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Influence of physicochemical properties and starch fine structure on the eating quality of hybrid rice with similar apparent amylose content

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ABSTRACT

In this study, we compared the physicochemical properties and starch structures of hybrid rice varieties with similar apparent amylose content but different taste values. In addition to the apparent amylose content, gel permeation chromatography analysis showed that the higher proportions of amylopectin short chains and relatively lower proportions of amylopectin long chains, which could lead to higher peak viscosity and breakdown value, as well as a softer and stickier texture of cooked rice, were the key factors in determining the eating quality of hybrid rice. High-performance anion-exchange chromatography analyses showed that the proportion of amylopectin short chains (degree of polymerization 6–10) and intermediate chains (degree of polymerization 13–24), which might affect the gelatinisation enthalpy and crystallinity, also contributed greatly to the eating quality of hybrid rice. Moreover, this study indicated that a greater diversity of forms and sizes of starch granules might influence the eating quality of hybrid rice.

1. Introduction

During the quality breeding of hybrid rice, the apparent amylose content (AAC) has been taken as a key factor affecting the eating and cooking qualities of hybrid rice grains (Tan et al., 1999). In general, a higher AAC is usually associated with a harder texture of cooked rice, and a slightly lower AAC is associated with a softer texture (Zhou et al., 2015). However, during our quality breeding process of hybrid rice, we found that hybrid rice varieties with similar AAC sometimes displayed differences in their eating quality. The edible part (endosperm) of a hybrid rice differs substantially from that of conventional rice because hybrid rice is an F_2 segregated population derived from hybrid F_1, and its eating quality is affected by both parents (Qian et al., 2016). Theoretically, hybrid rice with a lower AAC could also be bred from parents with different AAC. Thus, compared with that of hybrid rice obtained from parents with similar AAC, there is a greater separation in the AAC of the hybrid F_2 population and the eating quality might be poorer. This may serve as a possible reason for the quality differences among hybrid rice varieties with similar AAC. However, for some hybrid rice varieties, even though the AAC of their parents are comparable, obvious quality differences still exist in hybrid rice varieties with consistent and lower AAC. Thus, the factors contributing to the eating quality of hybrid rice must be investigated by excluding the major effect caused by the AAC.

The eating quality of cooked rice is a very complex trait, attributed to numerous factors (Shinada et al., 2015). As the main component of rice grains, starch is a branched glucose polymer that usually comprises two types of molecules: amylopectin and amylose (Syahariza et al., 2013). AAC has always been used as the key factor affecting the eating quality

Abbreviations: AAC, apparent amylose content; AP1, short-branch amylopectin chains; AP2, long-branch amylopectin chains; AM, amylose; DSC, differential scanning calorimeter; DP, degree of polymerization; GC, gel consistency; DMSO, dimethyl sulfoxide; HPAEC, high-performance anion-exchange chromatography; GPC, gel permeation chromatography; PT, pasting temperature; PC, protein content; ΔH, enthalpy of gelatinization; RVA, Rapid Visco Analyzer; SEM, scanning electron microscopy; XRD, X-ray powder diffraction.

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of rice grains (Takeda et al., 1990). Other physicochemical properties, such as the gelatinisation temperature (GT), gel consistency (GC), and protein content (PC), also play important roles in the evaluation of rice eating quality (Tian et al., 2009). The starch pasting viscosity, which can be measured with a Rapid Visco Analyser (RVA), serves as another important index for estimating the eating quality of rice (Hu et al., 2004). Many studies have shown that the RVA profile characteristics, especially the breakdown value and setback value, were significantly correlated with the eating quality of cooked rice (He et al., 2015). The rice textural properties of cooked rice are of prime importance to its eating quality. Except for amylose content, the amylopectin to amylose ratio and amylopectin structure significantly influence the texture of cooked rice (Li et al., 2016). The fine structure of starch is one of the main reasons for quality differences among rice varieties with similar AAC (He et al., 2010). Han and Hamaker (2001) and Ong and Blanshard (1995) indicated that the proportion of amylopectin long chains contributed greatly to the quality of conventional rice varieties with similar AAC. Recent studies have shown that regardless of amylose content, amylose molecular sizes and long amylose chains cause the differences in rice quality after cooking (Li et al., 2016).

The eating quality of rice refers to the comprehensive evaluation of sensory indexes such as smell, appearance (glossiness and shape), palatability, and taste of cooked rice (Zhang et al., 2009). The most direct method for determining the eating quality is based on sensory evaluation by trained panellists (Tomlins et al., 2010). However, as the number of samples increases, sensory evaluation is easily affected by subjective factors, and the sensitivity of a panellist's palate decreases. Thus, considerable effort has been expended to develop quicker and more convenient instrumental measurements to provide objective data related to the quality of cooked rice. A rice taste analyser based on near-infrared spectroscopy is a non-destructive method that has been applied to evaluate the taste value of milled or cooked rice (Bao et al., 2010). Previous studies have indicated that there are significant correlations between the sensory evaluation and taste value measured by the rice taste analyser (Lu et al., 2007). Moreover, the indica rice standard has been added to the rice taste analyser (Lv et al., 2006). However, until now, the standard curve of hybrid rice has not been developed for the currently used taste analyser. Therefore, to meet the evaluation requirements of varieties in different countries and regions, a taste analyser based on hybrid rice should be developed for wider applications.

