



## Starch digestion and physicochemical properties of a newly developed rice variety with low glycemic index

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### ABSTRACT

This study aimed to clarify the starch digestion characteristics and related physicochemical properties of the newly developed low-GI rice variety, Ditangliangyou 335 (D335), in comparison with two widely grown rice varieties, Xiangzaoxian 45 (X45) and Zhongzao 39 (Z39). The results showed that D335 had an active digestion duration (286 min) that was 101–190 % shorter, a glucose production rate ( $1.06 \text{ mg g}^{-1} \text{ min}^{-1}$ ) that was 57–73 % slower, and a total glucose production ( $303 \text{ mg g}^{-1}$ ) that was 11–19 % less than X45 and Z39. These differences were attributable to the distinct starch physicochemical properties, including amylose content, amylose-to-amylopectin ratio, starch granule size, amylopectin chain length, and starch molar mass, as well as the different pasting properties of rice flour, such as pasting temperature and breakdown viscosity. These findings reveal the starch digestion characteristics and the key physicochemical properties that determine these characteristics in the low-GI rice variety D335.

### 1. Introduction

Rice, a high-carbohydrate food, typically has a high or medium-high glycemic index (GI) (Ngo et al., 2023). China, the world's largest rice consumer, has an annual rice consumption of 150 million tons and a *per capita* consumption of 77 kg per year (OECD-FAO, 2022). The country also has the highest number of diabetics globally, with 141 million people diagnosed with diabetes, alongside 27 million individuals with impaired fasting glucose and 170 million with impaired glucose tolerance (IDF, 2021). Although the direct link between increased rice consumption and a higher risk of diabetes in China remains inconclusive (Huang, 2023), developing rice varieties with a lower GI is crucial for reducing the prevalence of diabetes in the country (Huang & Hu, 2021).

The starch digestion rate represents a major factor in determining the GI of foods, with slower starch digestion leading to a lower GI (Yang et al., 2023). Starch consists of two polysaccharides: amylose, a linear chain of glucose molecules connected by  $\alpha$ -1,4-glycosidic bonds, and amylopectin, a highly branched polymer made up of glucose molecules connected by both  $\alpha$ -1,4-glycosidic and  $\alpha$ -1,6-glycosidic bonds (Maji,

2019). Amylose is more resistant to digestion than amylopectin due to its more compact structure and its tendency to recrystallize, forming resistant starch that is not easily broken down by human pancreatic amylase in the small intestine (Fernandes et al., 2020; Trinh, 2015). As a result, rice varieties with higher amylose content generally have a slower starch digestion rate compared to those with lower amylose content (Huang et al., 2022). Additionally, the amylose-to-amylopectin ratio also significantly influence the digestion of starch. A higher amylose-to-amylopectin ratio can lead to an increased content of resistant starch by reducing starch swelling, which in turn decreases the accessibility of enzymes required to hydrolyze starch molecules (Zaman & Sarbini, 2016).

Other physicochemical properties of starch, such as starch granule size, amylopectin chain length, and starch molar mass, also play a critical role in determining starch digestion in rice (Khatun et al., 2019; Li, 2023). Starch granule size affects starch digestion by influencing the surface area available for digestive enzymes (Khatun et al., 2019). Larger starch granules generally exhibit a lower starch digestion rate because they provide a smaller surface area for enzyme action (Dhital

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et al., 2015; Kong et al., 2003). Amylopectin chain length impacts starch digestion because it affects enzymatic hydrolysis (Ramadoss et al., 2019). Longer amylopectin chains can form stable helices through hydrogen bonds, making the starch more resistant to digestion (Lehmann & Robin, 2007). Conversely, shorter amylopectin chains facilitate easier digestion. For instance, Shu et al. (2009) found that short amylopectin chains with a degree of polymerization (DP) of 8–12 showed a positive correlation with starch digestion. Starch molar mass also influences digestion by affecting starch reassembly during heat-moisture treatment (Chi et al., 2024). Tu et al. (2021) reported that rice starch molecules with low molar mass ( $0.60\text{--}1.50 \times 10^7 \text{ g mol}^{-1}$ ) tended to reassemble into more ordered multi-scale structures, including double helical and short-range ordered structures. These arrangements increased the content of slowly digestible starch.

Non-starch chemical constituents also affect starch digestion in rice (Khatun et al., 2019). Protein, a major non-starch component in rice, can influence starch digestion by altering the microstructure, crystal structure, and thermal stability of starch (Lu et al., 2022). In addition, proteins may interact with starch to form a protective layer around the starch granules, which limits the accessibility of digestive enzymes and inhibits starch digestion (Khatun et al., 2019). Generally, higher grain protein content is associated with a slower rate of starch digestion (Ye et al., 2018).

Pasting properties of rice flour, which are influenced by both starch and non-starch constituents, are closely related to starch digestion (Khatun et al., 2019; Li, 2023). Bonmoussa et al. (2007) observed a positive correlation between rapidly digestible starch content and breakdown viscosity, whereas a negative correlation between slowly digestible starch content and breakdown viscosity. Chung et al. (2011) found that slowly digestible starch and resistant starch content were positively correlated with pasting temperature as well as setback and final viscosities, while negatively correlated with peak and breakdown viscosities. Ouyang et al. (2024) observed that resistant starch content was negatively correlated with peak and breakdown viscosities in rice.

