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Comparisons of Appearance and Eating Quality Traits Between Early and Late Maturing Soft Rice

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Abstract

Soft *Japonica* rice is popular in Yangtze River Delta of China in recent years because of its high eating quality. In this study, high eating quality soft *Japonica* rice with early and late maturity was used to analyze their eating quality traits. The appearance, texture, and physicochemical characteristics, gelatinization and pasting properties, and fine structures between early and late maturing soft rice were compared. Early maturing soft rice had higher whiteness, chalkiness, gelatinization temperature, peak viscosity, breakdown viscosity, long branch-chain amylopectin, amylopectin chains (DP > 25), and molecular weight than late maturing soft rice. The higher gelatinization and pasting properties in early maturing soft rice probably be attributed to its higher amount of long branch-chain amylopectin, proportions of amylopectin chains (DP > 25) and molecular weight, compared with late maturing soft rice. Late maturing soft rice had higher amylose content, gel consistency, short branch-chain amylopectin, amylopectin short chains (DP 6–12), setback viscosity, and consistence viscosity than early maturing soft rice. The appearance and eating quality of late maturing soft rice were significantly higher than those of early maturing soft rice.

Keywords: appearance, eating quality, amylose, amylopectin, viscosity, gelatinization temperature

1. Introduction

Soft *Japonica* rice is popular in Yangtze River Delta of China in recent years because of its high eating quality (Zhu et al., 2015; Hu et al., 2020). Its hardness and stickiness is between *Japonica* rice and glutinous rice, and it is not easy to harden after cooling (Li et al., 2016). Eating quality, which is primarily determined by physicochemical properties through affecting hardness, stickiness, appearance, and taste, is the most important trait for rice grain-quality evaluation (Hori et al., 2016; Li et al., 2017).

Rice amylose content (AC) is widely recognized as the most important factor for eating quality (Tao et al., 2019; Zhu et al., 2021). Low AC rice generally has soft and sticky texture after cooking (Gong et al., 2023). Apparent amylose content (AAC) of soft rice (8-12%) is low and between *japonica* rice (13-19%) and glutinous rice (0-2%) (Zhang et al., 2021). Fine structures of starch probably play key roles in determining rice eating quality (Li et al., 2018; Zhang et al., 2021). Gel consistency (GC), protein content (PC), gelatinization temperature (GT), and pasting properties are also widely used for evaluating the eating quality of rice (Zhu et al., 2021). Pasting properties of starch and taste values of cooked rice suggest the eating quality to some extent.

Waxy (*Wx*) gene is the main gene controlling the AC of rice and then affecting its eating quality. Several alleles of *Wx* have been identified, including W_x^{lv}, W_x^{a}, W_x^{b}, W_x^{in}, W_x^{hp}, W_x^{mw}, and W_x^{mp} (Zhang et al., 2019; Gong et al., 2023). W_x^{b} mainly exists in traditional *Japonica* rice (13-19%). Wx^{mp} was introduced to China from Japan through Guandong 194 with low AAC, and then Nangeng 46 was cultivated in 2008 (Wang et al., 2009). Afterwards, a series of new Nangeng soft *Japonica* rice varieties with Wx^{mp} were gradually cultivated. Wx^{hp} was found in some soft *indica* rice of Yunnan (Zhang et al., 2019).

Many studies on soft rice eating quality were researched (Li et al., 2018; Wang et al., 2020; Yang et al., 2022), however, limited information can be found about the influence of heading date on the eating quality of soft rice. The eating quality of late maturing soft rice was generally better than early maturing soft rice. It was reported the eating quality of early-maturing rice cultivar by editing PHYC in Nanjing 46 via CRISPR/Cas9 was reduced (Li et al., 2021), while the reason is not clear. Thus, understanding the difference of eating quality between early and late maturing soft rice is important. In the present study, four soft *Japonica* rice varieties with Wx^{mp} of early and late maturity were used to study the influence of maturity on the eating quality of soft rice. The appearance, texture, and physicochemical properties, gelatinization and pasting characteristics, and fine structures between early and late maturing soft rice were compared.