During our quality breeding process of hybrid rice, we found that hybrid rice varieties with similar AAC sometimes displayed differences in their eating quality, even though their parental lines showed similar and lower AAC. Hence, in the present study, two groups of materials were selected for further research, in which each group included an indica temperature-sensitive genetic male sterile (TGMS) line, two indica restorer lines, and two indica hybrid combinations with similar AAC but vastly different taste values. By analysing the physicochemical properties and starch molecular structure related to the eating quality of the hybrid combinations, we determined the factors contributing to the eating quality of hybrid rice by excluding the major effect of AAC, which provided new information for the breeding of hybrid rice varieties with high eating quality.

2. Materials and methods

2.1. Materials

The rice accessions used in the present study were divided into two groups. Group A included an indica TGMS line S1, two indica restorer lines with similar AAC (R1 and R2), and two indica hybrid combinations with similar AAC but vastly different taste values ($F_{2\text{-}1}$ and $F_{2\text{-}2}$). Group B included an indica TGMS line S2, two indica restorer lines with similar AAC (R3 and R4), and two other indica hybrid combinations with similar AAC but vastly different taste values ($F_{2\text{-}3}$ and $F_{2\text{-}4}$). All materials were collected from the College of Agronomy, Hunan Agricultural University (China) and planted in the same field on an experimental farm in Chunhua town, Changsha, China (28°2592″ N, 113°2311″ E). The planting season was from June to October 2019. TGMS lines were treated with cold irrigation during the fertility thermosensitive period. Each hybrid rice line was planted in six rows per plot and six plants per row. The seeds were harvested at maturity from 20 plants at the centre of each plot, thoroughly mixed, and allowed to air-dry at room temperature (15–25 °C) for 2 months.

2.2. Flour and starch preparation

All mature seeds were de-husked using a rice huller (SY88-TH, Korea) and milled with a grain polisher (Kett, Tokyo, Japan). A portion of the polished rice samples was ground into flour in a mill (FOSS 1093 Cyclotec Sample Mill; Foss A/S, Hillerød, Denmark) and passed through a 100-mesh sieve. The remaining polished rice was stored in sealed bags at 4 °C for use in subsequent taste evaluations. Starch samples were extracted using a neutral protease method described previously (Zhang et al., 2013).

2.3. Sensory evaluation

Sensory evaluation was conducted according to GB/T 15682–2008 published by the Ministry of Agriculture of China. The sensory evaluation team comprised eight people of different sexes and ages who were professionally trained to identify taste. Four samples were evaluated at a time, including one indica reference sample (Huang Huazhan, with moderate eating quality) and three other samples. The tasting evaluation factors were fragrance, appearance, palatability (viscosity, hardness, and elasticity), taste, and cold rice texture. The comprehensive scores were as follows: ≤ 50, very poor; 51–60, poor; 61–70, average; 71–80, relatively good; 81–90, good; and > 90, very good.

2.4. Physicochemical analyses

The grain shape and chalkiness were measured with a ScanMaker grain appearance analyser (WSeen SC-E; Hangzhou WSeen Detection Technology Co. Ltd., Hangzhou, China). The AAC of the flour was determined following a modified method based on an iodine colorimetry method described by Man et al., (2012). The GC was measured according to a method described by Tan et al. (1999). The PC was measured using near-infrared spectroscopy. The viscosity was determined using an RVA (Newport Scientific PTY Ltd., Warriewood, Australia) (Zhu et al., 2010). The cooked rice texture was evaluated using a texture analyser (SATAKE RHS1A; Satake Corp., Hiroshima, Japan). The thermal properties were measured with differential scanning calorimetry (DSC200F3; Netzsch Instruments NA LLC, Burlington, MA, USA), as described previously by Zhang et al. (2017). All tests were performed in triplicate.

2.5. Scanning electron microscopy (SEM)

Starch samples were suspended in anhydrous ethanol and then applied to an aluminium stub using double-sided adhesive tape. After being coated vertically with gold using a sputter coater, the samples were observed and photographed at a magnification of 2000 × using an SEM (Zeiss Merlin Compact).

2.6. Starch size distribution

First, 100–200 mg of starch was placed in a clean eppendorf tube, and then 75% alcohol was added to the eppendorf tube and mixed well. The samples were measured with dynamic light scattering (Mastersizer 3000). All tests were performed in triplicate.

2.7. Gel permeation chromatography (GPC)

Purified rice starch was debranched with isoamylase (EC3.2.1.68, E-ISAMY; Megazyme, Bray, Ireland) and dissolved in dimethyl sulfoxide. The relative molecular weight distribution of the debranched starch was determined using GPC in a PLGPC 220 system (Polymer Laboratories Varian, Inc., Amherst, MA, USA). Based on dextrans of known molecular weights (2800, 18,500, 111,900, 192,410,000, 1,050,000, 2,900,000, and 6,300,000), the GPC data were transformed through integral equations. With the use of dextran standards, the GPC data are reported as dextran-equivalent molecular weight, denoted MW (Zhang et al., 2017). All tests were performed in triplicate.