In recent years, significant advancements have been made in developing rice varieties with a low GI (<55). The International Rice Research Institute (IRRI) has successfully developed two low-GI rice varieties, IRRI 125 and IRRI 147, which have already been released in the Philippines (IRRI, 2023). China has also developed several low-GI rice varieties. For instance, the Crop Breeding and Cultivation Research Institute of the Shanghai Academy of Agricultural Sciences released low-GI varieties Youtangdao 2 and Youtangdao 3 in 2019 and 2020, respectively (Yang et al., 2020; Yang et al., 2022). The Yuan Longping High-Tech Agriculture Co., Ltd. developed a low-GI variety Ditangliangyou 335 (D335), anticipated for release in 2024. Despite these developments, there is still a scarcity of data on the physicochemical properties of these low-GI rice varieties.

In our present study, we compared the starch digestion characteristics and associated physicochemical properties between the newly developed low-GI rice variety D335 and two widely grown rice varieties. The objectives of this study were to clarify the starch digestion characteristics of D335 and identify the key physicochemical properties linked to these characteristics.

## 2. Materials and methods

### 2.1. Rice varieties and crop management

Three rice varieties, including Xiangzaoxian 45 (X45), Zhongzao 39 (Z39), and D335, were used in this study. These three varieties belong to the *indica* early-season rice type, having a total growth duration of less than 115 d. X45 is characterized by its low amylose content and rapid starch digestion rate, whereas Z39 has a higher amylose content and a slower starch digestion rate (Hu et al., 2022). These two varieties have been widely grown by farmers. D335 is a newly developed low-GI rice variety. The three rice varieties were grown in a field experiment at

Pingtou Village (28°09' N, 113°37' E, 43 m asl), Yongan Town, Liuyang County, Hunan Province, China during the early rice-growing season in 2023. The field experiment employed a completely randomized block design with three replicates and a plot size of 15 m<sup>2</sup>.

Pre-germinated seeds were sown in a seedbed to raise seedlings on March 25. After 25 days, the seedlings were transplanted into plots with a hill spacing of 20 cm × 16.7 cm, placing three seedlings per hill. Urea was used as nitrogen fertilizer and applied in three splits: 75 kg N ha<sup>-1</sup> one day before transplanting, 45 kg N ha<sup>-1</sup> seven days after transplanting, and 30 kg N ha<sup>-1</sup> at the panicle initiation stage. Superphosphate was used as phosphorus fertilizer and applied at a rate of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> one day before transplanting. Potassium chloride was used as potassium fertilizer and applied in two splits: 75 kg K<sub>2</sub>O ha<sup>-1</sup> one day before transplanting and 75 kg K<sub>2</sub>O ha<sup>-1</sup> at the panicle initiation stage. A floodwater of 5–10 cm was maintained in all plots from transplanting until five days before the expected maturity, after which the plots were drained. Diseases, pests, weeds were controlled by chemicals as recommended by the local agricultural technology department.

### 2.2. Sampling and measurements

Approximately 500 g of rice grains were collected from each plot at maturity. The collected grains were sun-dried and then stored at room temperature. After a three-month storage period, the rice grains were milled using a laboratory mill. Head milled rice was selected from the milled rice for subsequent measurements.

#### 2.2.1. Measurements of starch digestion characteristics of cooked rice

About 10 g of head milled rice was cooked to measure the starch digestion characteristics, including active digestion duration (ADD), glucose production rate (GPR), and total glucose production (TGP). These measurements were conducted using a NutriScan GI20 automated *in vitro* digestion system (Next Instruments Pty Ltd., Condell Park, Australia), following the method outlined by Huang et al. (2022). In brief, the cooked rice was finely chopped 20 times using a hand-held food chopper. A precise 100 mg of the chopped rice was placed into the sample cups of the NutriScan GI20, which was maintained at a temperature of 37 °C. Three enzyme solutions were added to each cup to digest the samples: an  $\alpha$ -amylase solution, a pepsin solution, and a mixed solution of pancreatin and amyloglucosidase. The glucose released from the digested samples was measured at intervals of 15, 60, 120, 180, 240, and 300 min. The starch digestion kinetics of the cooked rice (as indicated by glucose production over time) were modeled using an exponential association model, expressed as  $y = a[1 - \text{EXP}(-bx)]$ , where  $y$  represents the estimated glucose produced per unit weight of cooked rice,  $x$  is the digestion time, and  $a$  and  $b$  are fitting parameters. The parameters for starch digestion were calculated using the following equations:  $\text{ADD} = \text{LN}(0.05)/-b$ ;  $\text{TGP} = 0.95a$ ; and  $\text{GPR} = \text{TGP}/\text{ADD}$ , with  $a$  and  $b$  derived from the fitting process.

#### 2.2.2. Measurements of chemical and pasting properties of rice flour

Approximately 20 g of head milled rice was ground into flour using a high-speed blender to analyze chemical properties, including total starch content, amylose content, amylopectin content, the amylose-to-amylopectin ratio, resistant starch content, and protein content. Additionally, the rice flour was also used to determine pasting properties, including pasting temperature and peak, trough, breakdown, final, setback, and consistency viscosities.

The total starch content was measured using a P850 Pro auto digital polarimeter (Hanon Instruments Co., Ltd., Jinan, China) following the stand procedure. The amylose content was determined using the iodine colorimetric method (Juliano, 1971), using standardized rice flour provided by the China National Rice Research Institute. The amylopectin content was calculated by subtracting the amylose content from the total starch content. The amylose-to-amylopectin ratio was derived by dividing the amylose content by the amylopectin content. The

resistant starch content was measured according to the protocol of Megazyme (2019). The protein content was determined by multiplying nitrogen content by a conversion factor of 5.95 (Juliano et al., 1971), with nitrogen content measured using a Skalar SAN Plus segmented flow analyzer (Skalar Inc., Breda, The Netherlands). The pasting properties were measured using an RVA-Super 4 rapid viscosity analyzer (Newport Scientific Pty Ltd., Warriewood, Australia) according to the standard methodology.

