sepharose Fast Flow were obtained from General Electric Company (Uppsala, Sweden). Electrophoresis reagents were purchased from Bio-Rad Laboratories (Shanghai) Co., Ltd. (Shanghai, China). All chemicals were reagent grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Cloning and preparation of 4αGT

The complete genome sequence of Acidothermus cellulolyticus 11B has been released in National Center for Biotechnology Information (GenBank accession number: CP000481.1). The full-length nucleotide sequence of 4αGT gene with locus tag AceI_1601 from the strain was synthesized and incorporated with Ndel and Xhol sites in 5’- and 3’-terminal of the gene, and then was cloned into the expression vector pET-22b(+). An in-frame 6 × histidine-tag sequence at the C-terminus was provided in the recombinant plasmid.

The recombinant Escherichia coli BL21 (DE3) harboring 4αGT gene was cultivated with shaking (200 rpm) in Luria-Bertani medium (LB medium: 1% Bacto-tryptone, 0.5% yeast extract, 0.5% NaCl) containing ampicillin (50 μg/ml) at 37 °C. The extracellular expression was performed at 28 °C for 8 h by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to 0.5 mM when the absorbance at 600 nm was 0.6. After induction, the cell sediment was collected by centrifugation (10,000 × g for 10 min) and then disrupted by sonication (pulsations of 3 s, amplify 90, 6 min) at 4 °C for the further protein purification. The target protein was expressed as 6 × his-tagged fusion protein and purified by Ni-NTA affinity chromatography (Novagen) according to manufacturer’s protocol (pET His Taq System, Novagen). Purity of the protein was confirmed electrophoretically using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and the optimum reaction temperature and pH of the enzyme were determined as 75 °C and 7.5, respectively. 4αGT activity was determined by measuring the optical change in iodine-staining during the conversion of amylose by the enzyme as suggested by Liebl et al. [22]. One unit of 4αGT activity was defined as the amount of enzyme which degrades 0.5 mg/ml of amylose per min under the assay conditions used.

2.3. Enzymatic modification of corn starch

The normal corn starch slurry (8%, w/v in pH 7.5, 50 mM Tris–HCl buffer solution) was cooked in a water bath at 95 °C for 60 min. The temperature of the starch sample was adjusted to 75 °C, and 4αGT (10 U/g dry weight of starch) was added to the solution. The enzymatic reaction was incubated for 0.5, 1, 1.5, 2, 4, 6, 10, or 12 h. Immediately after the reaction, the solutions were placed in an autoclave set at 121 °C for 30 min to stop reaction and cooled to room temperature, and then 1 volume of 95% ethanol (w/v) was added to facilitate the precipitation of the reactant. The precipitated starch was collected by centrifugation at 5000 × g for 10 min, washed with deionized water and collected by centrifugation twice, then freeze-dried. The collected material was ground to form a powder (120 mesh) and stored in a desiccator for further analysis. The enzymatically hydrolysed samples were named M51, M52, M53, M54, M55 and M56, respectively, based on the enzymatic reaction times listed above. The supernatant of the hydrolysed starch product, with the enzymatic reaction stopped at different time point, was collected for the determination of the degree of hydrolysis using the phenol–sulphuric acid method [23]. To 1.0 ml of the supernatant, 1.0 ml of phenol solution (6%) and 5.0 ml of concentrated sulphuric acid (95.5%) were added and then mixed well. After cooling to room temperature, the solution was put into a quartz cuvette, and its absorbance was determined at 490 nm using a UV/visible Spectrophotometer (UV-2102PC, Unico Instrument Co., Ltd., Shanghai, China).

2.4. Amylose content and iodine binding analysis

The amylose content of the starch sample was determined using the colorimetric method [25]. The iodine binding analysis was measured using a UV/visible Spectrophotometer (UV-2102PC, Unico Instrument Co., Ltd., Shanghai, China) according to the method described by Shen et al. [26]. An iodine reagent was prepared by adding 2 mg of I₂ and 20 mg of KI to 1 ml of deionised water. The reagent was stored in a non-acticin bottle at room temperature. The dried starch sample (50 mg) was dispersed in deionised water in a 10 ml screw-cap vial. The diluted solution (0.5 ml) was mixed with the iodine solution (1.5 ml) and then adjusted to a final volume of 2 ml with water. The absorbance spectra and the wavelength of maximum absorption (λmax) were analyzed over a wavelength scan of 500 to 800 nm.