2.8. High-performance anion-exchange chromatography (HPAEC)

Purified rice starch was debranched with isoamylase (EC3.2.1.68, E-ISAMY; Megazyme, Bray, Ireland) and dissolved in double-distilled water. The chain length distribution of the debranched starches was then quantitatively analyzed using HPAEC (Thermo ICS-5000; Thermo Corp., Sunnyvale, CA, USA) equipped with a pulsed amperometric detector, a guard column, a CarboPacTM PA200 analytical column, and an AS-DV autosampler according to our recent paper, as described previously (Zhang et al., 2017). All tests were performed in triplicate.

2.9. Powder X-ray diffraction (XRD)

The starch powders of all samples were treated with a saturated solution of NaCl for a week. The starches were investigated by XRD with a D8 ADVANCE X-ray diffractometer (D8, Bruker, Germany) and the test conditions were as follows: the voltage was 40 kV, current was 200 mA, two theta (2θ) angles ranged from 3° to 40°, and the scan duration was 10 min/sample. The relative crystallinity of the starches was calculated following the method described by Wei et al. (2010).

2.10. Statistical analysis

The experiments were carried out in triplicate, and the data were reported as mean values and standard deviations (±SD). The one-way ANOVA and Tukey'smultiple-comparison test were used to determine significant differences among the mean values by using SPSS 16.0 statistical software program.

3. Results and discussion

3.1. Taste value, AAC, and other components

There was no apparent difference in the AAC and taste values among all the parental lines in each group (Table 1). However, the hybrid rice in each group showed similar AAC but vastly different taste values. Our results showed that the PC varied slightly among the hybrid rice with lower and higher taste values in each group. However, hybrid rice with higher taste values exhibited a relatively higher GC than that did hybrid rice with lower taste values. Further, comparing the physicochemical characteristics of the corresponding restorer lines in each group, the GC of R1 was higher than that of the R2 in group A, which was similar to that of R3 and R4 in group B.

3.2. Textural properties

Textural properties, with hardness and stickiness as the most commonly determined parameters for cooked rice, contribute greatly to the eating quality of rice (Li et al., 2016). Previous studies have indicated that hardness is positively correlated with amylose content, whereas stickiness is negatively correlated with amylose content (Cameron and Wang, 2005; Patindol et al., 2010). In the present study, all hybrid rice varieties showed comparable AAC, and all varieties were cooked in the same rice/water ratio, considering that the amount of water added to the grain is one of the major factors influencing the cooked rice texture (Champagne et al., 1999). However, the hardness and stickiness differed significantly, and hybrid rice with higher taste values exhibited a relatively higher stickiness but lower hardness than that did hybrid rice with lower taste values in each group. The difference in stickiness was attributed to the proportion of short amylopectin chains (Cameron and Wang, 2005; Ong and Blanshard, 1995). An increase in the proportion of short amylopectin chains creates a greater opportunity for bonding and molecular interaction; therefore, more force is required to make the grains come apart, eventually leading to higher stickiness (Li and Gilbert, 2018). Hybrid rice with higher long amylopectin chains has a harder texture after cooking. The long amylopectin chains can interact with other components in rice grains, such as proteins, lipids, and non-starch polysaccharides, resulting in a firmer texture and restricting starch swelling (Ong and Blanshard, 1995; Radhika Reddy et al., 1993).

3.3. Pasting properties

Fig. 1A–B shows the RVA profile of the hybrid combinations and their parental lines in each group. Previous studies have indicated that the breakdown value and setback value from RVA can be used to evaluate the difference in the palatability of cooked rice with similar AAC (Wang et al., 2010). Our RVA data revealed that all the hybrid rice showed similar final viscosity; however, the hybrid combinations with good taste values showed a higher peak viscosity and breakdown value, and a slightly lower setback value than that did the hybrid combinations with poor taste values, which is consistent with previous studies (Wang et al., 2010). The final viscosity may be due to the reassociation of amylose molecules (Ashogbon and And, 2012). In the present study, all hybrid rice showed similar AAC, which might be one of the reasons for the small difference in the final viscosity. Peak viscosity is the maximum viscosity attained by gelatinised starch during heating in water (Shimelis et al., 2006), and the breakdown value is caused by the disruption of the gelatinise starch granule structure (Adebowale et al., 2005; Ashogbon et al., 2012). The higher peak viscosity and breakdown value of hybrid rice with good taste values could be attributed to its higher

Table 1
Statistical values of the physicochemical properties of hybrid rice and parental lines.

