Comparative study on the structure-properties relationships of native and debranched rice starch

Chuan Cao, Mingyu Shen, Jinwei Hu, Jun Qi, Peng Xie & Yibin Zhou

To cite this article: Chuan Cao, Mingyu Shen, Jinwei Hu, Jun Qi, Peng Xie & Yibin Zhou (2020) Comparative study on the structure-properties relationships of native and debranched rice starch, CyTA - Journal of Food, 18:1, 84-93, DOI: 10.1080/19476337.2019.1710261

To link to this article: https://doi.org/10.1080/19476337.2019.1710261
Comparative study on the structure-properties relationships of native and debranched rice starch

Chuan Cao, Mingyu Shen, Jinwei Hu, Jun Qi, Peng Xie and Yibin Zhou

*Anhui Engineering Laboratory for Agro-products Processing, Anhui Agricultural University, Hefei, China; †Department of Food Engineering, Anhui Vocational College of Grain Engineering, Hefei, China; ‡Institute of Food Economics of NJUE, Nanjing University of Finance & Economics, NanJing, China

ABSTRACT
The structure-properties relationships of native and debranched starch (DBS) were investigated by analyzing the results of DSC, XRD, NMR, HPAEC, FT-IR, SEM, hydrolysis, and digestibility properties. After debranching of starch in waxy rice, japonica rice, and indica rice, the linear short-chain molecules formed were easier to alignment and aggregation, and associate into a double helix. The crystalline structure of the rice starch after the pullulanase treatment was transformed from the type A to the type V crystalline by XRD measurement. Based on FT-IR and 13C NMR observations, molecular rearrangement and degree of order in starch granules increased. The DSC curve showed an increase in gelatinization temperature of debranched starch compared to native starch. Meanwhile, Solubility, water holding capacity and resistant starch content of DBS also raised. The study of structure-properties relationship provides a theoretical foundation for the development of foods or drugs with targeted functional properties.

Abbreviations
- WS: waxy rice starch; JS: Japonica rice starch; IS: Indica rice starch; DBWS: debranched waxy rice starch; DBJS: debranched Japonica rice starch; DBIS: debranched Indica rice starch; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; DP: degree of polymerization; DSC: differential scanning calorimetry; Gelatinization temperature at onset (T_o), peak (T_p), and end (T_c); ΔH: transition enthalpy; HPSEC: high-performance size-exclusion chromatography; ATR-FTIR: Attenuated total reflectance Fourier transform infrared spectroscopy; NMR: nuclear magnetic resonance; XRD: X-ray diffraction; SEM: Scanning electron microscopy; WHC: Water holding capacity.

1. Introduction
Rice was one of the most important starchy foods consumed throughout Asia (Chang et al., 2018), and widely used on large-scale in China. With the improvement of rice breeding technology, rice production was gradually increasing, which was widely used in the food industry besides daily
consumption. Starch was one of the main components in rice, and affects the quality and texture of rice products (Chen et al., 2017). However, the lack of stability of native rice starch under extreme conditions (heating, shearing, and pH) limits its use in processed foods (Lee, Lee, & Lee, 2010). Thus, the chemical and biotechnological modifications was significant for extending the applications of native starches. The debranched starch obtained by enzymatical modification can be used as substitute for fats and proteins, coating of cereal breakfast food, embedding drugs and active substances(Liu, Gu, Hong, Cheng, & Li, 2017).

The molecular structure of starch had important effect on the quality of starchy food (Jang et al., 2016; Li, Wen, Wang, & Sun, 2018). Currently, many techniques have been widely used to characterize the structural changes of starch in different processing. XRD was used to detect the crystalline structure of different starch and DBS fractions, Solid-state $^{13}$C NMR and FT-IR were used to characterize the sensitive behavior of short-range structural features of helix structures (Ma, Yin, Chang, Hu, & Boye, 2018), and enthalpic transitions of DSC reflected the thermal energy required for crystallite melting and double helix dissociation (Chen, Ren, Zhang, Tong, & Rashed, 2015; Martinez et al., 2018). The amyllose–amylopectin ratio and amylopectin branch-chains distribution influence the physicochemical properties of starch and their susceptibility to pullulanase hydrolysis (Lin et al., 2018). After debranching, the amount of short amylose and the amylopectin molecule with a certain degree of polymerization (DP) of chain length was increased. Meanwhile, the molecules had a greater chance of aligning and aggregating to form double helix (Ma et al., 2018), thereby promoting the formation of resistant starch by self-assembly (Liu et al., 2018). These changes significantly affected the particle size, crystal, and helical structure as well as functional properties of the starch (Liu et al., 2015; Yang et al., 2016). Recently, many researches engaged with rice starch and debranched starch have focused on the structure of rice varieties and growth location affecting the structure parameters (Li & Liu, 2019), and the structure-digestion relationship of starch after gelatinization (Martinez et al., 2018) and effects of pullulan on rice starch gel during cold storage (Chen et al., 2017). Despite extensive studies were conducted on enzymatic hydrolysis of rice starch, besides the structural and physicochemical properties of a debranched rice starch, there was no information available on comparative studies of three widely used rice starches before and after debranching.