### 2.2.3. Measurements of starch granule size, chain length, and molar mass

Approximately 10 g of head milled rice flour was used to starch extraction to determine starch granule size, chain length, and molar mass. The extraction process followed the procedure described by Deng et al. (2021). The starch granule diameter was measured using a Mastersizer 3000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Malvern, UK). Distributions of short and long branch-chains of amylopectin and amylose were analyzed using a gel chromatography-differential system equipped with a U3000 liquid system (Thermo Fisher Scientific Inc., Waltham, USA) and an Optilab T-rEX differential detector (Wyatt Technology Inc., Santa Barbara, USA). The distribution of amylopectin chain length was determined using an ICS-5000+ high-performance anion-exchange chromatograph (Thermo Fisher Scientific Inc., Waltham, USA) with a Dionex™ arboPac™ PA200 anion-exchange column. The starch molar mass was measured using a gel chromatography-differential-multiangle laser light scattering system, featuring a U3000 liquid system, an Optilab T-rEX differential detector, and a DAWN HELEOS II laser light scattering detector (Wyatt Technology Inc., Santa Barbara, USA). All the measurements were conducted in accordance with the standard protocols established by the Sanshubio Co., Ltd., Shanghai, China (<http://www.sanshubio.com/home>).

### 2.3. Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and the means of varieties were compared using the least significant difference (LSD) test at  $p < 0.05$ . Both the ANOVA and LSD test were performed in Statistix 8.0 software (Analytical Software Inc., Tallahassee, USA).

## 3. Results

### 3.1. Varietal differences in starch digestion characteristics of cooked rice

The starch digestion characteristics of D335 differed significantly from those of X45 and Z39 (Fig. 1A–C). D335 had an ADD of 286 min, which was 190 % and 101 % longer compared to that of X45 and Z39, respectively (Fig. 1A). The GPR of D335 was  $1.06 \text{ mg g}^{-1} \text{ min}^{-1}$ , representing a 73 % reduction compared to X45 and a 57 % reduction compared to Z39 (Fig. 1B). D335 exhibited a TGP of  $303 \text{ mg g}^{-1}$ , which was 19 % less than X45 and 11 % less than Z39 (Fig. 1C).

### 3.2. Varietal differences in chemical properties of rice flour

The amylose content in D335 exceeded 30 %, which was 150 % higher than X45 and 20 % higher than Z39 (Table 1). In contrast, D335 had 31 % and 21 % lower amylopectin content compared to X45 and Z39, respectively. The total starch content in D335 was similar to that in X45 but 7 % lower than that in Z39. D335 had an amylose-to-amylopectin ratio that was 3.60 and 1.50 times that in X45 and Z39, respectively. The resistant starch content in D335 was 5.95 times that in X45 and 4.03 times that in Z39. D335 had 13 % lower protein content than X45, while the difference in protein content was not significant

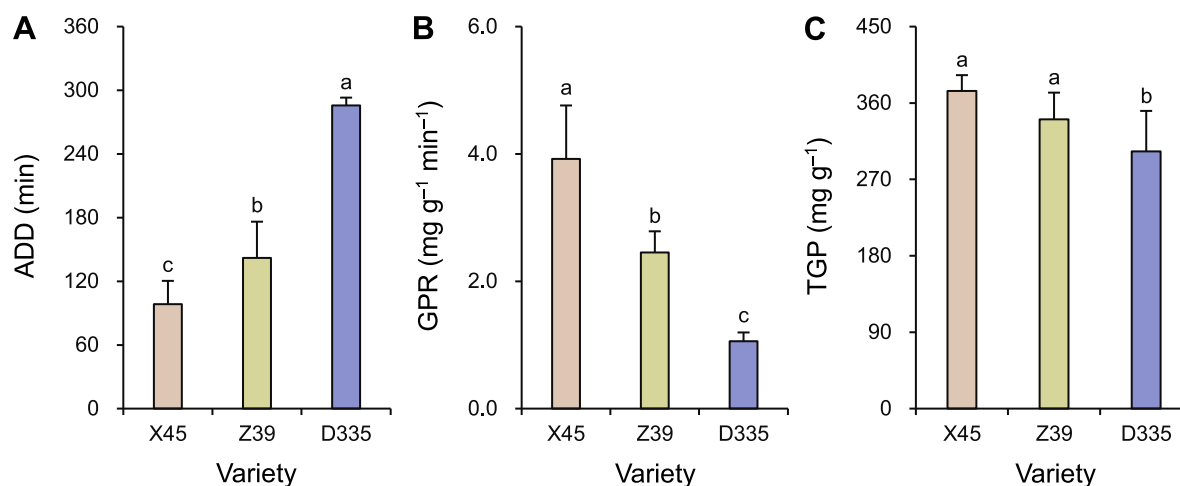
**Table 1**  
Chemical properties of milled rice flour of three varieties.