	Samples	Taste value(points)	AC(%)	GC(mm)	PC(%)	Hardness	Stickiness
A	S1	83.33 ± 2.89^{b}	15.40 ± 0.36^{b}	$76.67 \pm 4.16^{c,d}$	$7.73 \pm 0.02^{a,b}$	2.35 ± 0.06^{b}	0.31 ± 0.03^{c}
	R1	84.67 ± 4.04^{b}	13.23 ± 0.32^{a}	79.33 ± 5.69^{d}	7.63 ± 0.12^{a}	2.02 ± 0.08^{a}	0.34 ± 0.02^{c}
	$F_{2\text{-}1}$	79.00 ± 3.61^{b}	15.30 ± 0.30^{b}	65.00 ± 3.61^{b}	7.67 ± 0.06^{a}	2.26 ± 0.15^{b}	0.21 ± 0.01^{b}
	R2	81.33 ± 4.16^{b}	15.07 ± 0.21^{b}	$69.33 \pm 2.65^{b,c}$	7.84 ± 0.10^{b}	1.86 ± 0.09^{a}	0.12 ± 0.01^{a}
	$F_{2\text{-}2}$	60.00 ± 5.00^{a}	15.98 ± 0.09^{c}	47.67 ± 4.73^{a}	7.81 ± 0.15^{b}	3.95 ± 0.14^{c}	0.15 ± 0.01^{a}
B	S2	81.67 ± 3.51^{b}	13.32 ± 0.30^{a}	$82.67 \pm 4.16^{b,c}$	7.66 ± 0.12^{a}	1.89 ± 0.06^{a}	0.40 ± 0.02^{d}
	R3	80.33 ± 3.79^{b}	14.17 ± 0.32^{b}	88.67 ± 4.16^{c}	7.94 ± 0.06^{a}	2.35 ± 0.10^{b}	0.35 ± 0.01^{c}
	$F_{2\text{-}3}$	80.67 ± 5.13^{b}	15.91 ± 0.20^{d}	101.27 ± 4.93^{d}	8.01 ± 0.06^{a}	2.84 ± 0.08^{c}	0.41 ± 0.02^{d}
	R4	86.00 ± 1.73^{b}	15.29 ± 0.34^{c}	76.33 ± 4.73^{b}	7.82 ± 0.12^{a}	1.99 ± 0.06^{a}	0.15 ± 0.01^{b}
	$F_{2\text{-}4}$	61.67 ± 2.89^{a}	16.01 ± 0.13^{d}	49.67 ± 3.21^{a}	7.86 ± 0.12^{a}	3.00 ± 0.07^{d}	0.13 ± 0.01^{a}

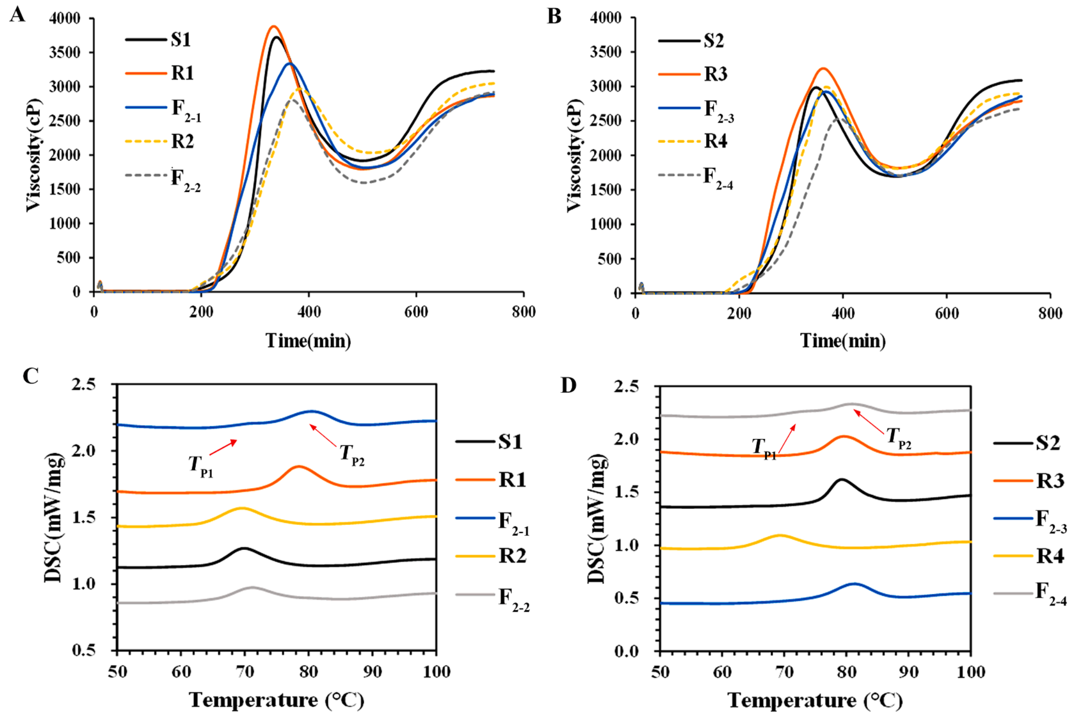

Fig. 1. Rapid viscosity profiles (A-B), and gelatinization properties (C-D) of hybrid combinations and parental lines. T_{p1} and T_{p2} represent peak temperature1 and peak temperature2, respectively.

proportion of short chains in starch, which allows easier swelling of starch granules due to weaker binding forces (Han and Hamaker, 2001; Srichuwong and Jane, 2007). The proportion of long chains in amylopectin mimics amylose to form helical complexes with lipids and intertwines with other branch chains to maintain the integrity of starch granules during heating and shearing, resulting in a lower peak viscosity and breakdown value (Farhan et al., 2019; Jane et al., 1999). The setback value is a measure of the recrystallisation of gelatinised starch during cooling (Chang et al., 2014). Together with the effects of the amylose content, the amylopectin molecular structure and degree of randomly limited branching in the amylose play a critical role in the setback of different starches (Jane et al., 1999). The variations in the pasting characteristics of hybrid rice with different taste values could also be attributed to the differences in protein, fat, and mineral content, because these components might interact with rice starch to varying degrees and influence its pasting properties (Farhan et al., 2019).

