Preparation of Slowly Digested Corn Starch Using Branching Enzyme and Immobilized α-Amylase

Ruomin Li, Huanxin Zhang,* Saikun Pan, Mengwei Zhu, and Yi Zheng

ABSTRACT: The aim of this study was to modify the digestibility and structure of corn starch by treatment with compound enzymes. Corn starch was treated with two enzymes (α-amylase, which catalyzes hydrolysis, and branching enzyme, a transglycosidase that catalyzes branch formation), and the reaction was monitored by determining the content of slowly digestible starch in the reaction product. The fine structure and physical and chemical properties of enzyme-modified starch samples were analyzed using scanning electron microscopy, gel chromatography, and X-ray diffraction methods; modified starch has a high degree of branching, a high proportion of short-chain branched structures, and greatly improved solubility. The results show that the slow digestion performance of corn starch was significantly improved after hydrolysis by α-amylase for 4 h and treatment with branching enzyme for 6 h. These results show that enzymatic modification of corn starch can improve its slow digestibility properties.

INTRODUCTION

Starch is the most important source of carbohydrate and energy for humans. According to the speed of digestion in the body and release of glucose, starch can be divided into rapidly digestible starch (RDS, digested within 20 min), slowly digestible starch (SDS, digested within 20–120 min), and resistant starch (RS, digested after 120 min). The RDS is easily hydrolyzed by acid or enzymes into oligosaccharides or monosaccharides and is completely digested and absorbed by the small intestine. After eating, RDS causes the blood sugar level to rise rapidly, which is undesirable, especially for those with metabolic diseases such as diabetes. SDS is a kind of starch with high amount of short-chain branch structures and a low crystalline state; because of its special structural state, SDS can be completely digested and absorbed by the human body, but its digestion rate is slow. Thus, SDS does not cause large fluctuations in blood glucose levels after consumption; thus, it has the effect of regulating and controlling diabetes, obesity, and cardiovascular diseases. As a type of functional food with health care benefits and a wide range of potential applications, SDS has attracted the attention of both researchers and health care benefactors, including the global food industry to produce starch products that are easier to digest and absorb.

Most previous studies have used a single enzyme to modify starch to prepare SDS, but a few have used a double-enzyme method. Jiang et al. treated ordinary corn starch with 4-α-glucosyltransferase and branching enzyme (BE) to prepare slowly digested starch and found that the double-enzyme method was significantly better than the single-enzyme method. They found that there was a significantly higher SDS content in starch modified by the double-enzyme method in comparison to that modified using a single enzyme. Li et al. used glutinous rice starch as the raw material and treated it with α-amylase and BE to produce modified starch with good physical and chemical properties. As an endoacting enzyme, α-amylase can randomly cut α-1,4-glycosidic bonds across branch points to produce more linear chains, thereby greatly reducing the production cost and reducing the use of chemical reagents. It is also safer for the human body when it is used in the food industry to produce starch products that are easier to digest and absorb.
can effectively increase the ratio of α-1,6-glycosidic bonds, generating products with a higher ratio of branched and short chains; that is, branched-chain glycogen molecules. Studies on the enzymatic modification of amylose have shown that a higher proportion of α-1,6-bonds significantly improves the slow digestibility of modified starch.

As an important food crop worldwide, corn is an abundant resource that is cheap and readily available. Using enzymes to modify starch can improve its inherent defects and extend the industrial chain by creating a product with slow digestion properties. The purpose of this research was therefore to modify corn starch using a compound enzymatic method designed to modify the carbohydrate chain length and branching degree and alter the crystallinity to increase the SDS content. Further, we aimed to explore the relationships among starch granule morphology, physical and chemical properties, and digestibility.

