

Enhancing Enzymatic Resistance in Corn Starch through RS3 Formation, Guar Gum Complexation, and Heat-Moisture Treatment

By Ruochu Chen

AUTHOR BIOGRAPHY

Ruochu Chen is a high school student at the Second High School Attached to Beijing Normal University in Beijing, China. She is interested in food science, nutrition, and public health and hopes to continue exploring how food science and nutrition can contribute to human health.

ABSTRACT

Diabetes is a major public-health problem, and the rapid digestion of starch-rich foods is relevant to postprandial glucose exposure. This study tested whether a sequence of type III resistant starch (RS3) formation, guar gum complexation, and heat-moisture treatment could make native corn starch more resistant to in vitro digestion. Native corn starch was gelatinized, debranched with pullulanase, and recrystallized to form RS3. Guar gum was added at 20% of the RS3 dry mass, followed by heat-moisture treatment. In vitro digestion was used to measure rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). X-ray diffraction (XRD), scanning electron microscopy (SEM), and differential scanning calorimetry (DSC) were used to examine structural and thermal changes. Mean RS content increased from 16.36% in native corn starch to 32.22% in RS3, 45.00% after guar gum addition, and 57.10% after heat-moisture treatment. RDS fell from 77.13% to 36.64%. The crystal pattern changed from A-type to mixed B+V-type, and relative crystallinity increased from 16.90% to 86.8%. SEM and DSC also showed changes in particle form and thermal behavior. These results link structural changes in the starch-guar gum system with stronger resistance to in vitro digestion. Glycemic index and postprandial blood glucose were not measured, so in vivo studies are still needed.

Keywords: corn starch, type III resistant starch (RS3), resistant starch, guar gum, heat-moisture treatment, in vitro digestion

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Diabetes is a major global public-health problem. A pooled analysis estimated that more than 800 million adults were living with diabetes in 2022 (NCD Risk Factor Collaboration, 2024). Cereal staples provide a large share of dietary carbohydrate, so the rate of starch digestion matters for postprandial glucose exposure (Birt et al., 2013). Resistant starch (RS) escapes digestion in the small intestine. This study tested whether a sequence of enzymatic, hydrocolloid-based, and physical treatments could increase the fraction of corn starch that resists in vitro digestion (Birt et al., 2013).

A repeatable modification method may help produce starch-based ingredients with slower digestion profiles. Still, a higher in vitro RS value does not by itself prove a lower glycemic index or reduced postprandial blood glucose. Those effects also depend on the final food and must be tested in vivo (Birt et al., 2013).

Pullulanase debranching and recrystallization can form RS3 and change the structure and digestibility of maize starch (Miao et al., 2009). Guar gum can slow starch hydrolysis by changing rheology and limiting enzyme access (Dartois et al., 2010). Heat-moisture treatment can also change crystallinity, thermal behavior, and digestibility (Fu et al., 2015). The present study measured how each step changed RDS, SDS, RS, XRD, SEM, and DSC results.

METHODS

Materials and Instruments

Food-grade native corn starch was used as the base material. Pullulanase (≥ 1000 U/mL), food-grade guar gum, ethanol, sodium hydroxide, sodium acetate, and deionized or distilled water were used. Porcine pancreatic α -amylase and amyloglucosidase from *Aspergillus niger* were obtained from Sigma. The GOPOD assay kit was obtained from Megazyme (Ireland). Main equipment included an AUX220 balance (Shimadzu, Japan), an IKA RCT stirrer (IKA Group, Germany), a DHG-9076A drying oven (Shanghai Jinghong Experimental Equipment Co., Ltd., China), an HWS-26 water bath (Shanghai Shupeixi Experimental Equipment Co., Ltd., China), an Arium comfort II ultrapure-water system (Sartorius, Germany), an SWB-2000 thermostatic water-bath shaker (Tianjin Auto Science Instrument Co., Ltd., China), a YNX 4000 refrigerated high-speed centrifuge (Thermo Scientific, USA), a UV-2800A ultraviolet-visible spectrophotometer (UNICO, Shanghai, China), a D8 ADVANCE X-ray diffractometer (Bruker, Germany), an SU8020 field-emission scanning electron microscope (Hitachi, Japan), and a Discovery DSC 25 differential scanning calorimeter (TA Instruments, USA).