2.5. In vitro digestion using the Englyst assay

The digestibility of the starch was analyzed according to the procedure of Englyst et al. [24] with a slight modification. To prepare Enzyme Solution I, amyloligosidase solution (0.14 ml) was diluted to 6.0 ml with deionised water. Enzyme Solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 ml) with magnetic stirring for 10 min, then centrifuging the mixture for 10 min at 1500 × g. Finally, a portion (54.0 ml) of the supernatant was transferred into a beaker. Enzyme Solution III was prepared immediately before use by mixing Enzyme Solution I (6.0 ml), Enzyme Solution II (54.0 ml) and water (4.0 ml).

The starch sample (200 mg) was dispersed in 15 ml of phosphate buffer (0.2 M, pH 5.2) by vortexing. After the solution was equilibrated at 37 °C for 5 min, seven glass balls (10 mm diameter) and Enzyme Solution III (5.0 ml) were added. Then, the samples were shaken in a 37 °C water bath at 150 rpm. Aliquots of hydrolysed solution (0.5 ml) were taken at different time intervals and mixed with 4 ml of absolute ethanol to denature the enzymes. The glucose content of the hydrolysate was determined using the glucose oxidase/peroxidase assay kits. The percentage of hydrolysed starch was calculated by multiplying the glucose content by a factor of 0.9. Each sample was analyzed in triplicate.

The values of the different carbohydrate nutritional fractions (RDS, SDS and RS) were obtained by combining the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose), and TG (total glucose) using the following formulas:

\[%RDS = \left( G_{120} - FG \right) \times 0.9 \times 100 \]
\[%SDS = \left( G_{120} - G_{20} \right) \times 0.9 \times 100 \]
\[%RS = \left( TG - FG \right) \times 0.9 \times 100 - \%RDS - \%SDS \]
2.6. High-performance size-exclusion chromatography (HPSEC) analysis

The starch samples (10 mg) were added to 5 ml of deionised water and boiled with stirring for 15 min to completely dissolve the samples using DMSO with 50 mM LiBr at a concentration of 2% (w/v). The dissolved samples, filtered through 5 μm cellulose acetate filters (Whatman, Maidstone, UK), were injected into a high performance size exclusion chromatography system with a multi-angle laser light scattering detector and a refractive index detector (HPSEC-MALLS-RID) [Wyatt Technology, Santa Barbara, CA]. Two series tandem columns (300 x 8 mm, Shodex OH-pak SB-806 and 804, Showa Denko K.K., Tokyo, Japan) with an OH-pak SB-G guard column, a DAWN HELEOS II laser photometer fitted with a He–Ne laser (\( \lambda = 632.8 \) nm) with a K-5 flow cell, and an OPTILAB\textsuperscript{®} T-rEX interferometric Refractometer were used. The flow rate was set at 0.5 ml/min with a mobile phase of distilled-deionised water (pH 6.8, 18.2 MΩ cm) containing 0.02% NaN\(_3\). A dn/dc value of 0.138 was used in molecular weight calculations, and data processing was performed using ASTRA software (Version 5.3.4.14, Wyatt Technology, USA).

2.7. High-performance anion-exchange chromatography (HPAEC) analysis

The chain length distribution of starch was determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The samples (10 mg) were dissolved with 2 ml of NaNO\(_3\) solution (pH 4.0, 0.1 M) and heated in a boiling water bath for 10 min. Isoamylase (0.5 U) was added to each dispersion, and the mixtures were incubated at 40 °C with shaking for 24 h. Then, the solution was heated in a boiling water bath for 10 min to deactivate the enzyme. The debranched samples were filtered through a 0.45-μm membrane filter and then injected into the HPAEC-PAD system (50 μl sample loop). The HPAEC-PAD system consisted of a Dionex DX 600 equipped with an ED 50 electrochemical detector with a gold working electrode, GP 50 gradient pump, LC 30 chromatography oven, and AS 40 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, with the following periods and pulse potentials: \( T_1 = 0.40 \) s, with 0.20 s sampling time, \( E_1 = 0.05 \) V; \( T_2 = 0.20 \) s, \( E_2 = 0.75 \) V; \( T_3 = 0.40 \) s, \( E_3 = -0.15 \) V. The data were collected using Chromeleon software, version 6.50 (Dionex Corporation, Sunnyvale, CA, USA). Eluents were prepared in distilled deionised water with helium sparging: eluent A was 150 mM NaOH, and eluent B was 50 mM sodium acetate in 150 mM NaOH. Linear components were separated on a Dionex CarboPac\textsuperscript{TM} PA1 column with gradient elution (40% of eluent B at 0 min, 50% at 2 min, 60% at 10 min, and 80% at 40 min) at 30 °C and a flow rate of 1 ml/min.