**MATERIALS AND METHODS**

**Materials.** Corn starch was purchased from the Shanghai Fengwei Industrial Co., Ltd. (Shanghai, China). α-Amylase (3700 U/g) was purchased from the Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China), and isoamylase (240 U/mL) was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). BE from *Bacillus stearothermophilus* (200 U/mL) was produced as a recombinant protein by *Escherichia coli* BL21(DE3) harboring the plasmid pET22(+) (Novagen). The glucose determination kit was purchased from Changchun Huili Biotech Co., Ltd. (Changchun, China). All chemicals were of reagent grade and were obtained from the Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

**Preparation of Enzyme-Modified Corn Starch.** Corn starch (20%, w/v) was dispersed in 10 mM Na2HPO4−NaH2PO4 buffer (pH 6.5). The starch slurry was gelatinized in a water bath at 90 °C for 25 min. The temperature of the starch sample was adjusted to 50 °C, and then 200 U/g α-amylase was added and the mixture was incubated for a further 4 h. Immediately after the reaction, the solution was heated at 100 °C for 20 min to deactivate the reaction. The temperature was lowered to 75 °C by incubating the mixture in a water bath for 20 min, and then the pH was adjusted to 7.0. Then, BE at a concentration of 300 U/g dry weight of starch was added to the solution. The enzymatic reaction mixture was incubated for 12 h and sampled at 0, 2, 4, 6, and 12 h. At each sampling time, the reaction was stopped by heating the reaction mixture at 100 °C for 20 min to inactivate the BE activity. The solution was then cooled to room temperature, and two volumes of absolute ethanol was added to precipitate the reactant. The mixture was centrifuged at 8000 r/min for 20 min to collect the starch precipitate, which was washed with deionized water. This step was repeated twice, and the precipitated starch was collected and freeze-dried. The collected material was stored in a desiccator until further analysis.

**Morphology of Starch Granules.** A scanning electron microscope (SEM; S-4800, Hitachi, Tokyo, Japan) was used to observe the microscopic features of the starch samples digested for different time periods. The samples were mounted onto a metal sample stage covered with carbon tape and then coated with a thin layer of gold. The images were examined under the SEM at an acceleration voltage of 20 kV.

**Determination of Starch Molecular Weight.** Corn starch samples (0.2%, w/v) were dissolved in deionized water, boiled for 20 min, and then centrifuged at 6000 r/min for 5 min. The supernatant was analyzed using a high-performance size exclusion column chromatography (HPSEC)-multilangle laser light scattering (MALLS) system connected to a refractive index (RI) detector. The columns used in this system were as follows: Shodex OH-pak SB-805 and SB-806. The mobile phase was an aqueous 0.1 M NaNO3 solution. The injection volume and flow rate were 30 μL and 0.5 mL/min, respectively. The experimental data collected from the a DAWN DSP/OptiLab system were processed with Abstra V software (Version 4.09.07).

**Determination of Starch Chain Length Distribution.** Each completely dissolved starch sample (20 mg of starch in 10.0 mL of 100 mM, pH 4.5 NaAc-HAc buffer) was incubated in a water bath at 100 °C for 15 min and then at 40 °C for a further 20 min. Then, 3 μL of isoamylase was added and the mixture was shaken at 30 r/min for 24 h at a constant-temperature water oscillator. The solution was heated at 100 °C for 15 min to stop the reaction. The debranched sample was centrifuged at 8000 r/min for 10 min and filtered through a 0.45 μm filter. An aliquot of the filtered sample (30 μL) was injected into a high-performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) system equipped with an electrochemical detector and a CarboPac PA-200 anion-exchange column (4 × 250 mm, Dionex, Sunnyvale, CA, USA). All samples were analyzed at 25 °C. The solvent system consisted of 250 mM sodium hydroxide (elucent A) and a 1 M sodium acetate solution containing 100 mM sodium hydroxide (elucent B) supplied at a flow rate of 0.5 mL/min.

**Branch Density Determination.** The relative content of α-1,6 glycosidic bonds in starch samples was determined from the 1H NMR spectrum. For this analysis, 30 mg of modified starch was mixed with 500 μL of D2O (99.9%) to produce a 60 mg/mL slurry. The emulsion was then heated, with shaking, in a boiling water bath for 30 min to completely dissolve the starch, and then the mixture was freeze-dried. A 20 mg portion of the freeze-dried sample was placed in a clean NMR tube and redissolved in D2O. Then, nuclear magnetic spectrometry analyses were performed using an AVANCE III-400 MHz proton nuclear magnetic resonance spectrometer with the following settings: scan width, 8223 Hz; relaxation time, 1 s; probe rotor, 5 mm; pulse sequence, zg30; test temperature, 80 °C. The ratio of α-1,4-glycosidic bonds to α-1,6-glycosidic bonds was obtained by a 1H map analysis.