Experimental Procedures

Preparation of RS3

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The RS3 method was based on published enzymatic debranching and retrogradation procedures, with modifications (Arp et al., 2020). Native corn starch was dispersed in water and gelatinized at 100 °C for 10 min. After cooling, the pH was adjusted to 5.0. Pullulanase was added at 80 U/g dry starch, and the sample was kept at 50 °C for 12 h. The enzyme was inactivated at 100 °C for 10 min.

The debranched starch was kept at 4 °C for 24 h. It was washed three times with ethanol and centrifuged for 10 min after each wash. The sample was dried at 105 °C for 2 h, ground, and passed through an 80-mesh sieve.

Preparation of the Guar Gum–Starch Composite

Guar gum was added at 20% of the dry mass of RS3, or 0.20 g guar gum per 1.00 g RS3. The mixture was dispersed in deionized water and stirred at 50 °C for 6 h. The mixture was dried at 105 °C for 2 h and ground.

Heat-Moisture Treatment

Deionized water equal to 30% of the dry mass of the RS3-guar gum composite was added. The sample was mixed well and treated at 120 °C for 30 min. After treatment, the sample was cooled, dried at 105 °C for 2 h, ground, and passed through a 100-mesh sieve.

In Vitro Digestion Assay

The digestion assay followed the RDS, SDS, and RS framework of Englyst et al. (1992), with modifications. For each test, 0.300 g of dry-basis sample was mixed with 7.5 mL water and heated in a boiling-water bath for 10 min. The tube was shaken about every 3 min. After cooling, 2.5 mL of 0.1 M sodium acetate buffer (pH 5.2) was added. The sample was held at 37 °C and 160 rpm for 15 min. Digestion started when 2.5 mL of mixed enzyme solution was added.

At 20 and 120 min, 250 µL of hydrolysate was transferred into 10 mL of 66% ethanol to stop hydrolysis. The samples were centrifuged at 4100 rpm for 10 min. A 10 µL portion of supernatant was mixed with 200 µL of GOPOD reagent, held at 37 °C for 30 min, and measured at 510 nm.

$$\text{RDS (\%)} = (\text{G20} - \text{FG}) \times 0.9 / \text{m} \times 100$$

$$\text{SDS (\%)} = (\text{G120} - \text{G20}) \times 0.9 / \text{m} \times 100$$

$$\text{RS (\%)} = 97.50 - \text{RDS (\%)} - \text{SDS (\%)}$$

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FG represents the glucose content of the blank control; G20 and G120 represent the glucose amounts measured after 20 and 120 min of digestion, respectively. The term *m* represents the dry-basis sample mass in milligrams. The total starch content of the samples was 97.50%; therefore, RDS, SDS, and RS were expressed on a whole-sample dry-mass basis and summed to 97.50%.

The four groups were native corn starch, RS3, the RS3-guar gum composite, and the heat-moisture-treated composite. Each group was tested three times with 300 mg per test. These were parallel measurements from the same prepared batch. They were used to reduce variation from sampling, weighing, color development, and the instrument.

STATISTICAL ANALYSIS

Digestibility results are reported as mean $\pm$ SD from three parallel measurements. Group differences were tested by one-way ANOVA followed by Tukey's HSD test in IBM SPSS Statistics. A value of $p < 0.05$ was considered significant. Since all replicates came from one prepared batch, the analysis compares the prepared samples within that batch and does not show between-batch reproducibility.

STRUCTURAL CHARACTERIZATION

Each prepared sample was tested three times by XRD. Patterns were collected with Cu K α radiation at 40 kV and 40 mA over 5°-40° 2 θ at 4°/min. Relative crystallinity was calculated in MDI Jade 9.0 as the crystalline peak area divided by the total diffraction area (Dome et al., 2020).

SEM samples were fixed on conductive adhesive, gold-coated, and imaged at 5.0 kV with an SU8020 microscope. The figure shows representative fields of view.

DSC measurements were performed three times with a Discovery DSC 25 differential scanning calorimeter (TA Instruments, USA). About 3 mg of sample and 9 μ L of water were sealed in an aluminum pan and left overnight to equilibrate. The samples were heated from 30 °C to 150 °C at 10 °C/min.