In this study, waxy rice, japonica rice, and indica rice were debranching to compare with the different properties between three native rice starches and DBS. The relationship between the structure and physicochemical properties of the three native rice starches and the DBS was also evaluated. The obtained results can be useful for the improved applications of rice starch particularly, intensifying the utilization of DBS in the food industry.

2. Materials and methods

2.1. Materials

Waxy rice starch (proteins: 0.66%, lipids: 0.051%, amyllose content: 1.97%, and moisture: 4.4%, w/w); Indica rice starch (proteins: 0.49%, lipids: 0.045%, amyllose content: 25.65%, and moisture: 6.02%, w/w), and Japonica rice starch (proteins: 0.41%, lipid: 0.046%, amyllose content: 17.23%, and moisture: 5.34%, w/w) were obtained from Guangming Huaixiang Co., Ltd. (Hefei, Anhui province, China). Pullulanase (1498 NPUN/g) was obtained from Sigma Aldrich (Chemical Co. St. Louis, MO, USA). Amyloglucosidase (100000U/g) from Aspergillus niger and a-amylase (13U/g) from Pancreatin were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China).

All the other chemicals and reagents used in the experiments were of analytical grade procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample preparation

As per the previous method with some modifications for DBS preparation (Liu, Hong, Gu, Li, & Cheng, 2015), Rice starch (5% w/v) and acetic acid buffer (0.01 M, pH 5.5) were added into a 500mL Erlenmeyer flask, boiled and stirred for 30 min, and transferred to vertical heating pressure steam sterilizer (LDZX-50BFS; Shengen Medical Instrument Factory, Shanghai, P. R. China). The above mixture was incubated for 1 h at 121°C, cooled to 55°C, then pullulanase (100U/g) was added followed by further incubation for 12 h at 55°C and pH 5.5. Subsequently, ethanol precipitation was carried out for 12h at ambient temperature followed by centrifuging at 5000r/min for 10 min (JW-1016; Jiaven Equipment Industry Co., Ltd., Jiangsu, P. R. China). After centrifugation, sediments were washed with anhydrous ethanol and vacuum dried (DGT-G-C; Darth carter Experimental Instrument Co., Ltd., Shanghai, P.R. China) at 40°C to obtain DBS.

2.3. Structural properties

2.3.1. Chain length distribution

Chain length distribution from rice starches was determined by High-performance anion-exchange chromatography (HPAEC-PAD) (Bertoft et al., 2016) (Dionex ICS-5000, Dionex Corp., Sunnyvale, CA, USA). The mobile phase consisted of three parts: A phase: dd H$_2$O; B phase: 100mM NaOH; C phase: 100 mM, NaOH, 500 mM NaAC and the flow rate of 0.5 mL/min.

2.3.2. FT-IR spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was determined by a previously reported method (Sun et al., 2017) with some modifications. The samples were analyzed using FT-IR spectra (ISS0, Thermo Nicolet Corporation, America). The obtained infrared spectra were processed by OMNIC 8.0. Baseline calibration and deconvolution were performed in the range of 950-1075cm$^{-1}$ to calculate the absorbance ratios of 1047/1022 cm$^{-1}$ and 995/1022 cm$^{-1}$.

2.3.3. Solid-state NMR

$^{13}$CCP/MAS nuclear magnetic resonance (NMR) of three kinds of starches before and after debranching were measured using NMR spectrometer (BRUKER AVANCE III 400WB, Swiss, France) based on the previously described procedure (Yang et al., 2016).