2. Materials and Methods

2.1 Plant Materials and Sample Preparation

Two groups of four soft rice varieties Hu-zao-xiang-ruan 1 (HZXR1), Song-zao-xiang 1 (SZX1), Song-xiang-geng 1018 (SXG1018), and Shang-shi-da 19 (SSD19) were used in this study. All varieties were grown and harvested in the Zhuanghang experimental field of Shanghai Academy of Agricultural Science (Shanghai, China) under management conditions as previously described (Zhang et al., 2019). HZXR1 and SZX1 with early maturity were grown during the summer months from May to the end of September, 2021. SXG1018 and SSD19 were grown from May to November in 2021. The rice grains were harvested and air-dried. Rice flours were prepared from polished rice and then stored at 4 °C.

2.2 Appearance and Scanning Electron Microscopy (SEM) Analysis

The appearance of polished rice grains was measured using a Grain Rice Analyzer (Vibe Imaging Analytics, QM3, Bnei-Brak, Israel). The rice grain transverse sections were checked with scanning electron microscope (Hitachi, TM4000Plus, Tokyo, Japan) as previously described (Zhang et al., 2019).

2.3 Physicochemical Properties Measurement

AAC, GC, and PC were measured as previously reported (Zhang et al., 2019). The moisture and lipid content of rice starch were measured in accordance with a previous method (Shen et al., 2021). The colour of soft rice was determined using colorimeter (CM-5, Konica Minolta, Tokyo, Japan). Colour readings were expressed by values for L*, a*, and b*. Whiteness (W) was converted by the following formula as previously reported (Zhu et al., 2013).

$$W = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]\cdot 1/2 \quad (1)$$

2.4 Pasting and Thermal Properties Measurement

The pasting properties of rice flours were measured with a Rapid Visco Analyzer (RVA, TecMaster, Newport Science, Warriewood, Australia). The RVA indexes included pasting temperature (PAT), peak viscosity time (PVT), peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity [BDV (PKV-HPV)], setback viscosity [SBV (CPV-PKV)], and consistence viscosity [CSV (CPV-HPV)], were analyzed. Rapid viscosity units (RVUs) were used as their outputs. The thermal properties of starch were determined by differential scanning calorimetry (DSC, Q2000, TA Instruments, USA). The DSC characteristics included onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and the difference in enthalpy (ΔH).

2.5 Texture Characteristics and Eating Quality Analysis of Cooked Rice

35.0g polished rice grains was washed, and water is added to 38.5g (polished rice: water = 1:1.1). Then they were cooked with the same rice cooker for 45 min and cooled at room temperature for 30 min. The hardness, stickiness and comprehensive evaluation of cooked rice was measured with a Tensipresser texture analyzer (MyBoyII, Takemoto Electric Co. Tokyo, Japan).The cooked rice taste was evaluated by near-infrared micro-light transmission with a cooked-rice taste meter (STA1B, SATAKE, Hiroshima, Japan).

2.6 Starch Fine Structure Analysis

Rice starch was isolated and then debranched with isoamylase as previous report (Zhang et al., 2019). The relative molecular weight distribution of whole and debranched starch was determined with a high-temperature gel permeation chromatography (GPC) system (Wyatt, Santa Barbara, CA, USA) as previously described (Deng et al., 2021). The chain length distribution of amylopectin was quantitatively measured in the Ion Chromatography system (ICS5000+, Thermo Fisher Scientific, MA, USA) according to previous study (Deng et al., 2021).

2.7 Statistical Analysis

Tukey's multiple-range tests analysis were conducted using SPSS 20 software with $p < 0.05$ as the significance level. All samples were analyzed in three biological replicates.

3. Results and Discussion

3.1 Appearance and Physicochemical Characteristics of Rice Grains

Two groups of early and late maturing soft *Japonica* rice were used (Table 1). The early maturing soft rice included HZXR1 and SZX1, and the late maturing soft rice included SXG1018 and SSD19. HZXR1 and SXG1018 had short grain, whereas SZX1 and SSD19 had long grain (Figure 1). The grains in the late maturing soft rice showed better transparency than the early maturing soft rice (Figure 1). SEM analysis was performed to investigate starch structure of rice grain, and no obvious differences were found between early and late maturing soft rice (Figure 1). Appearance quality is important for customer choice in market.