RESULTS

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In Vitro Digestibility

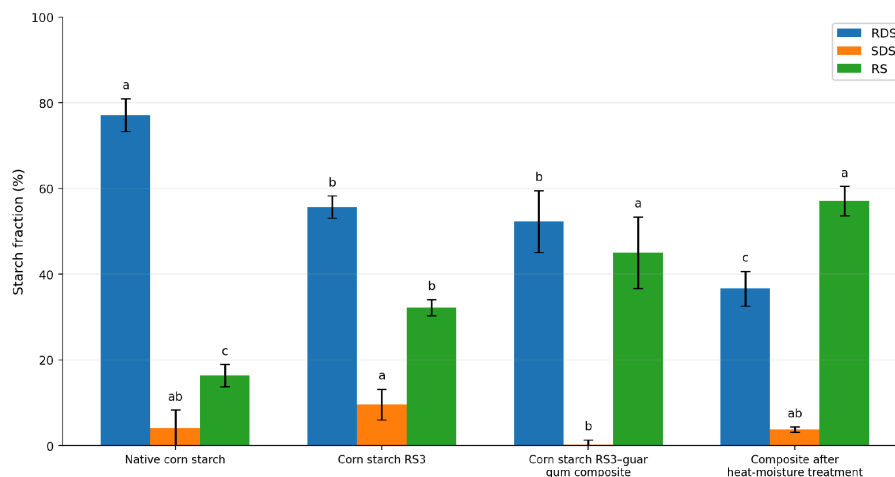
Table 1 and Figure 1 show how starch digestibility changed after each treatment.

Table 1

Rapidly Digestible, Slowly Digestible, and Resistant Starch Contents of Untreated and Sequentially Modified Samples

Sample	RDS (%)	SDS (%)	RS (%)
Native corn starch	77.13 ± 3.85 ^a	4.01 ± 4.32 ^{ab}	16.36 ± 2.60 ^c
RS3	55.67 ± 2.57 ^b	9.62 ± 3.59 ^a	32.22 ± 1.86 ^b
Corn starch RS3–guar gum composite	52.28 ± 7.26 ^b	0.22 ± 1.14 ^b	45.00 ± 8.37 ^a
Heat-moisture-treated RS3–guar gum composite	36.64 ± 4.06 ^c	3.76 ± 0.65 ^{ab}	57.10 ± 3.44 ^a

Note. Values are mean ± SD from three analytical replicates of one prepared batch. Fractions are expressed on a whole-sample basis and sum to the total starch content of 97.50%. Within each fraction, values without a shared superscript differ at $p < .05$ by one-way ANOVA and Tukey HSD. Created by author, 2026.



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Figure 1

RDS, SDS, and RS Contents of Untreated and Sequentially Modified Corn Starch Samples

Note. Bars show mean $\pm$ SD ($n = 3$ analytical replicates); colors denote RDS, SDS, and RS. Letters indicate Tukey HSD groupings within each fraction ($p < .05$). Created by author, 2026.

RS3 formation reduced RDS from 77.13% to 55.67% and increased RS from 16.36% to 32.22% ($p < 0.05$).

Adding 20% guar gum raised RS to 45.00%. RDS fell slightly to 52.28%, but it did not differ significantly from the RS3 group.

The final composite had the lowest RDS value, 36.64%, and the highest mean RS value, 57.10%. Heat-moisture treatment significantly reduced RDS. The rise in RS from 45.00% to 57.10% was not statistically significant.

Across the full treatment sequence, RDS fell by 40.49 percentage points and RS rose by 40.74 percentage points.

Crystalline Structure (XRD)

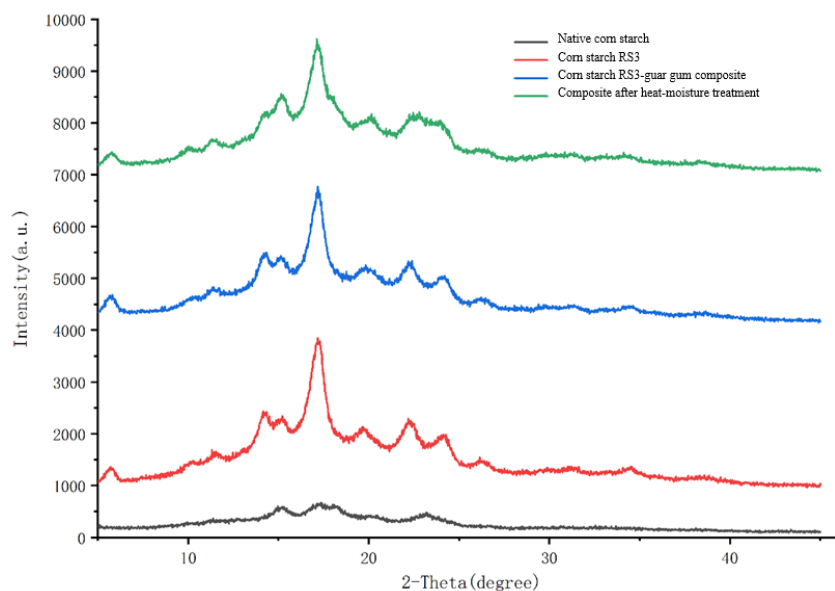


Figure 2

X-Ray Diffraction Patterns of Untreated and Sequentially Modified Corn Starch Samples