2.3.4. X-ray diffraction (XRD)

The crystallinity of natural starch and debranched starch was determined by X-ray diffractometry (TTR-III, Rigaku, Japan) as per the previously reported method (Yang et al., 2016).
2.3.5. Scanning electron microscopy (SEM)
Morphology of starch granules was determined by Hitachi field emission SEM (SEM S-4800, Hitachi Japan). The microscopic morphology and structure of the starch granules were observed. Sample spraying treatment was performed according to the previous method (Yang et al., 2018) with an accelerating voltage of 1.0kV and an amplification factor of 6000 times.

2.4. Physicochemical properties

2.4.1. Thermal performance analysis
Evaluation of the thermal properties of native starch and DBS were conducted using Differential Scanning Calorimeter (DSC) (DSC2000, Perkin Elmer Instruments Company Ltd., Shanghai, China). For the measurement of thermal properties (Yang et al., 2016), each sample (3.0 mg) was mixed with 9 μl of distilled water in an aluminum vessel and hermetically sealed, and kept at ambient temperature (25 ± 1°C) for 24 h to equilibrate moisture before DSC test. The sample crucibles were heated from 30 to 130°C at a heating rate of 10°C/min. The blank control was used as an empty aluminum crucible. The $T_o$, $T_p$, $T_c$, and $\Delta H$ were determined from the data recording software.

2.4.2. Water holding capacity (WHC)
WHC of starch samples was determined according to the previous method with few modifications (Liu et al., 2015).

2.4.3. Solubility and expansion properties
Starch slurry (5%) was completely gelatinized and 10 ml of the emulsion was used in a centrifuge test tube, centrifuged at 3000 r/min for 30 min, and precipitated. The supernatant was placed in the surface pan and dried at 105°C for constant weight, and the water-soluble starch was weighed (Tang, Liu, Li, & Dong, 2015). Solubility and expansibility are calculated according to the following formulas.

\[
\text{Solubility} \, (\%) \, S = \frac{A}{W} \times 100
\]

\[
\text{Expansion capacity} \, (\%) = \frac{P \times 100}{W \times (100 - S)}
\]

Where, A represents supernatant after steaming and constant weight. W is the quality of the fine sample. P is the centrifugal tube weight.

Figure 1. Distribution of chain length of rice starches. (a) Waxy rice starch (b) Japonica rice starch (c) Indica rice starch.

Figura 1. Distribución de la longitud de la cadena de los almidones de arroz. (a) Almidón de arroz ceroso (b) Almidón de arroz japonica (c) Almidón de arroz indica.
2.5. **In vitro digestion properties**

The digestibility of native starches and DBS samples were analyzed based on the previously reported method with modification (Englyst, Kingman, & Cummings, 1992). The sample (200 mg) and 15 mL of sodium acetate buffer (0.2 M, pH 5.2) were added to a 50 mL Erlenmeyer flask. Few glass beads were added to allow gelatinization for 30 minutes followed by stirring in a water bath (150 rpm) at 37°C for 5 min. Pancreatin α-amylase (290 U/ml) in 10 mL and Amyloglucosidase (160 U/ml) in 1 mL of sodium acetate buffer mixture were added into each tube and tubes were incubated at 37°C. Subsequently, 1 mL from each tube was removed at 0, 20, 40, 60, 80, 120, 180 min, and mixed with 2 mL of absolute alcohol. Then, 1 mL from the resulting solution was centrifuged (5000 r/min for 5 min) and 0.5 mL of supernatant was used for the absorbance measurement using the 3,5-dinitrosalicylic acid method. Different starch components (RDS, SDS, and RS) were calculated on the basis of previous reports (Chen et al., 2017; Meng-Na, Ying, Han-Qing, & Bao, 2019).

2.6. **Statistical analyses**

Significant differences among the average values ($P < 0.05$) were analyzed by Duncan’s multiple ranges evaluated using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. **Structural properties**

3.1.1. **Chain length distribution**

The chain length distribution of waxy, Japonica, and Indica rice starches was shown in Figure 1. According to the chain length of the degree of polymerization (DP), starch branches were classified into four types: A (6–12), B$_1$ (13–24), B$_2$ (25–36), and B$_3$ (≥37) chain (Kennedy & Mistry, 2003).

The result of chain length distribution showed that amylopectin of Indica rice starches has longer branches (B$_2$ and B$_3$ chains: 27.47%), in comparison with waxy rice starch and Japonica rice starch. Whereas, the waxy rice starch has a low proportion of long-chain (B$_2$ and B$_3$ chains: 22.08%).