Table 1. Appearance and texture characteristics within two groups

Groups	Samples	Chalkiness (%)	Whiteness	Hardness (gw/cm^2)	Stickiness (gw cm/cm^2)
Early maturing	HZXR1	1.9±0.2a	68.9±0.3a	87.2±3.6a	54.1±2.5d
Soft rice	SZX1	2.1±0.1a	68.6±0.5a	71.7±1.5b	66.0±1.9c
Late maturing	SXG1018	0.9±0.3b	64.4±0.1b	50.8±2.8d	79.1±1.3b
Soft rice	SSD19	1.2±0.2b	63.2±0.3c	58.8±5.3c	84.9±3.7a

Note. Data represent means±standard deviations. n = 3. Different letters in the same column indicate significant differences ($p < 0.05$).

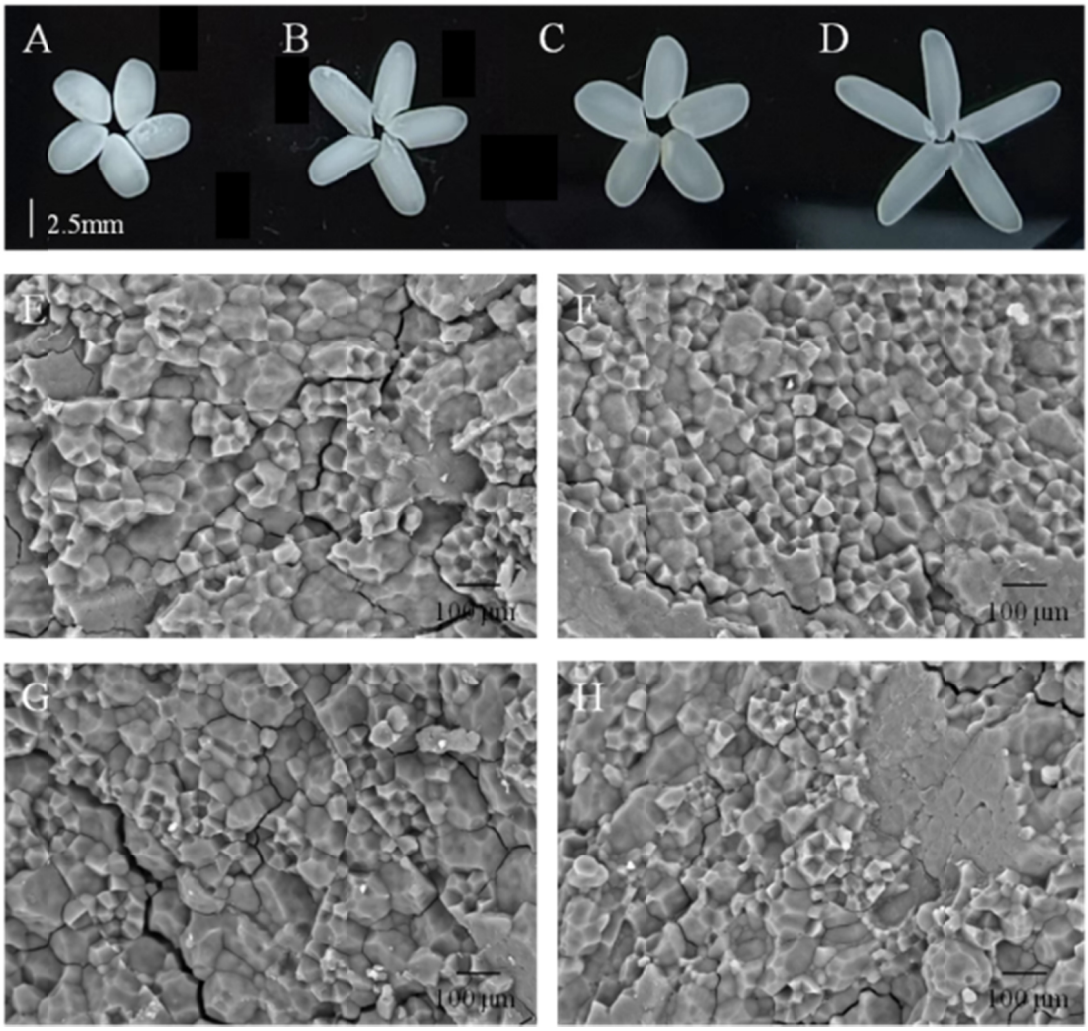

Figure 1. Grain appearance of rice varieties (A) HZXR1, (B) SZX1, (C) SXG1018 and (D) SSD19 and micrographs of transverse sections for varieties(E) HZXR1, (F) SZX1, (G) SXG1018 and (H) SSD19