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Note. Black, red, blue, and green curves represent untreated native corn starch, RS3, the RS3–guar gum composite, and the final heat-moisture-treated composite, respectively. The horizontal axis shows diffraction angle (2θ , degrees), and the vertical axis shows diffraction intensity in arbitrary units (a.u.). Native corn starch displayed characteristic A-type peaks near 15.3° , 17.2° , 18.0° , and 23.0° . Modified samples displayed a mixed B+V-type pattern, including peaks near 17.0° , 20.0° , and 22.5° . Curves are vertically offset for clarity; baseline positions should not be interpreted as absolute intensity differences. Created by author, 2026.

Native corn starch had peaks near 15.3° , 17.2° , 18.0° , and 23.0° . This pattern is consistent with A-type starch (Dome et al., 2020). Its relative crystallinity was 16.90%.

Debranching and recrystallization weakened the A-type peaks. Peaks near 17.0° , 20.0° , and 22.5° showed a mixed B+V-type pattern, which agrees with earlier work on pullulanase debranching and recrystallization (Miao et al., 2009). Relative crystallinity rose to 62.8%.

The mixed B+V-type pattern remained after guar gum addition and heat-moisture treatment. Relative crystallinity increased to 82.1% and 86.8%, respectively.

Microstructure (SEM)

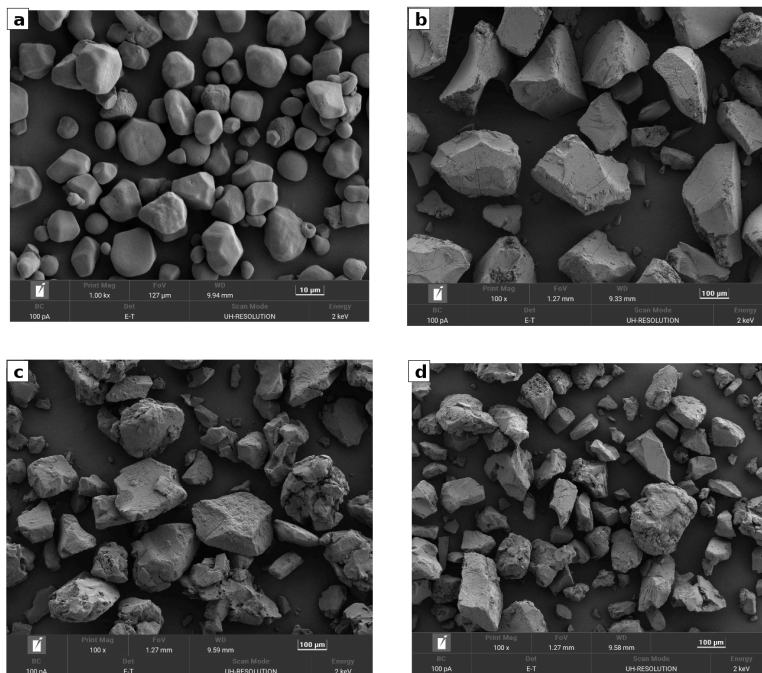


Figure 3

SEM Micrographs of Untreated and Sequentially Modified Corn Starch Samples

Note. (a) Native starch; (b) RS3; (c) RS3–guar gum; (d) heat-moisture-treated composite. Panel (a): 1.00 k $\times$, 10 μ m; panels (b)–(d): 100 $\times$, 100 μ m. Apparent particle sizes should not be directly compared across magnifications. Created by author, 2026.

Native starch granules were round and smooth. RS3 particles were rougher and more fractured.

The particles became more aggregated after guar gum addition and heat-moisture treatment. Their boundaries were also less clear, which matched the matrix changes seen in the treated samples.