2.8. Rheological properties analysis

The starch suspension (6%, v/v) was cooked in a boiling water bath for 30 min. The rheological properties were determined using a stress-controlled rheometer (AR2000, TA Instrument, New Castle, DE, USA) fitted with a stainless steel cone of 40 mm diameter and 2° angle and solvent trap in steady-state flow parameters. The measurement temperature was kept at 25 °C using a circulating bath and a controlled peltier system. The apparent viscosity of starch was determined using a range of shear rates of 0.1–100 s\(^{-1}\) and the resulting flow curves analyzed.

2.9. Statistical analysis

All data were analyzed by the Duncan test using the statistical analysis system (SAS Institute, Cary, NC). A level of 0.05 was set to determine statistical significance.

3. Results and discussion

3.1. 4oGT treatment

The hydrolysis profile of normal corn starch during the 4oGT treatment over 12 h is presented in Fig. 1. The degree of hydrolysis of normal corn starch over time increased substantially up to 30.67% in the first 4 h, and then incrementally increased to 36.65% at 12 h. Combined with the data of amylose (Table 1) and digestibility (Fig. 2), this result indicated that starch molecules were broken down and modified as well, in line with the report of Cho et al. [12]. They found that the hydrolyzed products from starch increased during enzyme treatment, and was consist of amylose and small amylpectin clusters by hydrolysis and disproportionation of 4oGT. Normal corn starch subjected to 4oGT treatment led to degrade amylose and amylpectin macromolecules, resulting in a range of small clustered amylpectins and oligosaccharides.

3.2. In vitro starch digestibility

The enzymatic digestibility of the 4oGT treated starch sample is shown in Fig. 2. The Englyst assay is a classical method to categorize starch fractions based on starch digestion rates. RDS,

**Table 1** Amylose and molecular weight of 4oGT treated starches.

<table>
<thead>
<tr>
<th>Amylose (%)</th>
<th>( M_w )</th>
<th>Peak A (10(^5) g/mol)</th>
<th>Peak B (10(^5) g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.6 ± 1.0(^a)</td>
<td>3.16 ± 0.06(^a)</td>
<td>0.56 ± 0.21(^a)</td>
</tr>
<tr>
<td>MS1</td>
<td>30.9 ± 0.3(^a)</td>
<td>1.35 ± 0.12(^b)</td>
<td>0.64 ± 0.09(^b)</td>
</tr>
<tr>
<td>MS2</td>
<td>28.1 ± 0.7(^c)</td>
<td>0.58 ± 0.00(^d)</td>
<td>1.08 ± 0.06(^d)</td>
</tr>
<tr>
<td>MS3</td>
<td>28.2 ± 1.3(^e)</td>
<td>0.31 ± 0.04(^e)</td>
<td>1.14 ± 0.08(^e)</td>
</tr>
<tr>
<td>MS4</td>
<td>27.2 ± 0.8(^f)</td>
<td>0.23 ± 0.07(^f)</td>
<td>1.22 ± 0.00(^f)</td>
</tr>
<tr>
<td>MS5</td>
<td>27.1 ± 0.0(^g)</td>
<td>0.12 ± 0.10(^g)</td>
<td>1.93 ± 0.07(^g)</td>
</tr>
<tr>
<td>MS6</td>
<td>26.8 ± 1.5(^h)</td>
<td>0.11 ± 0.01(^h)</td>
<td>3.57 ± 0.15(^h)</td>
</tr>
</tbody>
</table>

Significant difference in each column is expressed as different superscript letters (\( p<0.05 \)).
SDS and RS, ordered consecutively by reaction times, represent the three different starch materials found in cooked starches. The 4αGT treated starch samples had an increase in slow digestion property, which was significantly different from the control starch sample. The cooked normal corn starch (control sample) had 80.08% RDS, 9.40% SDS and 10.52% RS. Compared to the control sample, the percentage of RDS in all of the 4αGT treated starch samples was 77.01%, 72.97%, 69.02%, 61.97% and 60.40% for MS1, MS2, MS3, MS4, MS5 and MS6, respectively; the content of SDS was 11.91%, 14.55%, 17.08%, 20.92%, 19.00%, 18.26% for the six samples, respectively. 4αGT treated starch also had a greater RS than the control sample, but the RS was composed of different content in range of 11.08–21.34%. SDS content increased as the time increased, reaching a maximum at about 4 h, and decreasing thereafter, whereas the RS content increased. Lee and coworkers [27] reported that the hydrolysis rate of 4αGT-treated starch was lower than that of the control starch, presumably due to an increase in crystallinity and the cycloamylose formation. In general, the cycloamylose was less susceptible to amylose action than the linear oligosaccharide [28]. Meanwhile, an increased number of branched molecules and the re-arrangement of branches in amylpectin of 4αGT-modified starch influenced the crystalline structure. According to the studies of Takaha and Smith [9] and Cho et al. [12], 4αGT transfers the moiety of glycosyl residues to amylpectin and tentatively increases the length of branch chains. This enzyme can further rearrange the existing amylpectin branch chains, and during the prolonged period of reaction, certain long chains will associate with adjacent side chains and then spontaneously produce partial crystalline lamella. In some amylpectin molecule, however, the chain length was too short to associate with other chains. Inside these newly generated clustered amylpectin molecules, crystalline and amorphous regions may co-exist. Consequently, these molecules led to a more resistant property, which was consistent with the results obtained in this study.