The temperature during the early filling period is relatively high and previous reports showed chalkiness was closely related to high temperature (Abayawickrama et al., 2017; Lin et al., 2020). The chalkiness of early maturing soft rice was significantly higher than late maturing soft rice (Table 1), which probably related to its higher temperature during the early filling period. A high amount of chalkiness decreases the appearance quality. The whiteness of late maturing soft rice was significantly lower than that of early maturing soft rice, indicating the higher appearance quality of late maturing soft rice.

3.2 Physicochemical Characteristics of Rice Grains

The texture of cooked rice was measured with Tensipresser MyBoyII texture analyzer. The hardness of early maturing soft rice was significantly higher than that of late maturing soft rice, while the stickiness of early maturing soft rice was significantly lower than that of late maturing soft rice. The late maturing varieties had lower hardness and higher stickiness (Table 1), which was commonly considered high eating quality. The taste values of late maturing soft rice were significantly higher than those of early maturing soft rice (Table 2), indicating higher eating quality of late maturing soft rice. The AAC of early-season rice is usually high (Huang et al., 2021). In this study, the AAC of early maturing soft rice was significantly higher than that of late maturing soft rice and its GC was significantly lower. The PC of HZXR1 was significantly lower than other varieties. Meanwhile, the moisture content and lipid content were similar between the two groups (Table 2).

Table 2. Taste values and physicochemical characteristics within two groups

Samples	Taste value (points)	AAC (%)	GC (mm)	PC (%)	Moisture content (%)	Lipid content (%)
HZXR1	75.2±0.6c	11.0±0.3a	75.0±0.9c	6.7±0.2b	15.0±0.0a	0.8±0.1a
SZX1	76.8±1.0c	10.5±0.2a	71.5±0.5d	7.5±0.1a	14.9±0.1a	0.7±0.0a
SXG1018	85.8±0.0a	9.6±0.2b	85.0±1.5a	7.3±0.2a	14.9±0.0a	0.7±0.1a
SSD19	80.5±0.6b	9.8±0.0b	80.5±0.3b	7.4±0.0a	14.8±0.1a	0.8±0.1a

Note. Data represent means±standard deviations. n = 3. Different letters in the same column indicate significant differences ($p < 0.05$). AAC: apparent amylose content; GC: gel consistency; PC: protein content.

3.3 Fine Structures of Starch

Fine structures of starch were essential for rice eating quality (Yu et al., 2019). Whole starch relative molecular weight distribution was performed to analyze the starch fine structure. Molecular parameters including number average molecular weight (Mn), peak molecular weight (Mp), weight average molecular weight (Mw), average molecular weight (Mz), PDI, and polydispersity index (Mw/Mn) were compared in Table 3. The Mn, Mp, Mw, and Mz of fully branched starch in early maturing soft rice was significantly higher than those in late maturing soft rice. The PDI between the two groups was similar, which indicated the broadness of their molecular weight distribution.

Table 3. Comparisons of molecular weight within two groups

Samples	Mn (10^4 kDa)	Mp (10^4 kDa)	Mw (10^4 kDa)	Mz (10^4 kDa)	PDI (Mw/Mn)
HZXR1	6.7±0.3a	9.3±0.3a	7.7±0.1a	9.0±0.0a	1.2±0.3a
SZX1	6.8±0.3a	8.7±0.1b	7.7±0.3a	8.8±0.2a	1.1±0.0a
SXG1018	5.1±0.1c	8.0±0.4c	6.7±0.3b	8.3±0.1b	1.3±0.1a
SSD19	5.6±0.4b	8.2±0.1c	7.0±0.4b	8.3±0.3b	1.2±0.1a

Note. Data represent means±standard deviations. n = 3. Different letters in the same column indicate significant differences ($p < 0.05$). Mn: number average molecular weight; Mp: peak molecular weight; Mw: weight average molecular weight; Mz: Z average molecular weight; PDI: polydispersity index (Mw/Mn).