![Figure 2. FTIR spectra (a) and ATR-FTIR spectra (b) of native and debranched rice starches.](image-url)

**Figure 2.** Espectros FTIR (a) y espectros ATR-FTIR (b) de almidones de arroz nativo y desramificado. WS: almidón de arroz ceroso; JS: almidón de arroz japonica; IS: almidón de arroz indica; DBWS: almidón de arroz ceroso desramificado; DBJS: almidón de arroz japonica desramificado; DBIS: almidón de arroz indica desramificado.
sequence of A-chain percentage was as follows: waxy (32.61%) > Indica (26.19%) > Japonica (25.75%). The average chain length of waxy rice starch was the shortest (DP: 19.27), and it was the longest (DP: 21.34) for Indica rice starch. Liu reported that pullulanase can selectively hydrolyze alpha-1,6-D-glycoside bonds, theoretically, pullulanase was more difficult to cleave longer lateral chains (Liu et al., 2015), thus amylopectin with more short side chains could be effectively hydrolyzed (Srichuwong, Isono, Mishima, & Hisamatsu, 2005). The differences in chain length distribution of branching structure would affect the hydrolysis and physicochemical properties of DBS. This could be concluded waxy rice more effective for the hydrolysis of pullulanase based on more short side chains in it.

3.1.2. ATR-FTIR

The FTIR spectra of native rice starches and DBS were shown in Figure 2(a). No significant difference in peak shape was found for debranching samples as compared to the native rice starches. However, Figure 2(b) showed that peak shifts from 1022 to 1024 cm\(^{-1}\) after debranching. The absorption bands observed near 1045 and 1022 cm\(^{-1}\) were corresponding to the signal region of the native rice starch (Chavez-Murillo, Orona-Padilla, & de la Rosa Millan, 2019). The peaks around 995 cm\(^{-1}\) were attributed to the vibration of C-OH of starches, which was associated with the hydrogen bonding interaction between starch and water.

The ratio of 1045/1022 obtained from the deconvoluted ATR-FTIR was used to characterize the degree of order in starch granules (Sun et al., 2017). Pullulanase treatment tends to increase the ratio of 1045/1022 in the native starch, and the sequential increase in starch granules was related to the amorphous lamellae density and the content of the amorphous and amorphous structures of starch (Chavez-Murillo, Orona-Padilla, & de la Rosa Millan, 2019). The peaks around 995 cm\(^{-1}\) were observed in the C\(_1\) region, which was attributed to the hydrogen bonding interaction between starch and water.

The ratio of 1045/1022 obtained from the deconvoluted ATR-FTIR was used to characterize the degree of order in starch granules (Sun et al., 2017). Pullulanase treatment tends to increase the ratio of 1045/1022 in the native starch, and the sequential increase in starch granules was related to the amorphous lamellae density and the content of the amorphous and amorphous structures of starch (Chavez-Murillo, Orona-Padilla, & de la Rosa Millan, 2019). The peaks around 995 cm\(^{-1}\) were observed in the C\(_1\) region, which was attributed to the hydrogen bonding interaction between starch and water.

The ratio of 1045/1022 obtained from the deconvoluted ATR-FTIR was used to characterize the degree of order in starch granules (Sun et al., 2017). Pullulanase treatment tends to increase the ratio of 1045/1022 in the native starch, and the sequential increase in starch granules was related to the amorphous lamellae density and the content of the amorphous and amorphous structures of starch (Chavez-Murillo, Orona-Padilla, & de la Rosa Millan, 2019). The peaks around 995 cm\(^{-1}\) were observed in the C\(_1\) region, which was attributed to the hydrogen bonding interaction between starch and water.

3.1.3. \(^{13}\text{C}\) NMR

Solid-state NMR of native and debranched rice starches were shown in Figure 3. Chemical shifts of carbon in starch were identified: C\(_1\) (106–96 ppm), C\(_2\), C\(_3\) and C\(_4\) (70–73 ppm), C\(_5\) (79–83 ppm) and C\(_6\) (59–62 ppm). Triplet peaks were observed in the C\(_1\) region, which indicated that the native rice starch was an A-type starch (Delval et al., 2010). It can be noticed that the C\(_1\) signal region of the native starch had distinct triplet peaks which were consistent with the results of previous studies (Fan et al., 2013). The two broad peaks in C\(_1\) were close to 101 ppm and 81 ppm in the C\(_2\) region. Peaks in C\(_1\) revealed the crystalline and amorphous structure of the starch, the C\(_4\) signals denoted the V-type single-helix crystal and amorphous structure of the starch. The chemical shifts of the four major peaks of the three rice starches were identical.