Chemical property	Variety <sup>a</sup>		
	X45	Z39	D335
Amylose content (%)	12.26 ± 1.36 c	25.41 ± 1.20 b	30.59 ± 1.11 a
Amylopectin content (%)	61.69 ± 1.68 a	53.35 ± 0.33 b	42.39 ± 1.60 c
Total starch content (%)	73.95 ± 0.35 b	78.76 ± 0.89 a	72.98 ± 0.55 b
Amylose-to-amylopectin ratio	0.20 ± 0.03 c	0.48 ± 0.03 b	0.72 ± 0.05 a
Resistant starch content (%)	0.44 ± 0.07 c	0.65 ± 0.04 b	2.62 ± 0.10 a
Protein content (%)	7.31 ± 0.26 a	5.97 ± 0.40 b	6.33 ± 0.62 b

Data are mean ± SD of three replicates.

Within a row, data sharing the same letters are not significantly different at  $p < 0.05$ .

<sup>a</sup> X45, Z39, and D335 are rice varieties Xiangzaoxian 45, Zhongzao 39, and Ditangliangyou 335, respectively.



**Fig. 1.** Starch digestion properties, including (A) active digestion duration (ADD), (B) glucose production rate (GPR), and (C) total glucose production (TGP), in three rice varieties. X45, Z39, and D335 are rice varieties Xiangzaoxian 45, Zhongzao 39, Ditangliangyou 335, respectively. Data are presented as mean and SD of three replicates. Data sharing the same letters are not significantly different at  $p < 0.05$ .

between D335 and Z39.

### 3.3. Varietal differences in starch granule size, chain length, and molar mass

All the distributions of quantity, surface area, and volume for starch granule diameters indicated that D335 had a higher proportion of starch granules larger than 9  $\mu\text{m}$  in diameter compared to X45 and Z39 (Fig. 2A–C). In particular, the total surface and volume distributions of starch granules larger than 9  $\mu\text{m}$  in D335 (11–22 %) were 83–100 % higher than those in X45 (6–11 %) and 57–69 % higher than those in Z39 (7–13 %) (Fig. 2B and C).

The content of short branch-chains of amylopectin (*i.e.*, the percentage of peak 1's area relative to the total area of three peaks) in D335 (49 %) was 29 % lower compared to X45 (69 %) and 16 % lower compared to Z39 (58 %) (Fig. 3A). In contrast, the content of long-branch chains of amylopectin (*i.e.*, the percentage of peak 2's area relative to the total area of three peaks) in D335 (26 %) was 44 % higher

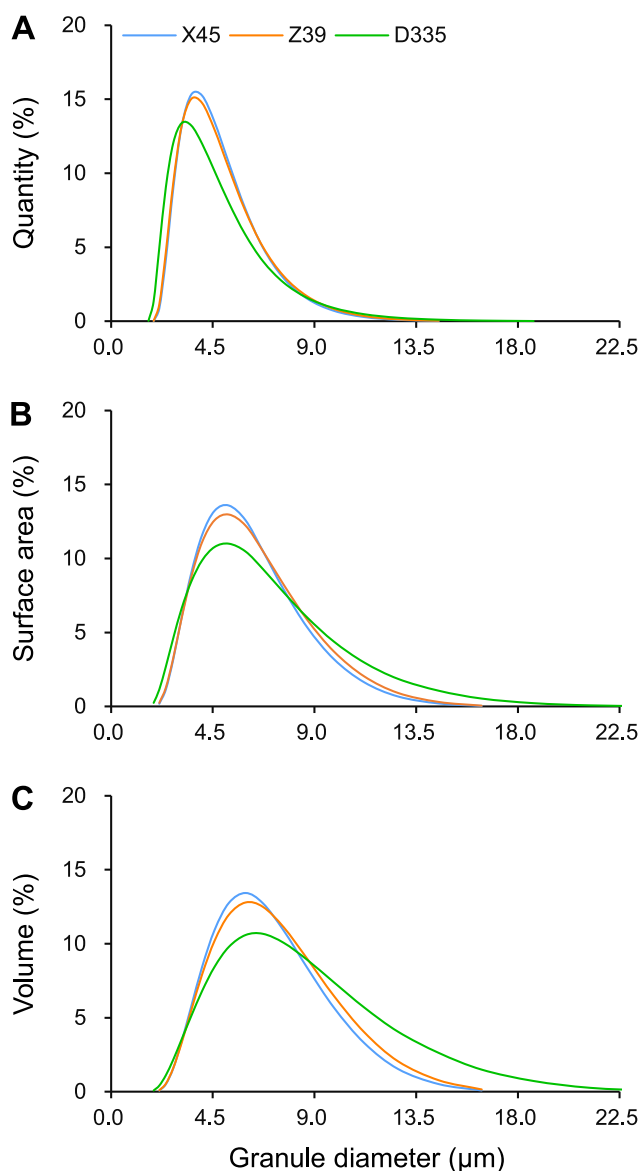


Fig. 2. (A) Quantity, (B) surface area, and (C) volume distributions of starch granule diameters of three rice varieties. X45, Z39, and D335 are rice varieties Xiangzaoxian 45, Zhongzao 39, Ditangliangyou 335, respectively.