3.3. Amylose content and iodine binding

Based on the colorimetric iodine method, the percentage of amylose in normal corn starch was 32.6% (Table 1). For starch subjected to 4αGT treatment, the content of amylose decreased from 30.9% for MS1 to 26.8% for MS6. The decrease in amylose during 4αGT treatment was caused by a preferential hydrolysis on amylose molecules, which was in agreement with the research of Do et al. [29]. They also reported that there were several possible fates of amylose on 4αGT treatment: (a) segments of amylose chains could be transferred to amylpectin branch chains by inter-molecular transglycosylation, (b) cyclic glucans could form by intra-molecular transglycosylation, and (c) smaller linear chains could remain through (a) and (b).

Both amylose and amylpectin can bind iodine to form a color complex, which is measured spectrophotometrically to estimate the affinity of the polysaccharide structure for iodine. The absorbance and the wavelength of the maximum absorbance (λmax) varies in the spectrophotometric method depending on the degree of polymerization (DP) and the structural properties of the starch molecules, which determine the composition of the polyiodine chains complexed with the linear chain helices [26,30]. Amylose has high iodine absorption values and amylopectin with many short linear chains displays low values. As shown in Fig. 3, the iodine binding with the control starch showed λmax of approximately 600 nm and absorbance of 0.71, while both λmax and absorbance reduced substantially with initial 4αGT treatment. The blue color developed for the enzyme modified starch, however, included the blue color of iodine binding with the long branch-chains of amylpectin. Only linear branches of amylpectin that are long enough to form helix can develop a complex with iodine in the same manner of amylose; but from the short length of these branches originates unstable purple to red color complexes [28]. The absorbance value of amylopectin-iodine is much lower than that of amylose. The iodine-binding capacity of pure amylose was reported to be 19–22% of its weight, whereas for amylpectin it was about 1% [26]. As the length of the starch chain decreased with the extent of enzymatic modification (Fig. 4B), the number of helical turns reduced, and the number of iodine molecules which could be accommodated decreases as well with a resulting lower iodine binding capacity (absorbance reduced from 0.71 to 0.23). This decrease binding resulted in a shift in the λmax (from 600 nm to 570 nm), similar with the data reported by Lee et al. [27]. Do et al. [29] found that λmax of amylpectin was around 567 nm, which was close to that of 4αGT-modified starch, indicating that 4αGT disassembled long-chain amylose into a large number of shorter double helical glucans through disproportionation. Bailey and Whelan [30] demonstrated that 1/λmax has been shown to be directly proportional to 1/DP up to a DP of about 100 in linear chains and the absorbance value of the complex decreases with decreased molecular weight of α-1,4 linked glucan. These above observations revealed that the decrease in the amylose content and the branched chain length in enzyme modified starch.
3.4. Molecular weight analysis

The data of molecular weight (Mw) of 4αGT-modified starch analyzed by HPSEC-MALLS-RI are present in Table 1. The HPSEC profiles of normal corn starch presented two molecular weight distribution peaks; peak A represented larger amylopectin molecule (3.16 × 10^8 g/mol) and peak B indicated smaller amylose molecule (0.56 × 10^5 g/mol). The 4αGT has been noted that for its action of degrading starch component into smaller molecules as previously reported [8,9]. As illustrated in Table 1, the Peak A gradually decreased as the modified time increased, whereas Peak B increased substantially during the enzyme treatment. It
appeared that the decreased portion of amylpectin (Peak A) moved to a new fraction (Peak B). This new fraction was likely originated from the 4αGT-degraded amylpectin and amylose such as smaller amylpectin clusters and cyclic glucans through cleaving and reorganization of starch molecules [12]. Therefore, our data (Table 1 and Fig. 4) implied that the content of amylose and higher Mw of amylpectin were decreased and long branched-chains of amylpectin were degraded and rearranged during 4αGT reaction.