After debranching, the signal in the C\(_1\) and C\(_4\) regions representing the amorphous state gradually increased, whereas the triplet peaks in the crystalline state steadily

![Figure 3. \(^{13}\text{C}\)CP/MAS NMR spectra of native and debranched rice starches.](image)

**Figure 3.** \(^{13}\text{C}\)CP/MAS NMR spectra of native and debranched rice starches.

**Figura 3.** Espectros de \(^{13}\text{C}\)CP/MAS NMR de almidones de arroz nativos y desramificados.
decreased and almost disappeared. The relative intensities of starch treated with pullulanase showed an increase in the C$_4$ region, indicating an increase in the amorphous content of the three DBS. Combined with the results of FTIR and $^{13}$C solid-state NMR analysis, it can be concluded that native rice starch treated with pullulanase could damage the crystalline region.

### 3.1.4. X-ray diffraction (XRD)

The effects of pullulanase on the crystalline structure of rice starch were determined by XRD. As shown in Figure 4, the positions of three rice starch diffraction peaks were mainly concentrated on the diffraction angles 2$\theta$ of 10°-27°, and strong diffraction peaks appeared at the diffraction angles 2$\theta$ of 15°, 17°, 18°, and 23° have displayed typical type A diffraction pattern (Farooq, Dhital, Li, Zhang, & Huang, 2017). The V-type crystalline structure has a large hollow structure, which was formed by forming an inclusion complex between linear short chain and polar organic molecules (Thérien-Aubin & Zhu, 2009). After pullulanase treatment, the position of some diffraction peak of the debranched starch was changed, V-shaped patterns were observed. The relative crystallinity for DBWS, DBJS and DBIS were 12.25%, 13.51% and 14.91% respectively, which showed decrease compared to WS (26.75%), JS (25.22%) and IS (24.70%). This confirms the fact that pullulanase treatment tended to decrease the relative crystallinity and increase the chances of linear chains reassociate into crystal structures (Liu et al., 2017). The characteristic diffraction peak intensity of DBWS after debranching was lower than that of DBJS and DBIS due to the generation of more linear short chains. This can be explained on the basis of the fact that the distribution of chain length, the content of amylose, and the branching pattern of branches can affect the crystal structure of DBS.

Figure 4. X-ray diffraction patterns of native and debranched rice starches.

Figure 4. Patrones de difracción de rayos X de almidones de arroz nativos y desramificados.

Figure 5. Scanning electron micrographs of three varieties of native and debranched rice starches. The magnification range of raw starch and debranched starch was 6000x.

Figure 5. Micrografías electrónicas de barrido de tres variedades de almidones de arroz nativo y desramificado. Rango de aumento de almidón crudo y almidón desramificado fue 6000x.
3.1.5. Starch granule morphology
The effect of debranching on the morphology of three types of starch can be seen from Figure 5. Native rice starches granules displayed regular polyhedrons with distinct edges and smooth surfaces, and there were no pinholes noticed on the particles (Corgneau et al., 2019). After debranching, visible changes on the surface of the particles of DBS, the absence of particles to form agglomerates were observed. Loss of structural integrity of the starch granules, indicating that the ordered crystal structure was destroyed (Yang et al., 2016). Ma thought that more short linear chain in colloid and a certain concentration of liquid, the amount of short linear chain molecule could form double helices (Ma et al., 2018). The reassociation and recrystallization of starch molecules usually lead to matrix shrinkage, which lead to synergies and a more denser network.