3.4. Thermal properties

GT is important for the selection of desirable rice cultivars with specific physiochemical properties of starch (Morales-Martínez et al., 2014). Therefore, in the present study, a DSC was used to determine the GT of all hybrid combinations and their parental lines in the two groups (Fig. 1C–D). A previous study indicated that a relatively lower GT might be a vital contributor to obtaining soft rice with a good eating and cooking quality (Chun et al., 2015). However, the findings of the present study showed that hybrid rice $F_{2\text{-}3}$ with a good taste value had a higher GT than that did hybrid rice $F_{2\text{-}2}$ with a poor taste value (Table 2). Even if the hybrid rice $F_{2\text{-}1}$ exhibited a distinct two-peak thermal curve due to the segregation of GT in the hybrid F_2 generation, it still showed a higher taste value than that did the hybrid combination with a low and consistent GT ($F_{2\text{-}2}$). To the best of our knowledge, by examining previous studies, no obvious relationship between the GT and taste value of hybrid rice has been shown; however, more research is required before firm conclusions can be drawn. In the present study, hybrids with good taste values exhibited higher gelatinisation enthalpy (ΔH_{gel}) values than

Table 2
Thermal properties of the different samples as determined by differential scanning calorimetry.

	Samples	T_o (°C)	T_p (°C)	T_{p1} (°C)	T_{p2} (°C)	T_c (°C)	ΔH (J/g)
A	S1	65.0 ± 0.40^b	69.9 ± 0.35^a			76.4 ± 0.25^{a,b}	5.97 ± 0.13^b
	R1	73.7 ± 0.45^c	78.4 ± 0.25^c			84.3 ± 0.35^c	7.09 ± 0.12^d
	$F_{2\text{-}1}$	75.2 ± 0.15^d		71.4 ± 0.3	80.4 ± 0.3	86.1 ± 0.26^d	6.06 ± 0.10^b
	R2	63.6 ± 0.45^a	69.9 ± 0.35^a			76.1 ± 0.25^a	6.32 ± 0.19^c
	$F_{2\text{-}2}$	65.5 ± 0.30^b	71.7 ± 0.25^b			76.9 ± 0.45^b	5.15 ± 0.11^a
B	S2	72.6 ± 0.30^b	76.3 ± 0.30^b			81.5 ± 0.25^b	7.92 ± 0.20^c
	R3	74.7 ± 0.06^c	79.6 ± 0.15^c			85.4 ± 0.25^c	7.66 ± 0.32^c
	$F_{2\text{-}3}$	75.1 ± 0.26^c	81.2 ± 0.40^d			86.4 ± 0.26^d	6.73 ± 0.23^b
	R4	63.5 ± 0.15^a	69.3 ± 0.25^a			76.1 ± 0.21^a	5.88 ± 0.22^a
	$F_{2\text{-}4}$	75.7 ± 0.15^d		74.0 ± 0.15	80.8 ± 0.35	86.8 ± 0.5^d	5.89 ± 0.29^a

that did the hybrids with poor taste values in the same group, even if they had a similar AAC. It has been suggested that ΔH_{gel} values primarily reflect the loss of double-helical order rather than the loss of crystalline register (Cooke and Gidley, 1992; Wani et al., 2012). However, Tester et al. (1990a) postulated that ΔH_{gel} reflects the overall crystallinity of amylopectin. ΔH_{gel} mainly corresponds to the distribution of short-chain amylopectin (degree of polymerisation [DP] 6 to 11) rather than the amylose content (Noda et al., 1996). In the present study, the lower ΔH_{gel} value of hybrid rice with the poor taste value could be attributed to the higher proportion of short chains (DP 6 to 10) in starch, which could decrease the efficiency of packing in starch crystallinity (Vandeputte et al., 2003).

3.5. Morphology and size distribution of starch

In the present study, an SEM was used to investigate the sub-microscopic shape and surface characteristics of starch granules in hybrid rice with different taste values. Fig. 2 (A–J) shows the SEM micrographs of all the samples. All parental lines showed typical polyhedral and irregular shapes together with a smooth surface, and there was no significant difference between the overall appearance and size of the starch granules. However, the starch granules of the hybrid rice showed significant variations in size and shape, as determined by the SEM analysis. Many small spherical starch granules were observed around the large starch granules, possibly because the starch granules observed were a mixed sample, and the development of different grains of starch granules was unequal due to the segregation of the character of the hybrid F_2. The greater diversity of the forms and sizes of the starch granules in the hybrid rice might lead to relatively large spaces between the granules.

We compared the differences between the starch granule number, volume, and surface area distributions of all the hybrid samples (Fig. 2A–C). The distribution of the starch granule number and surface area displayed a typical unimodal-peak curve, whereas the starch volume distribution showed a bimodal-peak curve for all hybrid rice samples. From the SEM images, a small number of starch granules with extreme sizes were found in the hybrid rice. Thus, we speculate that large starch granules would have a greater influence on the starch volume distribution than that would small starch granules, and the distribution of the starch volume would more likely shift towards large starch granules. In addition, the starch volume distribution showed that the bimodal-peak curves of hybrid rice $F_{2\text{-}2}$ and $F_{2\text{-}4}$ were more obvious than those of $F_{2\text{-}1}$ and $F_{2\text{-}3}$ (Fig. 2C), probably because of the small number of large starch granules with approximate maximum diameters existing in them ($F_{2\text{-}1}$), which might be one of the reasons for the different taste values of the hybrid rice with similar AAC.