**Determination of Relative Starch Crystallinity.** The crystalline characteristics of the starches were evaluated using an angle X-ray diffractometer (D2 PHASER, Bruker, Karlsruhe, Germany) operated at 40 kV and 40 mA. The starch powder was packed tightly in a round cell and scanned over the range of 5–35° Bragg angles at a rate of 2°/min at room temperature. The relative crystallinity was calculated as the ratio of the crystalline peak area to the total diffraction area using MDI Jade software (Version 6.5).

**Starch Solubility Determination.** The solubility was determined using the method of Ning, Cui, and Yuan with some modifications. Each starch sample (50.00 mg, W1) was suspended in 1.0 mL of water in a centrifuge tube and mixed for 0.5 min using a vortex mixer. The suspension was heated in a water bath at 95 °C for 30 min and then cooled to room temperature and centrifuged at 5000 r/min for 10 min. The supernatant was separated from the precipitate and dried to
constant weight in hot air. The solubility \((S)\) was calculated using the equation

\[
S(\%) = \left( \frac{A}{W} \times 4 \right) \times 100\%
\]

where \(W\) is the starch quality before and after modification and \(A\) is the supernatant quality.

**Starch Transparency Determination.** Each sample was used to prepare a 1% starch slurry, which was heated and stirred in a 100 °C water bath. The volume of the starch slurry was monitored to ensure that it remained unchanged. Each starch slurry was cooled to 25 °C, and then its absorbance was measured at 650 nm against distilled water as a reference using an ultraviolet spectrophotometer. The \(A_{650}\) value was used to calculate the transparency \((T)\) of the starch paste as follows:

\[
T(\%) = \frac{1}{A}
\]

**Determination of Starch Rheological Properties.** A MARS III rotational rheometer was used to measure the shear force characteristics of native corn starch and samples treated with BE for 12 h. Each sample was used to prepare 3% (w/v) and 5% (w/v) starch slurries. The parameters used to measure the change in apparent viscosity were as follows: parallel plate mold with a diameter of 40 mm; gap set to 1 mm; increase in shear rate from 0 to 100 per second at 25 °C.

**In Vitro Digestion Using Englyst Assay.** The *in vitro* digestibility of starch samples was analyzed according to the procedure of Periago, Englyst, and Hudson with a slight modification. To prepare enzyme solution A, a glucosidase solution (40 μL) was diluted to 2.0 mL with deionized water. Enzyme solution B was prepared by mixing porcine pancreas \(\alpha\)-amylase (4.0 g) with water (26 mL) by magnetic stirring at 4 °C for 10 min and then centrifuging the solution and collecting the supernatant. Enzyme solution C was prepared immediately before use by mixing enzyme solution A (1.8 mL), enzyme solution B (18 mL), and deionized water (1.2 mL).

Each starch sample (200 mg) was dispersed in 15 mL of phosphate buffer (200 mM, pH 5.2) by vortexing. After the solution was equilibrated at 37 °C for 5 min, five glass balls and enzyme solution C (5.0 mL) were added. Then, the mixture was shaken in a water bath at 37 °C at 200 r/min. Aliquots of the hydrolyzed solution (0.5 mL) were taken at different time intervals, and four volumes of ethanol was added to each sample to denature the enzyme. The glucose content of the hydrolysate was determined using a glucose assay kit. The proportions of the three nutritional fractions (RDS, SDS, and RS) were calculated from the values of \(G_{20}\) and \(G_{120}\) (amount of glucose released by hydrolysis of starch for 20 and 120 min, respectively), \(FG\) (free glucose), and \(TS\) (total glucose) using the following formulas:

\[
\begin{align*}
RDS & = (G_{20} - FG) \times 0.9 \\
SDS & = (G_{120} - G_{20}) \times 0.9 \\
RS & = TS - (RDS + SDS)
\end{align*}
\]