3.2. Physicochemical properties
3.2.1. Thermal properties
As shown in Figure 6 and Table 1, the initial (To), peak (Tp) and conclusion (Tc) of different rice starches determined by DSC were analyzed. The previous study reported that the content of amylose was the major factor that can influence the thermal properties of starches (Ahmad et al., 1999). Our results indicated that waxy rice starch had a low melting temperature, in comparison with Japonica rice starch and Indica rice starch, due to the increased gelatinization temperature with increasing amylose contents, which was consistent with the previous report (Chung, Liu, Lee, & Wei, 2011). The melting temperature of the starch was related to the chain length. According to the distribution of chain length of rice starch, the relative content of amylpectin chains with the degree of A chains (DP 6–12) were negatively correlated with To, Tp, and Tc (Vandeputte, Vermeylen, Geeroms, & Delcour, 2003). The enthalpy for waxy rice starch was the lowest, due to the low ratios of longer side amylpectin chains (Kohyama, Matsuki, Yasui, & Sasaki, 2004).

The gelatinization temperature of DBS significantly increased when compared with the native starch. DBS had a higher melting temperature range, indicating that DBS had a wider distribution of crystallite sizes. A possible reason for this difference was attributed to the higher content of short linear chains in DBS, the molecular easier to alignment and aggregation, and associating the more linear short-chain starches into a double helix (Chen et al., 2015). For enthalpy values, DBS exhibited a slight decrease, it was probably because the relative crystallinity in DBS was lower, resulting in the melting enthalpy was decreased due to the decreased crystallinity.

3.2.2. WHC, solubility and expansion properties
As shown in Figure 7, water holding capacity and solubility were improved, and the degree of expansion was weakened after debranching. Native starches were insoluble in water and their water holding capacity was weak (Aberle et al., 1997). The water holding capacity of waxy rice starch was higher than those of Japonica rice starch and Indica rice starch which was attributed to the amylose content, side-chain length (Liu et al., 2015).

After debranching, the amylose content increased, resulting in an increase in double helix content, and increased its solubility. There was no significant discrepancy observed in the expansion of the three kinds of DBS. DBS samples showed better solubility and water holding capacity due to linear short glucan chains (Kim, Chen, & Shin, 2015). Therefore, DBS was useful for the preparation of novel hydrophilic material.

DBWS had a lower solubility than DBJS and DBIS, the reason may be the more short starch chains in DBWS. Whereas, linear

Table 1. Thermal characteristics of native and debranched rice starches.

| Sample | T(°C) | ΔT(°C) | ΔH(J·g⁻¹) |
|--------|------|--------|-----------|
| WS     | 61.94 ± 0.23 | 73.21 ± 0.42 | 13.9905 ± 0.38 |
| JS     | 63.65 ± 0.29 | 75.15 ± 0.51 | 14.8728 ± 0.32 |
| IS     | 64.26 ± 0.42 | 78.93 ± 0.18 | 14.9831 ± 0.47 |
| DBWS   | 83.47 ± 0.31 | 97.69 ± 0.29 | 99.46 ± 0.82 |
| DBJS   | 85.79 ± 0.18 | 99.46 ± 0.82 | 105.72 ± 0.53 |
| DBIS   | 89.85 ± 0.22 | 105.72 ± 0.53 | 15.87 ± 0.65 |

The data in the table is the average of three experiments. Assays were performed in duplicates. Values with different superscripts within a column are significantly different (P < 0.05).

To: onset temperature; Tp: peak temperature; Tc: conclusion temperature; WS: waxy rice starch; JS: Japonica rice starch; IS: Indica rice starch;
DBWS: debranched waxy rice starch; DBJS: debranched Japonica rice starch; DBIS: debranched Indica rice starch.

Los datos en la tabla son el promedio de tres experimentos. Los ensayos se realizaron por duplicado. Los valores con diferentes superíndices dentro de una columna son significativamente diferentes (P < 0.05).

To: temperatura de inicio; Tp: temperatura pico; Tc: temperatura de conclusión; WS: almidón de arroz ceroso; JS: almidón de arroz japonica; IS: almidón de arroz indica; DBWS: almidón de arroz ceroso desramificado; DBJS: almidón de arroz japonica desramificado; DBIS: almidón de arroz indica desramificado.

Figure 6. Thermal characteristics of native and debranched starches.

Figure 6. Características térmicas de los almidones nativos y desramificados.
short glucan chains resulted in decreased starch solubility, due to their tendency to aggregate and entangled.