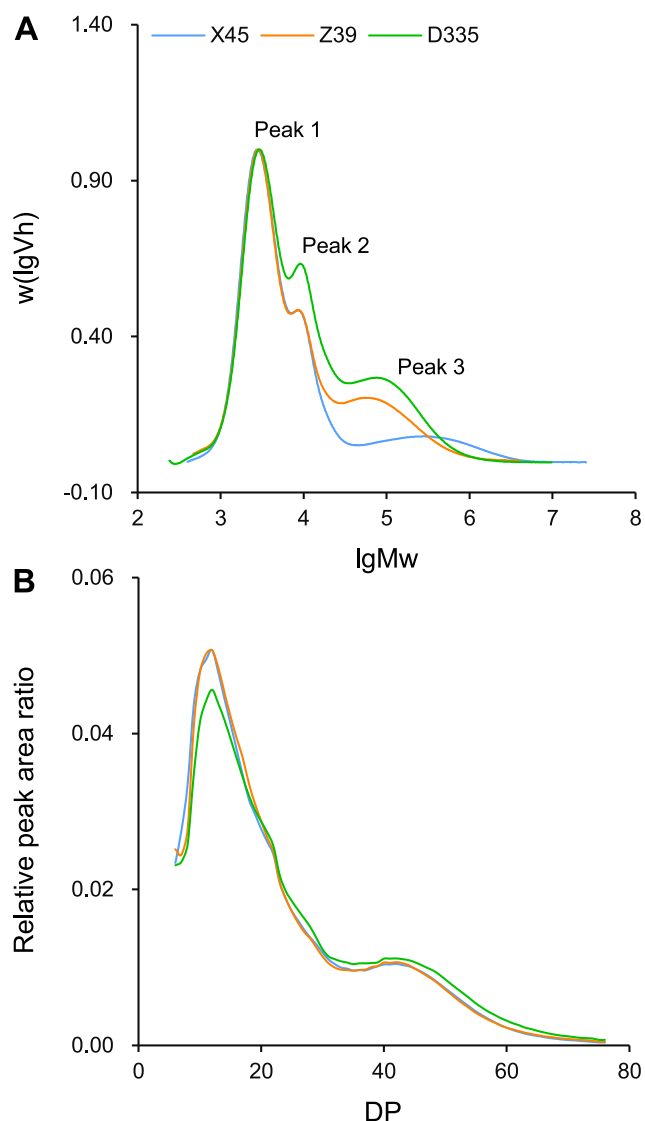
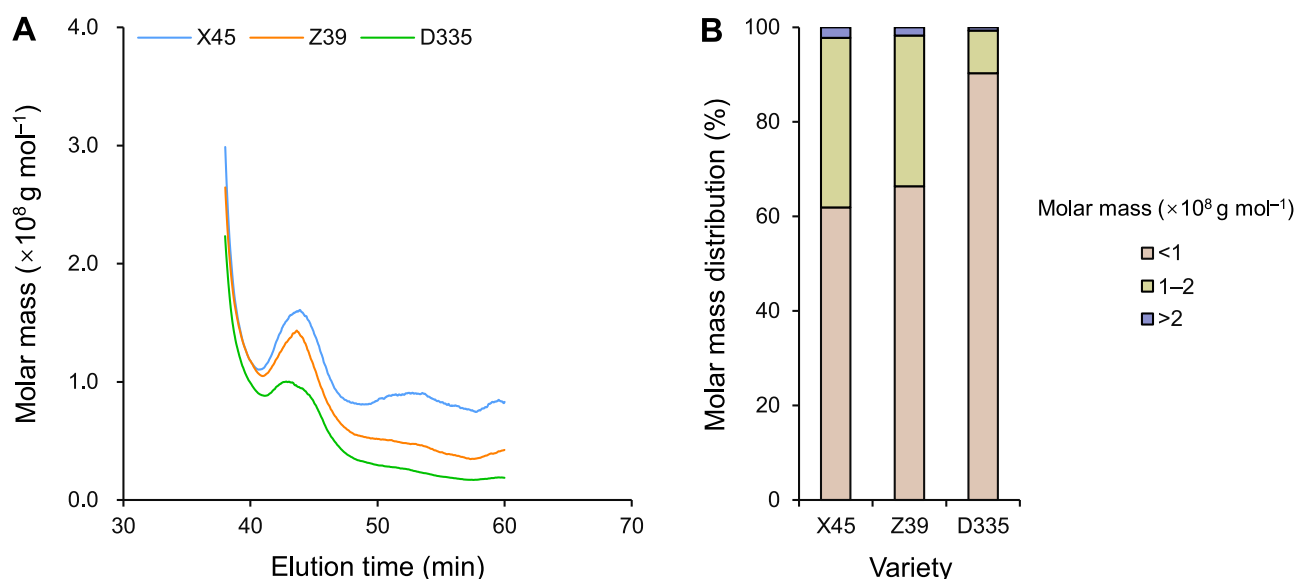


Fig. 3. (A) Distributions of short branch-chains of amylopectin (Peak 1), long-branch chains of amylopectin (Peak 2), and amylose (Peak 3) and (B) the chain length (the degree of polymerization, DP) distribution of amylopectin in starches of three rice varieties. X45, Z39, and D335 are rice varieties Xiangzaoxian 45, Zhongzao 39, Ditangliangyou 335, respectively.

than in X45 (18 %) and 30 % higher than in Z39 (20 %). The average DP of amylopectin chains in D335 was 24.5, which was about 7 % greater than in X45 (22.9) and Z39 (23.0) (Fig. 3B). Additionally, the content of short amylopectin chains (*i.e.*, the sum of relative peak area ratios) with a DP of 6–17 in D335 (0.434) was 10 % lower compared to X45 (0.483) and 9 % lower compared to Z39 (0.479).