**RESULTS AND DISCUSSION**

**Changes in Morphology of Starch Granules.** Changes in the morphology of corn starch granules during degradation by enzymes were analyzed by SEM. We acquired SEM images (2 and 5 kV) of corn starch granules before and after enzyme hydrolysis (\(\alpha\)-amylase treatment followed by 0–12 h of BE treatment) (Figure 1B–F). As shown in Figure 1A, the native starch granules had a relatively smooth and compact surface with typical pores. Most of the granules were polyhedral with rounded edge, and a few were spherical. Similar observations of native cornstarch granules were reported by Leyva-López et al. The process of gelatinization is known to cause hydration of the raw cells and physicochemical changes to intracellular components. As shown in Figure 1B–F, gelatinized starch had completely lost its particle morphology. The enzymatic treatment modified...
the surface of starch, with the outer part of the particles being hydrolyzed and degraded. The enzyme was able to penetrate into the particles through small pores and hydrolyze the molecules inside the particles. After BE treatment (Figure 1B), the particle surface remained relatively intact, but several particle fragments were visible on the surface. The reaction time affected the degree of hydrolysis; a longer reaction time led to larger changes in particle morphology. As indicated by the two arrows, the pore sizes on granules and channels into the granule interior became larger with increasing reaction time, which resulted in a more porous morphological appearance.23

Changes in Molecular Mass of Modified Starch. Using HPSEC-MALLS-RI to quantitatively analyze the relative molecular masses of substrates in the process of enzyme catalysis, the principle is that, when components with different sizes pass through the gel chromatographic column with the mobile phase, the molecules of components larger than the pore size of the packing cannot enter the gel column. The colloid cavity is repelled and flows out of the column first; the smaller the component, the more penetrates into the cavity, the later the elution time, and finally the separation is achieved.24 The molecular weight distribution peaks obtained from the HPSEC curve were integrated with the combined multangle laser light scattering and differential detector. The molecular weight (Mn) and number-average molecular weight (Mw) of the starch samples determined are shown in Table 1. According to Table 1, in the enzyme-treated starch samples, the Mn and Mw values of both amylose and amylopectin were decreased under the action of enzymes. With increasing reaction time, the polydispersity (Mw/Mn) of amylopectin tended to increase in the enzyme-modified starch, in comparison with that of natural corn starch, while that of amylose (Mw/Mn) showed a decreasing trend. Because enzymatic hydrolysis of transglycosides tends to produce shorter free chain segments, there may be multiple connection mechanisms when amylopectin is the receptor, resulting in a more uneven relative molecular mass distribution.25 During the process of starch hydrolysis, the high-DP fragments of amylopectin and amylose are hydrolyzed, and the short chains with low DP are reconnected. The amylose content gradually decreased, while the amylopectin content gradually increased with an increase in the enzymatic modification time. In this way, highly branched amylopectin clusters form. These changes are reflected by the sharp decrease in the relative Mn value of starch. Other studies have shown that the transglycosidation effect of BE can not only reduce the relative Mn value but also increase the branching points of the chain segments and make the internal molecular distribution more uneven. The experimental results obtained in this study are consistent with the changes in various parameters reported by Ren,26 who used gelatinized corn starch as a substrate for hyperbranching modification. Thus, on the basis of the changes in the relative Mn value and the degree of dispersion, we conclude that BE successfully hydrolyzed and transglycosylated corn starch.

Changes in Chain Length Distribution. The branch length distribution of the BE-treated debranched products was measured by HPAEC-PAD. As shown in Figure 2, the degree of polymerization is shown on the X axis and the relative peak area is shown on the Y axis. The proportion of short-branch chains was higher in enzyme-modified starch samples than in gelatinized corn starch. Table 2 summarizes the proportions of fractions A (DP ≤ 13), B (13 < DP < 30), and C (DP ≥ 30) in the linear chains of enzyme-treated and native starch samples. As shown in Table 2, most of the natural corn starch chain segments were B chains and C chains, and the content of A chains was relatively small. As the enzymatic reaction time increased, the chain length distribution shifted toward the A chain, and the ratio of B to C chains was significantly reduced.