Meanwhile, debranched starch relative molecular weight distribution of the two groups was shown in Figure 2. The starch samples contained short branch-chain amylopectin (AP1), long branch-chain amylopectin (AP2), and AM chains as described previously (Zhang et al., 2019). The AM and AP1 distributions were significantly lower than those in late maturing soft rice, and the AP2 fraction was significantly higher in early maturing soft rice (Table 4).

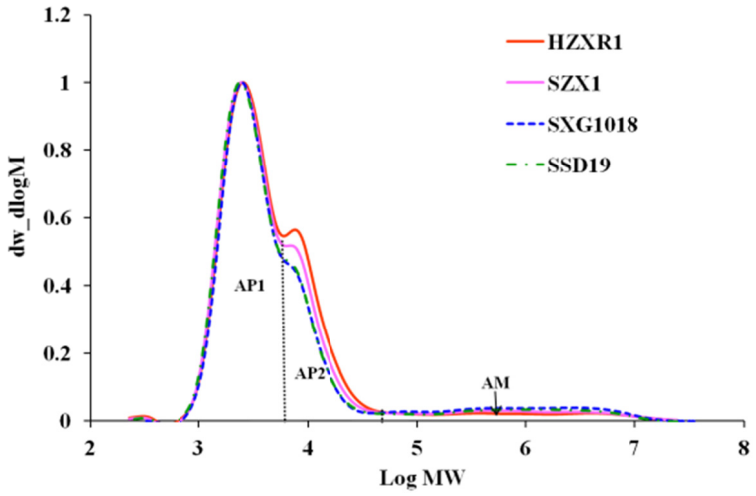

Figure 2. Relative molecular distributions of amylose and amylopectin within two groups. MW represents the apparent molecular weight relative to the standards

Table 4. Comparisons of amylose and amylopectin distributions within two groups

Samples	AM (%)	AP1 (%)	AP2 (%)	DP(6-12) (%)	DP(13-24) (%)	DP(25-36) (%)	DP > 36 (%)
HZXR1	7.2±0.6c	67.6±0.4b	25.2±0.3a	25.2±0.1d	47.9±0.0bc	12.5±0.3a	14.4±0.1a
SZX1	8.1±0.2b	68.2±0.1b	23.7±0.7b	27.5±0.4c	47.5±0.1c	12.1±0.1b	12.9±0.3b
SXG1018	9.1±0.4a	70.3±0.2a	20.6±0.3c	29.9±0.1a	47.9±0.4ab	10.7±0.2c	10.9±0.4d
SSD19	8.7±0.2a	70.5±0.4a	20.8±0.2c	29.1±0.6b	48.4±0.2a	11.1±0.1c	11.4±0.3c

Note. Data represent means±standard deviations. n = 2. Different letters in the same column indicate significant differences ($p < 0.05$). AM: amylose; AP1: short branch-chain amylopectin; AP2: long branch-chain amylopectin; DP: degree of polymerization.

The chain length distribution of amylopectin in the two groups was further investigated. As shown in Figure 3 and Table 4, the proportion of amylopectin short chains [degree of polymerization (DP) 6-12] in early maturing soft rice was significantly lower than that in late maturing soft rice. Meanwhile, the proportions of amylopectin medium and long chains (DP 25-36) and long chains (DP > 36) in early maturing soft rice were significantly higher than those in late maturing soft rice.