The molar mass of starch molecules in D335 ranged from  $0.19 \times 10^8$  to  $2.23 \times 10^8 \text{ g mol}^{-1}$ , which was obviously lower than that in X45 (from  $0.83 \times 10^8$  to  $2.98 \times 10^8 \text{ g mol}^{-1}$ ) and Z39 (from  $0.43 \times 10^8$  to  $2.65 \times 10^8 \text{ g mol}^{-1}$ ) (Fig. 4A). The content of starch molecules with a molar mass below  $1 \times 10^8 \text{ g mol}^{-1}$  in D335 reached 90 %, which was 45 % higher than in X45 (62 %) and 36 % higher than in Z39 (66 %) (Fig. 4B). Conversely, the content of starch molecules with a molar mass between  $1 \times 10^8$  to  $2 \times 10^8 \text{ g mol}^{-1}$ , as well as those with a molar mass exceeding  $2 \times 10^8 \text{ g mol}^{-1}$ , was 72–75 % and 61–69 % lower in D335 compared to X45 and Z39, respectively.



**Fig. 4.** (A) Changes in molar mass with elution time and (B) the molar mass distribution of starch molecules of three rice varieties. X45, Z39, and D335 are rice varieties Xiangzaoxian 45, Zhongzao 39, Ditangliangyou 335, respectively.

### 3.4. Varietal differences in pasting properties of rice flour

D335 exhibited a significantly higher pasting temperature but lower viscosities, except for the setback viscosity, when compared to X45 and Z39 (Table 2). Specifically, D335 had a higher pasting temperature than X45 and Z39 by 5 % and 7 %, respectively. The peak viscosity in D335 was lower by 54 % compared to X45 and by 59 % compared to Z39. D335 had 34 % and 50 % lower trough viscosity than X45 and Z39, respectively. The breakdown viscosity in D335 was lower by 78 % relative to X45 and by 75 % relative to Z39. D335 displayed 44 % and 62 % lower final viscosity than X45 and Z39, respectively. The setback viscosity in D335 was 106 % higher compared to X45 but 91 % lower compared to Z39. D335 had 62 % and 79 % lower consistency viscosity than X45 and Z39, respectively.

## 4. Discussion

The newly developed low-GI rice variety D335 did not only has a slower starch digestion rate but also produced less glucose from starch digestion compared to X45 and Z39, which represent widely grown rice varieties with rapid and slow starch digestion rates, respectively (Hu et al., 2022). Although this outcome may seem predictable, the difference in starch digestion characteristics between D335 than Z39 suggests that

**Table 2**  
Pasting properties of milled rice flour of three varieties.

Pasting property	Variety <sup>a</sup>		
	X45	Z39	D335
Pasting temperature (°C)	84 ± 1 b	82 ± 0 c	88 ± 1 a
Peak viscosity (cP)	3775 ± 155 b	4211 ± 179 a	1733 ± 111 c
Trough viscosity (cP)	2085 ± 97 b	2755 ± 122 a	1368 ± 86 c
Breakdown viscosity (cP)	1690 ± 249 a	1456 ± 64 a	365 ± 28 b
Final viscosity (cP)	3140 ± 116 b	4648 ± 94 a	1774 ± 113 c
Setback viscosity (cP)	-635 ± 269 c	437 ± 87 a	41 ± 74 b
Consistency viscosity (cP)	1055 ± 20 b	1893 ± 48 a	406 ± 60 c

Data are mean ± SD of three replicates.

Within a row, data sharing the same letters are not significantly different at  $p < 0.05$ .

<sup>a</sup> X45, Z39, and D335 are rice varieties Xiangzaoxian 45, Zhongzao 39, and Ditangliangyou 335, respectively.

screening for low-GI rice varieties from existing widely grown varieties may not be straightforward and specialized breeding programs are necessary to develop low-GI rice varieties. The finding of this study also implies that the starch digestion parameters measured through the *in vitro* method employed in this study could serve as indicators for estimating GI values in rice. This indirectly addresses the concern regarding discrepancies between *in vitro* and *in vivo* results, which arise from the difficulty of accurately replicating the complex physicochemical and physiological processes of the human digestive system (Huang et al., 2022; Hur et al., 2011). It further underscores the need for additional research to establish quantitative relationships between GI value and starch digestion parameters determined *via* the *in vitro* method, using a range of rice varieties with varying GI values.

The slower starch digestion rate and less amount of glucose produced from starch digestion in D335 than in X45 and Z39 were attributable to several differences in starch physiological properties. First, D335 had a higher amylose content and a greater amylose-to-amylopectin ratio than X45 and Z39. This could contribute to a more compact starch structure and promote the formation of resistant starch in D335, making its starch more resistant to digestion (Fernandes et al., 2020; Trinh, 2015; Zaman & Sarbini, 2016). This was corroborated by the observation that D335 contained a higher resistant starch content compared to X45 and Z39. Second, D335 had a higher proportion of large starch granules, which could result in a smaller surface area available for starch digestive enzymes relative to X45 and Z39 (Dhital et al., 2015; Kong et al., 2003). Third, D335 had more long amylopectin chains, fewer short amylopectin chains, and a higher content of starch molecules with a low molar mass compared to X45 and Z39, leading to the formation of more stable helices and highly ordered multi-scale structures that are more resistant to digestion (Chi et al., 2024; Lehmann & Robin, 2007; Tu et al., 2021).

Although protein can slow starch digestion by modifying the microstructure, crystal structure, and thermal stability of starch, as well as by forming a protective barrier around starch granules to restrict enzyme access (Khatun et al., 2019; Lu et al., 2022), protein content did not account for the slower starch digestion rate and reduced glucose production observed in D335 compared to X45 and Z39 in this study. This is because D335 exhibited a significantly lower or comparable protein content than X45 and Z39. However, it remains uncertain whether protein was entirely unrelated to the varietal differences in starch digestion properties in this study, as starch digestion in rice may



be influenced more by protein composition than by protein content. Khatun et al. (2020) found no negative correlation between protein content and starch digestion in rice, but noted that incomplete extraction of glutelin and prolamin, possibly due to starch-protein interactions, showed a negative correlation with starch digestion. Therefore, further investigations are required to explore the role of protein composition in starch digestion in low-GI rice varieties.