The shorter chain segments included intermediate products with a higher degree of branching, more branched short chains, and shorter average chain lengths.27 After reaction with BE for 6 h, the proportions of B and C chains had decreased by 23.41% and 1.05%, respectively. This indicates that the gelatinization of the starch substrate leads to the destruction of the starch crystal structure, fully exposing the internal structure of the starch granules. Enzymes show higher rates of hydrolysis and transglycosylation when they act on branched-chain segments. The proportion of A chains after a 6 h reaction time with BE was 80.74% (25.09% higher than that of natural starch), indicating that more short-chain structures were produced under the action of double enzymes. Thus, the degree of hydrolysis and transglycosylation increased with increasing enzymatic reaction time. The activities of both α-amylase and BE resulted in the reduction in the DP of amylopectin and amylose fragments and reconnection of the short chains with smaller DP, which shortened the overall

Table 1. Changes in Relative Molecular Mass of Amylose and Amylopectin during Reaction with α-Amylase and Branching Enzyme*

| sample     | amylopectin | amylose |
|------------|-------------|---------|
|            | Mw (×10^6 g/mol) | Mn (×10^4 g/mol) | Mw/Mn | Mw (×10^6 g/mol) | Mn (×10^4 g/mol) | Mw/Mn |
| corn starch| 4.826 ± 0.11a | 4.781 ± 0.08a | 1.009 ± 0.01a | 6.108 ± 0.08a | 5.082 ± 0.04a | 1.202 ± 0.01a |
| 0 h        | 3.607 ± 0.02b | 3.278 ± 0.08b | 1.101 ± 0.03b | 5.762 ± 0.06b | 4.811 ± 0.01b | 1.198 ± 0.01b |
| 2 h        | 3.379 ± 0.02c | 3.013 ± 0.02c | 1.122 ± 0.00c | 4.901 ± 0.02c | 4.177 ± 0.04c | 1.173 ± 0.01c |
| 4 h        | 2.693 ± 0.01d | 2.360 ± 0.07d | 1.142 ± 0.03d | 4.628 ± 0.03d | 4.128 ± 0.03c | 1.121 ± 0.02c |
| 6 h        | 2.520 ± 0.01e | 1.864 ± 0.03e | 1.207 ± 0.01e | 3.483 ± 0.06e | 3.234 ± 0.09e | 1.077 ± 0.02e |
| 12 h       | 1.920 ± 0.03f | 1.547 ± 0.02f | 1.241 ± 0.03f | 2.894 ± 0.02f | 2.750 ± 0.06f | 1.053 ± 0.01f |

Notes: *The results are expressed as means ± sd. The different letters in the same column indicate a significant difference among means (p < 0.05).
chain length. The connection of more branches to relatively short amylopectin chains, and the connection of smaller DP fragments to relatively large amylopectin chains, resulted in the formation of a short-cluster structure. In addition, BE catalyzed the production of cyclic polymers with a lower DP in the segments, which eliminated the reducing ends of the free segments. This led to a significant increase in the ratio of cyclic polymers with a low DP to linear short chains. Our results reflect all of these processes.

To determine the structural properties of enzymatically modified starch, the α-1,6-linkages were analyzed by 1H NMR. As shown in Table 2, the α-1,6-bond content of natural corn starch was 11.50%. After 6 h of BE modification, the α-1,6-bond content was 20.68%, an increase of 9.18% in comparison with that of original starch. This is because the enzyme mainly degraded the α-1,4-glycosidic bonds of starch, resulting in a shorter linear chain length. This conclusion was consistent with the results of HPAEC-PAD. At the same time, the transfer of glycosyl residues immediately connected free glucose residues to the same or different linear and amylopectin molecules with α-1,6-bonds to form new branched chains, thereby increasing the branching density of starch.

Figure 2. Changes in distribution of substrate chain length during reaction with branching enzyme.
Table 2. Chain Distribution, α-1,6-Linkage Ratio, Relative Crystallinity, Solubility, Transparency, and Digestibility of Native Starch and Enzyme-Modified Starch