3.6. Relative molecular weight distributions

The relative molecular weight distributions of the isoamylase-debranched starches, as determined by GPC, are shown in Fig. 3A–B. The hybrid combinations with higher taste values showed significantly higher proportions of amylopectin short chains (AP1) than that did hybrids with lower taste values in each group. This finding is consistent with results of previous studies on conventional rice (Ayabe et al., 2009; Tao et al., 2019). GPC parameters also showed that the AP1 to AP2 area ratios for hybrids with good taste values were higher than those of hybrid samples with poor taste values. The ratio of AP1 to AP2 has been used as an index of the extent of branching of amylopectin; the higher the ratio, the higher the degree of branching (Wang et al., 1993). Thus, hybrids with higher taste values had higher branching degrees of amylopectin than that did hybrids with lower taste values. The present results showed that the amylose chains in hybrid combinations with higher taste values were slightly lower than those obtained by the iodine colorimetry method for each group (Table 1), which may be because the long outer chains of amylopectin could interact with iodine and result in a higher iodine affinity of the starch (Jane et al., 1999). The higher proportions of long chains in amylopectin inhibit the swelling and gelatinisation of starch granules (Farhan et al., 2019), leading to a poor taste value of the hybrid rice.

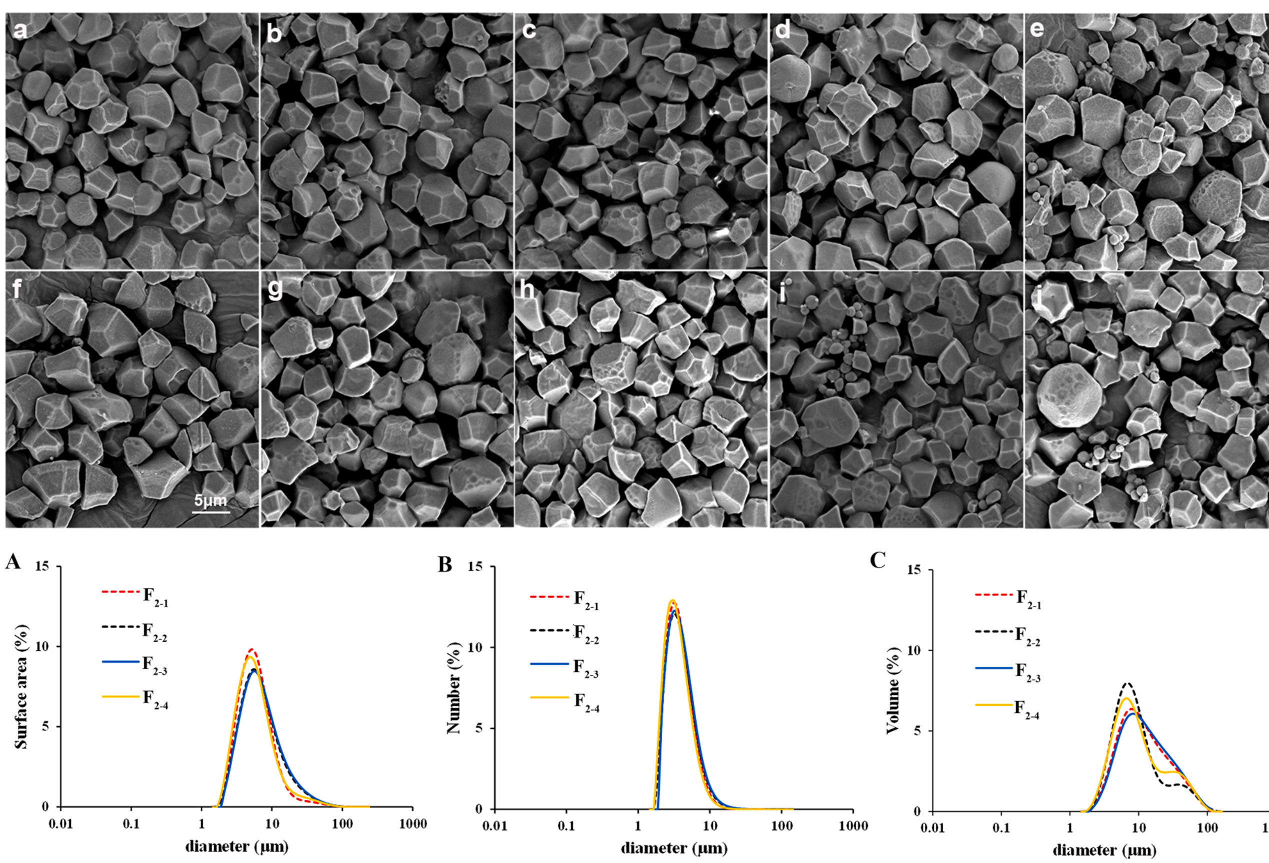

Fig. 2. Micrographs of purified starches, as obtained by scanning electron microscopy (a − j), and the size distribution of the starch granules (A-C). Panels a-e represent micrographs of S1, R1, R2, $F_{2\text{-}1}$, and $F_{2\text{-}1}$ in group A, respectively. Panels f-j represent micrographs of S2, R3, R4, $F_{2\text{-}3}$, and $F_{2\text{-}4}$ in group B, respectively. Panels A − C represent the starch granule surface area, number, and volume distributions for all the hybrid samples, respectively. Scale bar = 5 μm.