The pasting properties of D335 differed significantly from those of X45 and Z39. On one hand, D335 exhibited a higher pasting temperature than both X45 and Z39. This aligns with the finding of Chung et al. (2011), who observed a positive correlation between pasting temperature and the content of resistant and slowly digestible starch in rice. On the other hand, D335 demonstrated significantly lower viscosities overall—except for setback viscosity—compared to X45 and Z39. However, previous studies indicate that not all viscosity measurements are linked to starch digestion properties in rice. For example, Benmoussa et al. (2007) found a significant negative correlation between slowly digestible starch content and breakdown viscosity, but no such correlation with other viscosities. Chung et al. (2011) reported that slowly digestible and resistant starch content were positively correlated with setback and final viscosities, while negatively correlated with peak and breakdown viscosities. Ouyang et al. (2024) observed a negative correlation between resistant starch content and both peak and breakdown viscosities but did not observe correlations with other viscosities. Notably, both the present study and these previous studies consistently showed that higher content of resistant or slowly digestible starch was associated with lower breakdown viscosity. This suggests that breakdown viscosity is a dependable measurement for assessing starch digestion in rice.

## 5. Conclusions

This study clarifies the starch digestion characteristics and identifies the key physicochemical properties linked to these characteristics in the newly developed low-GI rice variety D335, in comparison to the widely grown rice varieties X45 and Z39. D335 exhibits a slower starch digestion rate and produces less glucose from starch digestion than X45 and Z39. These differences arise from the distinct starch physicochemical properties in D335, including its higher amylose content, larger amylose-to-amylopectin ratio, higher resistant starch content, more large starch granules, more long amylopectin chains, fewer short amylopectin chains, and more low-molar-mass starch molecules. Additionally, the differences in starch digestion characteristics are also associated with the different pasting properties of rice flour, with D335 having a higher pasting temperature and lower breakdown viscosity than X45 and Z39.

## CRedit authorship contribution statement

**Chengjing Liao:** Writing – original draft, Investigation, Formal analysis. **Fangbo Cao:** Investigation. **Min Huang:** Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Jiana Chen:** Supervision, Investigation. **Yuanzhu Yang:** Resources. **Chenjian Fu:** Resources. **Xinhui Zhao:** Resources. **Wei qin Wang:** Investigation. **Huabin Zheng:** Investigation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