| Sample                  | α-1,6 Linkage Ratio (%) | Branch Chain Distribution Ratio (%) | α-1,6 Branch Chain Distribution Ratio (%) | Relative Crystallinity (%) | Solubility (%) | Transparency (%) | RDS (%) | SDS (%) | RS (%) |
|-------------------------|-------------------------|------------------------------------|------------------------------------------|---------------------------|-----------------|-------------------|--------|--------|--------|
| Corn starch             | 11.50 ± 0.22            | 55.65 ± 0.85                      | A (DP < 13) 42.49 ± 0.34                | 1.86 ± 0.99              | 45.21 ± 0.58    | 7.27 ± 0.32       | 19.06 ± 0.27 | 64.05 ± 0.56 | 23.12 ± 0.69 | 12.83 ± 0.14 |
| α-amylase + BE          | 12.28 ± 0.31            | 64.27 ± 0.10                      | B (13 < DP < 30) 34.47 ± 0.23           | 1.25 ± 0.47              | 35.53 ± 0.19    | 15.18 ± 0.18      | 30.81 ± 0.57 | 58.74 ± 0.94 | 27.88 ± 0.46 | 13.38 ± 0.76 |
| 0 h                     | 13.79 ± 0.52            | 71.50 ± 0.65                      | C (DP ≥ 30) 1.86 ± 0.99                 | 0.97 ± 0.69              | 32.24 ± 0.41    | 20.77 ± 0.37      | 33.22 ± 0.47 | 55.19 ± 1.13 | 30.82 ± 1.01 | 13.99 ± 1.50 |
| 4 h                     | 15.97 ± 0.18            | 80.74 ± 0.24                      | 1.80 ± 0.20                           | 0.18 ± 0.49              | 27.11 ± 0.20    | 28.28 ± 0.34      | 39.11 ± 0.21 | 47.62 ± 0.44 | 27.88 ± 0.46 | 13.38 ± 0.76 |
| 6 h                     | 20.68 ± 0.12            | 86.76 ± 0.69                      | 1.84 ± 0.20                           | 0.08 ± 0.33              | 25.73 ± 0.30    | 29.79 ± 0.16      | 42.04 ± 0.20 | 46.33 ± 1.65 | 36.92 ± 1.00 | 16.75 ± 0.64 |
| 12 h                    | 22.03 ± 0.25            | 91.00 ± 0.69                      | 1.87 ± 0.20                           | 0.05 ± 0.33              | 27.74 ± 0.30    | 29.79 ± 0.16      | 42.04 ± 0.20 | 46.33 ± 1.65 | 36.92 ± 1.00 | 16.75 ± 0.64 |

The results are expressed as mean ± sd. The different letters in the same column indicate a significant difference among means (p < 0.05).
the starch solubility from 15.18% at 0 h to 21.01% at 6 h and 29.79% at 12 h due to the destruction of the double-helix structure of the starch crystal region during the gelatinization process. Enzyme modification of starch leads to more exposure of hydrogen bonds. The introduction of new branch points inhibits the recrystallization of amylose. The rearrangement of glucan chains and the increase in the number of hydrophilic groups enhances the hydration performance and the solubility of starch. Other studies have shown that highly branched polymers with low amylose content and a high ratio of short chains have good solubility.36

Transparency reflects the degree of retrogradation of starch: the higher the degree of aging, the lower the transparency. As shown in Table 2, the trends in the changes in transparency were similar to those of changes in solubility. The transparency of natural corn starch was 19.06%, and that of enzyme-modified starch samples was significantly higher. As the reaction time with BE increased, the transparency increased from 19.06% at 0 h to 39.11% at 6 h (an increase of 20.05%), because gelatinization made it more difficult for polymerization to occur inside starch granules. Enzyme modification reduced the amylose content in the starch and generated more short-range ordered structures.37 When starch is gelatinized, it becomes difficult for the molecules to polymerize. This means that the formation of gel bundles is inhibited after starch retrogradation, which results in a reduced starch aging rate and increased transparency of starch paste.38 The longer the reaction time, the lower the degree of aging and the higher the degree of transparency.

Analysis of Steady-State Rheological Properties.