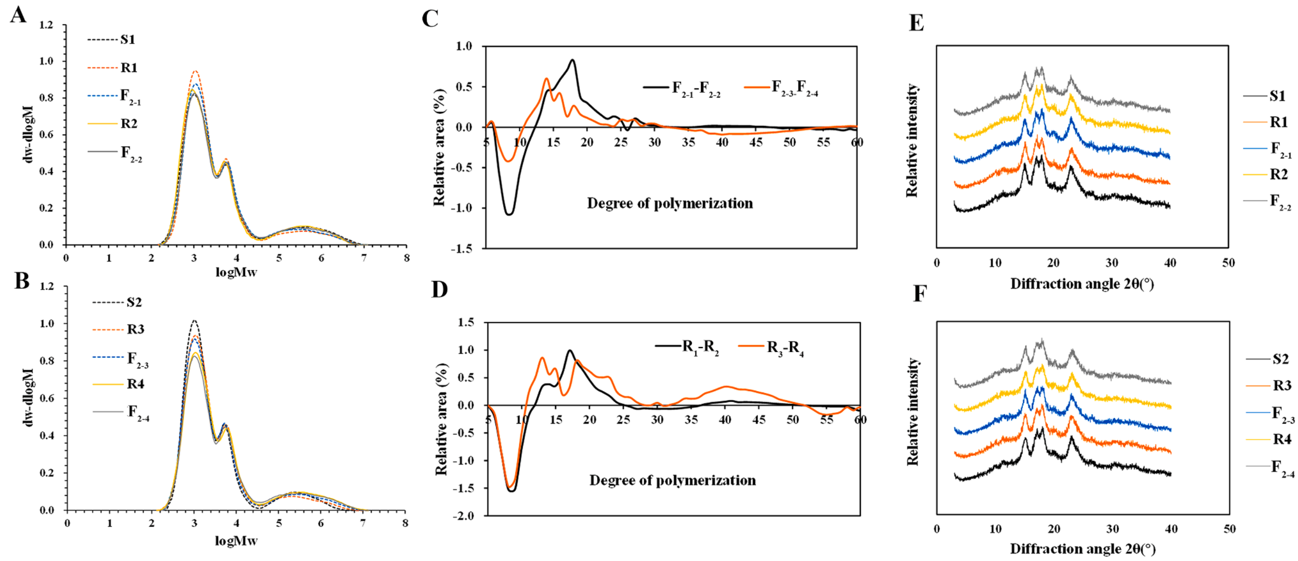

Fig. 3. The relative molecular weight distributions (A-B), chain length distributions of the debranched amylopectin (C-D) and XRD patterns (C-D) of hybrid combinations and parental lines. (C) Each curve with a unique colour represents the relative chain length distribution of amylopectin in the two hybrid combinations with different taste values in the same group. (D) Each curve with a unique colour represents the relative chain length distribution of amylopectin in the two restorer lines in the same group.

3.7. *Chain length distributions of the debranched amylopectin*

Compared with hybrid combinations with poor taste value, alterations in the DP of the chain length distribution of the hybrid starch combinations with good taste values exhibited the same tendencies in each group (Fig. 3C–D). The proportion of amylopectin short chains ranging from DP 6 to 10 for the hybrid combinations with good taste values was lower than that for the hybrid combinations with poor taste values. However, the proportion of intermediate chains ranging from DP 13 to 24 for the hybrid combinations with good taste values was higher than that for the hybrid combinations with poor taste values. Considering the two hybrid combinations with different taste values in each group were obtained by crossing the same sterile line and two different restorer lines, we also analysed the DP of the chain length distribution in the corresponding restorer lines of each group. Compared with the restorer lines corresponding to hybrid rice with poor taste values, those corresponding to hybrid rice with good taste values showed a similar curve in each group (Fig. 3D), with low amounts of short amylopectin chains (DP 6–11) and a high amount of intermediate chains (DP 12–24), which is consistent with the corresponding hybrid rice in each group. Therefore, for the same sterile line, the chain length distribution of restorer lines may determine the chain length distribution of hybrids, and the relative changes in the chain length distribution of amylopectin in the hybrid combinations in each group accounted for the substantial differences in the taste value with similar AAC.

3.8. *Crystalline structure*

In the present study, XRD was used to investigate the supramolecular structure of the rice starches in each group. Fig. 3E–F shows that all the materials displayed a typical A-type diffraction pattern, with strong diffraction peaks at 15°, 17°, 18°, 20°, and 23° 2*θ*. The relative crystallinity of all the hybrid rice and parental lines are shown in Table 3. The present results indicated that the relative crystallinity of hybrid rice starches with higher taste values was higher than that of hybrid rice starches with lower taste values, which could also explain the observed

Table 3
GPC parameters, degree of polymerization (DP) and relative crystallinity of the hybrid combinations and parental lines.