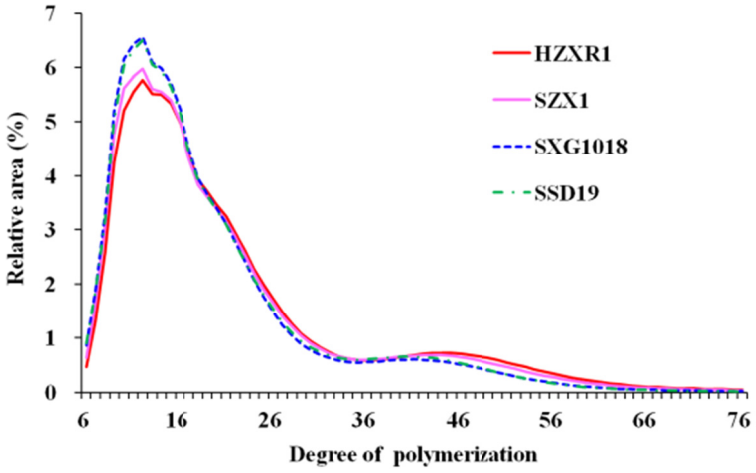

Figure 3. Chain length distribution of amylopectin within two groups

The high temperature during the early filling period can reduce the activity of grain bound starch synthase, and lead to a decrease in AC (Cao et al., 2015). In the present study, the AAC of early maturing soft rice was significantly higher than that of late maturing soft rice (Table 2). However, the real AC (Table 4) was lower in early maturing soft rice. As the AAC generally overestimates AC as part of amylopectin long chains have iodine affinity properties (Lin et al., 2016), the higher AAC probably be attributed to the significantly higher amylopectin long chains in early maturing soft rice. It was reported that the whiteness was negatively related to AC (Zhang et al., 2017). The whiteness of late maturing soft rice was significantly lower than that of early maturing soft rice (Table 1), which probably related to its higher AC.

Eating quality of late maturing soft rice was significantly higher than those in early maturing soft rice (Table 2). The AC, GC, proportion of AP1 and amylopectin short chains (DP 6-12) in late maturing soft rice were significantly higher than those in early maturing soft rice (Tables 2 and S2, Figures 3 and 4). Meanwhile, the proportion of AP2, amylopectin medium and long chains (DP 25-36), and amylopectin long chains (DP > 36), and the molecular weight were significantly higher in early maturing varieties (Tables 1, 2 and S2, Figures 2 and 4). Rice AM may limit starch swelling when cooking, causing a hardened texture (Li et al., 2018). Hardness is dominant in evaluating eating quality of cooked rice and positively correlated with AC (Wang et al., 2022). Stickiness is correlated with amylopectin amount, especially short amylopectin chains in the leachate (Li et al., 2019). In the present study, the late maturing varieties with higher AC had lower hardness and higher stickiness, which may be related to their higher amount of short branch-chain amylopectin and proportion of amylopectin

short chains (DP 6-12). The late maturing varieties with higher AC had lower hardness and higher stickiness (Table 1), contributing to their high eating quality (Table 2).

3.4 Pasting Properties of Rice Flour

The flour pasting properties of the two groups were shown in Figure 4 and Table 5. During rice cooking, the viscosity of starch increases through swelling (Wani et al., 2012). The soft rice with early maturity had significantly higher Pat, which is in agreement with the result of GT in DSC. The pasting properties, including PKV and BDV, were significantly higher in early maturing soft rice with lower AC than those in late maturing soft rice. The maximum viscosity PKV reflects the swelling ability of starch granules and BDV indicates the stability of hot paste in the pasting process (Chen et al., 2006). The higher PKV and BDV in early maturing soft rice suggested that its starch granules had the strongest swelling performance and ability to bind water. On the contrary, the SBV and CSV of early maturing soft rice were significantly lower than those of late maturing soft rice.

CPV denotes the viscosity of cold paste during cooling, and it involves reassociation of AM molecules. SBV is linked to recovery of heated starch suspension during cooling (Wani et al., 2012). CSV indicates retrogradation of starch. The higher the CSV is, the easier the starch regenerates (Wang et al., 2020). Starch retrogradation can cause quality deterioration, such as water retention loss, hardening, and difficulties in solubility (Wang et al., 2015).