Native corn starch and starch samples treated with α-amylase for 4 h and then BE for 6 h were made into starch slurries at concentrations of 3% and 5% (w/v, based on starch dry weight) to explore the influence of shear force on viscosity. The rheological properties of starch pastes under static shear force are shown in Figure 4. The apparent viscosity of all samples decreased significantly as the shear rate increased, showing typical shear thinning flow characteristics.39 The viscosity of the starch paste was proportional to the starch concentration, and 5% starch paste was more viscous than 3% starch paste. The apparent viscosity of enzyme-modified starches was generally lower than that of natural corn starch (<0.06 Pa s). The reduced viscosity of enzyme-modified starch samples may have been due to the reduced amylose content, increased branch density, and destruction of the internal structure of starch crystals by enzymes. The relatively scattered chain particles roll and shrink into clusters, reducing mutual hooks and thus reducing viscosity. Similar results were reported by Chen,40 who used BE-modified wax to reduce the viscosity of rice starch. Thus, this modified starch can be used at high concentrations in low-viscosity food products.

In Vitro Digestion Characteristics of Enzyme-Modified Starch. According to the digestion rate of starch in the body, starch can be divided into RDS, SDS, and RS. The SDS fraction can be completely and slowly digested and absorbed by the small intestine within 20–120 min and represents a new type of modified starch with a low glycemic index. It has attracted much attention from researchers and consumers because it helps to maintain blood sugar stability and the resulting products have a practical application value. In the present study, natural corn starch was used as the substrate. After compound enzymatic modification, the Englyst method with slight modifications was used to analyze the digestion performance of the modified starch samples in in vitro simulated digestion conditions. Other studies have shown that the digestibility of starch decreases with increased contents of α-1,6-glycosidic bonds and short chains and a higher branching degree and with decreased branching degree, relative molecular weight, amylose content, and average outer chain length.41 As shown in Table 2, natural corn starch was found to contain a high proportion of RDS, and the SDS content was only 23.12%. With increasing enzyme reaction time, the RDS content gradually decreased, and the SDS and RS contents increased significantly. After a reaction time of 6 h with BE, the RDS content was 16.43% lower than that in native corn starch, and the SDS content was 13.22% higher. Enzymatic treatment resulted in the formation of more short chains, shortened long chains, and cyclic polymers with short-cluster branches and low DP. This resulted in starches with a high branching density and a low digestion rate.42 This was because the starch modified by the enzymes not only had a lower amylose content but also had a higher amylopectin content as a result of hydrolysis and transglycosidation. Together, these changes increased the steric hindrance, weakened the continuity of enzyme digestion, and reduced the catalytic activity of the enzyme. Our results show that the
two-stage enzyme modification can greatly increase the proportion of SDS, thereby regulating starch digestibility.\textsuperscript{43} In comparison with the 6 h reaction time, the 12 h reaction time with BE did not significantly increase the SDS content of starch. This may be because the large number of shorter chain segments in the starch molecule resulted in the formation of strong intermolecular relationships, which makes it difficult for starch molecules to crystallize and thereby inhibits further SDS formation. On consideration of the energy consumption and cost of the experiment, the best conditions to prepare a corn starch product with a high SDS content and slow digestibility are 4 h treatment with $\alpha$-amylase followed by 6 h treatment with BE.

\section*{CONCLUSIONS}

In this study, $\alpha$-amylase and BE derived from \textit{E. coli} BL21 (DE3) were used to modify corn starch. Corn starch samples with a high SDS content were prepared via a two-stage modification treatment. The relationship between SDS content and starch fine structure was explored to reveal the mechanism of the enzymes and how their activity increased the SDS content and affected the physical and chemical properties of the treated samples. Other studies have shown that $\alpha$-amylase and BE can be used to prepare SDS products from corn starch. In this study, the results of the \textit{in vitro} digestion experiment showed that the proportion of SDS increased significantly with a longer reaction time and that the digestibility of the treated starch samples was much slower than that of native cornstarch. The SDS content was also affected by factors such as the short-chain structure and relative crystallinity of starch. Observations of the microstructure showed that the enzyme treatments significantly increased the number of pores in starch granules and resulted in uniform pore density. The fine structural analyses showed that, in comparison with native corn starch, the enzyme-modified starch had a lower relative molecular weight, more short chains, a higher degree of branching, and a lower degree of crystallinity. Further analyses of the physical and chemical properties of enzyme-modified starch revealed its good solubility, transparency, and improved apparent viscosity. Therefore, the modification of corn starch using this compound enzyme method has broad prospects in the food industry.

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\section*{Notes}
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