## References

- Bonmoussa, M., Moldenhauer, K. A. K., & Hamaker, B. R. (2007). Rice amylopectin fine structure variability affects starch digestion properties. *Journal of Agricultural and Food Chemistry*, *55*, 1475–1479.
- Chi, C., Ren, W., Yang, Y., Guo, X., Zhang, Y., Chen, B., He, Y., Chen, H., Zheng, X., & Wang, H. (2024). Starch ordered structures control starch reassembly behaviors during heat-moisture treatment for modulating its digestibility. *Food Chemistry*, *430*, Article 136966.
- Chung, H., Liu, Q., Lee, L., & Wei, D. (2011). Relationship between the structure, physicochemical properties and *in vitro* digestibility of rice starches with different amylose contents. *Food Hydrocolloids*, *25*, 968–975.
- Deng, F., Li, Q., Chen, H., Zeng, Y., Li, B., Zhong, X., Wang, L., & Ren, W. (2021). Relationship between chalkiness and the structural and thermal properties of rice starch after shading during grain-filling stage. *Carbohydrate Polymers*, *252*, Article 117212.
- Dhital, S., Butardo, V. M., Jobling, S. A., & Gidley, M. J. (2015). Rice starch granule amylolysis — Differentiating effects of particle size, morphology, thermal properties and crystalline polymorph. *Carbohydrate Polymers*, *115*, 305–316.
- Fernandes, J. M., Madalena, D. A., Pinheiro, A. C., & Vicente, A. A. (2020). Rice *in vitro* digestion: Application of INFOGEST harmonized protocol for glycemic index determination and starch morphological study. *Journal of Food Science and Technology*, *57*, 1393–1404.
- Hu, L., Cao, J., Liu, Y., Xiao, Z., Zhang, M., Chen, J., ... Huang, M. (2022). Multidimensional relationships of starch digestibility with physicochemical, pasting and textural properties of 30 rice varieties. *Agronomy*, *12*, 720.
- Huang, M. (2023). The association between the risk of diabetes and white rice consumption in China: Existing knowledge and new research directions from the crop perspective. *Journal of Integrative Agriculture*, *22*, 3234–3236.
- Huang, M., & Hu, L. (2021). Low glycemic index: The next target for rice production in China? *Journal of Integrative Agriculture*, *20*, 1727–1729.
- Huang, M., Hu, L., Chen, J., & Cao, F. (2022). *In vitro* testing indicates an accelerated rate of digestion of starch into glucose of cooked rice with the development of low amylose rice in China. *Food Chemistry: X*, *14*, Article 100278.
- Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). *In vitro* human digestion models for food applications. *Food Chemistry*, *125*, 1–12.
- IDF (International Diabetes Federation). (2021). IDF diabetes atlas. Retrieved from <https://diabetesatlas.org/atlas/tenth-edition> Accessed August 2, 2024.
- IRRI (International Rice Research Institute) (2023). IRRI reveals scientific breakthrough for low and ultra-low glycemic index rice. Retrieved from <https://www.irri.org/news-and-events/news/irri-reveals-scientific-breakthrough-low-and-ultra-low-glycemic-index-rice>. Accessed August 2, 2024.
- Juliano, B. O. (1971). A simplified assay for milled-rice amylose. *Cereal Science Today*, *12*, 334–360.
- Juliano, B. O., Bressani, R., & Elias, L. G. (1971). Evaluation of the protein quality and milled Rices differing in protein content. *Journal of Agricultural and Food Chemistry*, *19*, 1028–1034.
- Khatun, A., Waters, D. L. E., & Liu, L. (2019). A review of rice starch digestibility: Effect of composition and heat-moisture processing. *Starch*, *71*, 190090.
- Khatun, A., Waters, D. L. E., & Liu, L. (2020). The impact of rice protein on *in vitro* rice starch digestibility. *Food Hydrocolloids*, *109*, Article 106072.
- Kong, B., Kim, J., Kim, M., & Kim, J. C. (2003). Porcine pancreatic  $\alpha$ -amylase hydrolysis of native starch granules as a function of granule surface area. *Biotechnology Progress*, *19*, 1162–1166.
- Lehmann, U., & Robin, F. (2007). Slowly digestible starch—its structure and health implications: A review. *Trends Food Science and Technology*, *18*, 346–355.
- Li, C. (2023). Starch fine molecular structures: The basis for designer rice with slower digestibility and desirable texture properties. *Carbohydrate Polymers*, *299*, Article 120217.
- Lu, X., Ma, R., Zhan, J., Wang, F., & Tian, Y. (2022). The role of protein and its hydrolysates in regulating the digestive properties of starch: A review. *Trends in Food Science & Technology*, *125*, 54–65.
- Maji, B. (2019). Introduction to natural polysaccharides. In S. Maiti, & S. Jana (Eds.), *Functional polysaccharides for biomedical applications* (pp. 1–31). Woodhead Publishing.
- Megazyme. (2019). Resistant starch assay protocol. Retrieved from [https://d1kkinny8vk5e2.cloudfront.net/documents/Assay\\_Protocol/K-RSTAR\\_DATA.pdf](https://d1kkinny8vk5e2.cloudfront.net/documents/Assay_Protocol/K-RSTAR_DATA.pdf) Accessed June 15, 2024.
- Ngo, T. V., Kunyane, K., & Luangsakul, N. (2023). Insights into recent updates on factors and technologies that modulate the glycemic index of rice and its products. *Foods*, *12*, 3659.
- OECD-FAO (Organisation for Economic Co-operation and Development-Food and Agriculture Organization of the United Nations) (2022). OECD-FAO agricultural outlook 2022–2031. Retrieved from <https://doi.org/10.1787/f1b0b29c-en>. Accessed August 2, 2024.
- Ouyang, J., Wang, C., Huang, Q., Guan, Y., Zhu, Z., He, Y., Jiang, G., Xiong, Y., & Li, X. (2024). Correlation between *in vitro* starch digestibility and starch structure/physicochemical properties in rice. *International Journal of Biological Macromolecules*, *263*, Article 130316.

- Ramadoss, B. R., Gangola, M. P., Agasimani, S., Jaiswal, S., Venkatesan, T., Sundaram, G. R., & Chibbar, R. N. (2019). Starch granule size and amylopectin chain length influence starch in vitro enzymatic digestibility in selected rice mutants with similar amylose concentration. *Journal of Food Science and Technology*, *56*, 391–400.
- Shu, X., Jia, L., Ye, H., Li, C., & Wu, D. (2009). Slow digestion properties of rice different in resistant starch. *Journal of Agricultural and Food Chemistry*, *57*, 7552–7559.
- Trinh, K. S. (2015). Recrystallization of starches by hydrothermal treatment: Digestibility, structural, and physicochemical properties. *Journal of Food Science and Technology*, *52*, 7640–7654.
- Tu, Y., Huang, S., Chi, C., Lu, P., Chen, L., Li, L., & Li, X. (2021). Digestibility and structure changes of rice starch following co-fermentation of yeast and *Lactobacillus* strains. *International Journal of Biological Macromolecules*, *184*, 530–537.
- Yang, R., Bai, J., Wan, C., Tang, J., Gong, C., & Piao, Z. (2022). Breeding of new rice variety 'Youtangdao 3' with high resistant starch. *Acta Agriculturae Shanghai*, *38*, 6–9 (in Chinese with English abstract).
- Yang, R., Piao, Z., Wang, C., Lee, G. S., Gong, C., & Bai, J. (2020). Breeding and characteristics of new rice variety Youtangdao 2 with high resistant starch. *China Rice*, *26*, 94–95 (in Chinese with English abstract).
- Yang, Z., Zhang, Y., Wu, Y., & Ouyang, J. (2023). Factors influencing the starch digestibility of starchy foods: A review. *Food Chemistry*, *406*, Article 135009.
- Ye, J., Hu, X., Luo, S., McClements, D. J., Liang, L., & Liu, C. (2018). Effect of endogenous proteins and lipids on starch digestibility in rice flour. *Food Research International*, *106*, 404–409.
- Zaman, S. A., & Sarbini, S. R. (2016). The potential of resistant starch as a prebiotic. *Critical Reviews in Biotechnology*, *36*, 578–584.