3.5. Chain length analysis

The branch chain length distributions of 4αGT-treated corn starch were determined using HPAEC-PAD and the results are shown in Fig. 4. As can be seen in Fig. 4A, branch chain length distribution of 4αGT-modified starch greatly differed from that of the control starch. It was observed that the number of short and long chains increased as the reaction proceeded, whereas the number of medium chains decreased when compared with control sample. On the basis of cluster model, amylpectin molecules have A, B (B1–B4), and C chains, in which the fractions of DP <13 and DP 13–30 together compose the short chains and correspond to A + B1 chains, and the other remaining longer chain fractions (DP > 30) correspond to B2–B4 chains [31]. Fig. 4B displays an increasing in small (DP < 13) and longer (DP > 30) branch chains compared with the control starch, which was in accordance to the results of Kaper et al. [16]. They found that the potato starch after modification with Pyrobaculum aerophilum IM2 4αGT treatment resulted in a flatter branch chain distribution, displaying an increase in side chains smaller than DP6 and larger than DP35 up to DP50. The increase in the number of shorter side chains and the decrease in the number of medium side chains were possibly due to the partial hydrolysis and intramolecular rearrangement of branch chains of amylpectin (A and B1 chains). Meanwhile, the increase in the number of longer side chains might be due to the intermolecular transfer from amyllose to amylpectin that partially elongated the amylpectin side chains and the partial hydrolysis of inner long chains (probably long B chains). It was noteworthy that the amylpectin branch chain length distribution maintained stable state after 4αGT treatment for 2 h, whereas Mw continued to reduce as shown in Table 1. Hansen et al. [15] and Do et al. [29] also reported that the 4αGT modification reduced the Mw of starch without changing the chain structure of amylpectin. The reduction of Mw was not attributed to the modification of external branch chains, but to the cleaving internal chains of amylpectin by both hydrolysis and transglycosylation of 4αGT.

In some earlier studies, differences in the digestibility of starch has been attributed to the interplay of many factors, such as starch source, granule size, crystallinity, molecular fine structure, surface pore and interior channel [2,32,33]. Our data showed that 4αGT-modified starch sample MS4 with higher SDS content (20.92%) was related with higher proportion of short and long chains and after enzyme modification (Table 1). The increase in both short and long chain fractions was accompanied by a reduction of medium chain fraction with increasing hydrolysis time, which is related to the decrease in the hydrolysis rate and the increase in the SDS, as suggested by Zhang et al. [34]. They also found a parabolic relationship between starch structure and the slow digestion property of cooked starch, showing that starch with a high number of either short chains (DP < 13) or long chains (DP > 13) had a high content of SDS. Also, extensive cross-linking might occur for long chains, which results in the formation of a macro-molecular network eventually resulting in gelation as reported by our previously study [33]. These helices further aggregate leading to formation of ordered crystalline arrays which precipitate out of solution and are resistant to digestion. This trend was similar to the digestibility data of 4αGT-treated starches in this study.

3.6. Viscosity analysis

In order to access the rheological properties of 4αGT-modified starch, viscosities were measured and profiles are present in Fig. 5. The control or lesser enzyme treated starch had an appreciable viscosity noticed at low shear and the viscosity decreased with an increased of reaction time with 4αGT during starch processing. Thus, the viscosity was inversely related to the digestibility of the treated starches (Figs. 2 and 5). This result agrees well with the report by Gee and Johnson [35]. They found that viscosity declines over the course of a simulated digestion and that relative viscosity values can predict to what extent intact foods raise the viscosity of partially digested gut contents. Many researchers reported the formation of thermo-reversible gel after the treatment of starch with 4αGT [16,29]. The content of amylose and the Mw of amylpectin and amylose decreased to a certain level with 4αGT treatment, the modified starch could be large enough to form a rigid gel through re-association, which might significantly increase the thermal stability of the crystalline structure made up of longer chains that would otherwise decrease the solubility and hence viscosity.

4. Conclusions

Partial 4αGT treatment of normal corn starch reduced starch digestibility, which was related to the amylose, molecular weight, chain length and viscosity. A maximum SDS content (20.92%) was obtained using 4αGT hydrolysis over 4 h. The increment of both short chain fractions (DP < 13) and the long chain fractions (DP < 30) led to a more SDS and RS. Further work is underway to obtain structure–property relationships for the enzyme modification of starch. The results of the structure–property relationship studies would aid in the discovery and the development processes for designing a novel slowly digestible starch for controlling postprandial hyperglycaemia.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (31000764, 20976073, 31230057), the National High Technology Research and Development Program of China (2013AA102102) and the Science & Technology Pillar Program of Jiangsu Province (BY2012049, BE2012613, BE2013647).
References