		AP1/AP	AP2/AP	AM/(AM + AP1 + AP2)	AP1/AP2	DP1-DP12	DP13-DP24	DP25-DP36	DP37-DP60	Relative crystallinity (%)
A	S1	63.30 ± 0.16^b	21.61 ± 0.1^a	15.09 ± 0.06^d	2.93 ± 0.02^c	26.69 ± 0.08^d	47.10 ± 0.10^c	12.34 ± 0.05^c	12.80 ± 0.08^a	29.15 ± 0.36^b
	R1	65.20 ± 0.34^c	23.38 ± 0.58^b	11.43 ± 0.14^a	2.79 ± 0.08^b	21.56 ± 0.07^a	51.48 ± 0.13^e	12.46 ± 0.07^c	13.33 ± 0.12^c	33.29 ± 0.18^c
	F_{2-1}	63.66 ± 0.61^b	23.14 ± 0.41^b	13.20 ± 0.29^b	2.75 ± 0.07^{a,b}	22.53 ± 0.22^b	49.34 ± 0.25^d	11.56 ± 0.18^b	12.75 ± 0.08^a	29.62 ± 0.52^b
	R2	64.15 ± 0.62^{b,c}	21.62 ± 0.55^a	14.22 ± 0.29^c	2.97 ± 0.10^{b,c}	26.61 ± 0.09^d	46.16 ± 0.09^b	12.99 ± 0.06^d	13.06 ± 0.06^b	29.78 ± 0.59^b
	F_{2-2}	61.57 ± 0.57^a	23.47 ± 0.63^b	14.97 ± 0.60^d	2.62 ± 0.09^a	26.07 ± 0.16^c	44.88 ± 0.07^a	11.28 ± 0.08^a	12.88 ± 0.07^a	27.90 ± 0.25^a
B	S2	68.99 ± 0.04^e	20.65 ± 0.11^a	10.36 ± 0.15^a	3.34 ± 0.02^d	21.24 ± 0.12^a	51.85 ± 0.18^e	12.61 ± 0.13^e	12.85 ± 0.10^b	33.78 ± 0.36^d
	R3	67.10 ± 0.33^d	22.32 ± 0.14^b	10.57 ± 0.20^a	3.00 ± 0.03^c	22.58 ± 0.07^c	49.99 ± 0.06^c	11.96 ± 0.05^b	14.19 ± 0.05^c	29.05 ± 0.28^c
	F_{2-3}	64.68 ± 0.58^c	21.76 ± 0.34^b	13.56 ± 0.42^b	2.97 ± 0.02^{b,c}	21.85 ± 0.11^b	50.85 ± 0.42^d	12.41 ± 0.06^d	12.86 ± 0.08^b	28.56 ± 0.28^c
	R4	63.10 ± 0.70^b	21.82 ± 0.44^b	13.31 ± 0.21^b	2.89 ± 0.09^b	26.17 ± 0.04^d	43.51 ± 0.19^a	11.13 ± 0.05^a	12.21 ± 0.04^a	27.81 ± 0.21^b
	F_{2-4}	60.10 ± 0.33^a	25.14 ± 0.52^c	14.77 ± 0.26^c	2.39 ± 0.04^a	22.65 ± 0.09^c	48.32 ± 0.14^b	12.13 ± 0.08^c	14.45 ± 0.13^d	25.42 ± 0.42^a

difference in ΔH_{gel}, as determined by DSC. Bhat and Riar (2018) reported that rice cultivars with higher amylose content had higher crystallinity values and vice versa, whereas Zhang et al. (2017) and Chung et al. (2011) reported that amylose was negatively correlated with the degree of crystallinity, and amylopectin was responsible for starch crystallinity. Additionally, starches with abundant shorter amylopectin chains (DP < 11) can reduce the crystallinity, whereas an abundance of longer-chain amylopectin (such as DP 16–21) could contribute to a perfect crystalline structure (Chung et al., 2011; Vandeputte et al., 2003; Witt et al., 2012). In the present study, for hybrid rice with similar amylose content, the higher relative crystallinity of hybrid rice with higher taste values was attributed to the lower amounts of short amylopectin chains (DP 6–11), which were too short to form a double helix spanning the entire crystalline lamella, resulting in an unstable crystal structure, and a higher amount of intermediate chains (DP 12–24), which could form a double helix structure across the crystal lamella, making the crystal structure more stable.

4. Conclusion

In the present study, we compared the physicochemical properties and starch structures of hybrid rice with similar AAC but different taste values. GPC analysis indicated that the hybrid rice with good taste values had higher proportions of amylopectin short chains but relatively lower proportions of amylopectin long chains, which could lead to higher peak viscosity and breakdown value, and a softer and stickier texture of the cooked rice. HPAEC analysis indicated that the proportion of amylopectin short chains (DP 6–10) for the hybrid rice with good taste values was low, whereas the proportion of intermediate chains (DP 13–24) was high, which could lead to higher gelatinisation enthalpy and crystallinity. Moreover, for the first time, the present study indicated that the greater diversity of forms and sizes of starch granules might influence the eating quality of hybrid rice. Overall, the present study provides a theoretical basis for the improvement of the eating quality of hybrid rice with similar AAC.

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CRediT authorship contribution statement

Yan Peng: Investigation, Formal analysis, Writing - original draft. **Bigang Mao:** Resources, Investigation. **Changquan Zhang:** Methodology, Writing - review & editing. **Ye Shao:** Resources, Investigation. **Tianhao Wu:** Investigation. **Liming Hu:** Investigation. **Yuanyi Hu:** Software, Validation. **Li Tang:** Software, Validation. **Yaokui Li:** Software, Validation. **Wenbang Tang:** Methodology, Resources. **Yinghui Xiao:** Conceptualization, Methodology, Supervision. **Bingran Zhao:** Conceptualization, Methodology, Supervision.

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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