Thermal Properties (DSC)

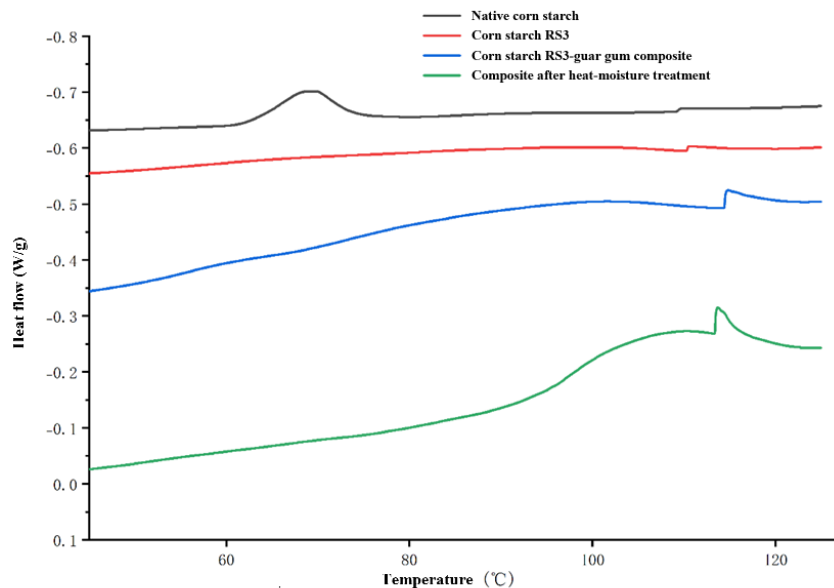


Figure 4

DSC Thermograms of Untreated and Sequentially Modified Corn Starch Samples

Note. Curves represent native starch, RS3, RS3–guar gum, and the heat-moisture-treated composite. Endothermic heat flow is upward; curves are vertically offset. Created by author, 2026.

Native corn starch had T_o , T_p , T_c , and ΔH values of 61.18 °C, 69.23 °C, 78.40 °C, and 2.85 J/g, respectively.

After guar gum was added, T_o , T_p , and T_c rose to 66.73 °C, 80.72 °C, and 96.45 °C. ΔH fell to about 1.90 J/g. Heat-moisture treatment shifted and broadened the main DSC peak further.

DISCUSSION

Effects of RS3 Formation on Digestibility and Structure

RS rose by 40.74 percentage points across the treatment sequence, while RDS fell by 40.49 percentage points. These changes show that the prepared samples behaved very differently under the same in vitro test conditions.

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The change after RS3 formation agrees with reports that pullulanase debranching and recrystallization can reorganize maize starch and make it less open to enzymatic attack (Miao et al., 2009).

Contribution of Guar Gum Complexation

Guar gum addition was linked to a higher RS fraction and stronger particle aggregation in the SEM images. Earlier work shows that guar gum can change viscosity and limit enzyme access (Dartois et al., 2010). This study did not measure viscosity or diffusion, so those explanations remain possible rather than confirmed.

Contribution of Heat-Moisture Treatment

Heat-moisture treatment produced the lowest RDS value and the highest mean RS value. It was applied after guar gum addition, so the experiment shows the combined result of the full sequence. It does not separate the effect of heat-moisture treatment from the earlier steps.

Relationship Among Digestibility and Structural Measurements

Lower RDS and higher mean RS appeared with several structural changes: the crystal pattern shifted from A-type to mixed B+V-type, relative crystallinity increased, particles became more aggregated, and DSC behavior changed. The same samples showed both structural and digestibility changes, but the data do not prove that one single mechanism caused them.

LIMITATIONS AND IMPLICATIONS

The three measurements were technical replicates from one prepared batch, not separate preparation batches. The statistical results therefore describe differences within the prepared samples. Future experiments should use independent batches and directly measure viscosity and enzyme access. In vivo testing is also needed before making claims about glycemic response.

CONCLUSION

Under the present in vitro conditions, the sequential modification of corn starch increased resistance to enzymatic digestion.

Mean RS increased from 16.36% to 57.10%, while RDS decreased from 77.13% to 36.64%. RS increased significantly after RS3 formation and guar gum addition, and final heat-moisture treatment significantly reduced RDS.

Together, the digestion and structural results suggest that RS3 formation, guar gum complexation, and heat-moisture treatment can meaningfully alter corn-starch digestibility, although independent preparation replicates and in vivo studies are needed before drawing stronger application conclusions.

AI USE DISCLOSURE

This English manuscript was translated in part and revised from the author's original Chinese manuscript with help from ChatGPT. AI was not used to generate experimental data, numerical results, references, or scientific conclusions. The author checked and approved the final manuscript.

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