3.3. In vitro digestion properties

The digestibility of native rice starches and DBS were shown in Figure 8. The hydrolysis rate of native starch was 80%, while that of debranched starch was 40%. Due to the inhibitory effect of pullulanase on starch gelatinization and the coating on the surface of starch granules, the access of the enzyme to its structure was limited, resulting in a decrease in the starch digestibility of DBS (Chen et al., 2017). Indica rice starch was more susceptible to hydrolysis by pancreatin because amylopectin with a larger molecular weight could retain more water, and the gel structure was easily

![Figure 7. Solubility, expansion capacity and water holding capacity of starches native and debranched rice starches.](image1)

![Figure 7. Solubilidad, capacidad de expansión y capacidad de retención de agua de almidones de arroz nativo y desramificado.](image2)

![Figure 8. Digestibility of native and debranched rice starches.](image3)

![Figure 8. Digestibilidad de almidones de arroz nativo y desramificado.](image4)
debranched by enzymes. DBS tends to resist the hydrolysis of pancreatin compared to native starches.

SDS was associated with stable glucose metabolism, a slow increase in postprandial blood glucose has a health advantage, and RS can prevent and control certain chronic diseases to improve human health (Pan et al., 2019; Sun et al., 2018). Pullulanase modification of rice starch led to a decrease in RDS content and an increase in SDS and RS content (Figure 9). The RS content of the DBWS sample was 17.29%, and it was higher than DBJS (13.69%) and DBIS (12.95%). After treatment by pullulanase, the external branch of amylopectin was converted into many linear chains with a certain DP, thereby promoting the formation of resistant starch by self-assembly. This result was in agreement with previous reports (Liu et al., 2018). Consequently, the anti-enzymolysis capability of native starch by the modification of the debranching enzyme was observed.

4. Conclusions

Three rice starches had different amylose contents and amylopectin structure, and their properties significantly varied before and after debranching. The crystalline structure of the rice starch after the pullulanase treatment was transformed from the type A to the type V crystalline by XRD measurement. Debranching pronounced destroyed the crystal structure of the starch granules were observed by SEM. Moreover, according to FT-IR and $^{13}$C NMR results, molecular rearrangement and degree of order in starch granules increased. Compared to native starch, the debranching treatment enhanced the thermal stability, increased solubility and WHC, and enhanced the anti-enzyme capacity. In addition, the amylopectin of WS has higher A chain (32.61%), in comparison with JS and IS. Meanwhile waxy starch with most intensified degradation which suggesting that the hydrolysis was more effective for shorter side chains. According to the above results, the study of structure-property relationship provides a theoretical foundation for the development of foods or drugs with targeted functional properties, thereby extending the use of starch in the food industry.

Disclosure statement

The authors declare no conflicts of interest.

Funding

This study was financially supported by a grant from the “Twelfth Five Year” Plan of Science and Technology of Anhui Province, China [No. 1401032009], and “Anhui Scientific and Technical Tackle-Key-Problem Plan” [grant 1704a07020098].

ORCID

Chuan Cao  http://orcid.org/0000-0001-6226-646X

References

Aberle, T., Burchard, W., Hanselmann, R., Michel, E., Klingler, R. W., & Galinsky, G. (1997). Particularities in the structure of amylopectin, amylose and some of their derivatives in solution. Macromolecular Symposia, 120(1), 47–63. doi:10.1002/masy.v120.1
Ahmad, F. B., Williams, P. A., Dobbler, J. L., & Buleon. (1999). Physico-chemical characterisation of sago starch. Carbohydrate Polymers, 38(4), 361–370. doi:10.1016/S0144-8617(98)00123-4
Bertoft, E., Annor, G. A., Shen, X., Rumpagaporn, P., Seetharaman, K., & Hamaker, B. R. (2016). Small differences in amylopectin fine structure may explain large functional differences of starch. Carbohydrate Polymers, 140, 113–121. doi:10.1016/j.carbpol.2015.12.025
Chang, R., Xiong, L., Li, M., Liu, J., Wang, Y., Chen, H., & Sun, Q. (2018). Fractionation of debranched starch with different molecular weights via edible alcohol precipitation. Food Hydrocolloids, 83(4), 430–437. doi:10.1016/j.foodhyd.2018.05.033
Chavez-Murillo, C. E., Orona-Padilla, J. L., & de la Rosa Millan, J. (2019). Physicochemical, functional properties and ATR-FTIR digestion analysis of thermally treated starches isolated from black and bayo beans. Starch/Staerke, 71(3–4), 1–10. doi:10.1002/star.2018000250
Chen, L., Ren, F., Zhang, Z., Tong, Q., & Rashed, M. M. A. (2015). Effect of pullulan on the short-term and long-term retrogradation of rice