Table 5. Pasting properties of rice flours within two groups

Samples	PAT (°C)	PKV (RVU)	HPV (RVU)	CPV (RVU)	BDV (RVU)	SBV (RVU)	CSV (RVU)
HZXR1	79.0±0.0a	394.0±0.6a	167.5±0.2a	216.5±0.6ab	226.5±0.4a	-177.5±0.1c	49.0±0.5b
SZX1	74.4±0.0b	314.5±0.5b	153.4±2.4c	207.4±1.1b	161.1±1.0b	-107.2±2.5b	54.0±0.6b
SXG1018	70.2±0.3c	270 ±1.0d	127.3±1.3d	187.8±0.9c	142.5±1.4c	-82.0±5.1a	61.2±2.0a
SSD19	70.1±0.5c	295.5±0.9c	159.2±2.1b	219.5±1.5a	136.3±1.6c	-76.0±4.0a	60.1±1.2a

Note. Different letters in the same column of each group indicate significant differences ($p < 0.05$). n = 3.

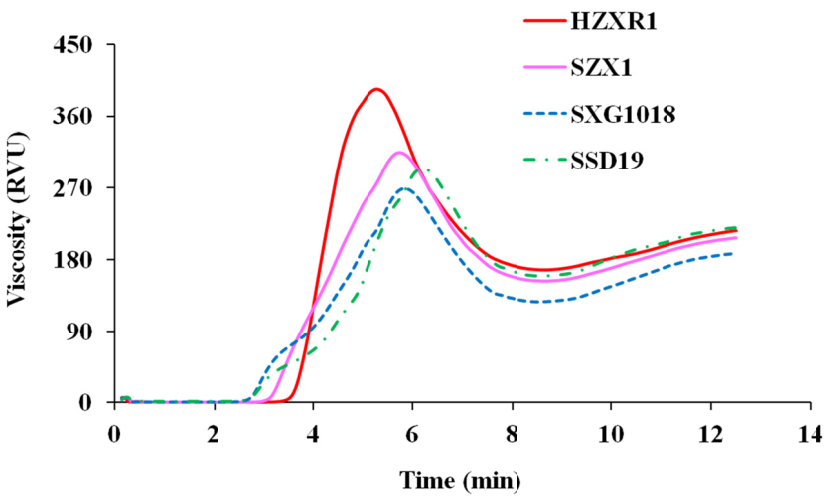

Figure 4. RVA pasting viscosity profile of the rice varieties within two groups

The significantly higher proportion of amylopectin long chains (DP > 36) in early maturing soft rice may form helical complexes and contribute to higher PKV and BDV, which is consistent with the previous results of rice starch with a higher amount of amylopectin long chains displaying higher PKV and BDV (Tao et al., 2019). Meanwhile, the higher PKV and BDV in early maturing soft rice were possibly related to its higher Mn, Mp, Mw, and Mz (Table 6). Mn and Mw were reported to be significantly positively correlated with the PKV and BDV of rice starch (Kowittaya et al., 2014). During cooling, cooked rice increases in hardness and viscosity (Chen et al., 2021). The retrogradation of amylopectin occurs slowly, and that of starch depends greatly on the AM (Iftikhar et al., 2019). The early maturing varieties with lower SBV had higher hardness, compared with late maturing

varieties. CSV was significantly lower in early maturing varieties with lower AC than in late maturing varieties, indicating they are not easy to harden after cooling, possibly due to their low AC.

3.5 Gelatinization Properties of Starch

Lower GT of rice is considered to be associated with higher eating quality (Zhang et al., 2019). T_o, T_p, T_c, and ΔH in gelatinization process were measured, as shown in Table 6. GT is typically expressed as T_p. The soft rice with early maturity showed significantly higher GT than its counterpart, containing T_o, T_p, T_c, and ΔH.

Table 6. Comparisons of gelatinization properties within two groups

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
HZXR1	70.6±0.1a	76.5±0.1a	88.5±0.1a	11.5±0.0a
SZX1	64.0±0.1b	69.7±0.1b	80.1±0.6b	10.1±0.2b
SXG1018	57.7±0.2c	64.7±0.2c	76.8±0.0d	8.3±0.2c
SSD19	57.8±0.1c	64.8±0.3c	78.4±0.3c	8.0±0.5c

Note. Data represent means±standard deviations. n = 3. Different letters in the same column indicate significant differences ($p < 0.05$). T_o: onset temperature; T_p: peak temperature; T_c: conclusion temperature; ΔH: difference in enthalpy.

Previous studies showed that the low pasting temperatures and GT of rice may be ascribed to their low AM and high amylopectin short-chain content (Wang et al., 2020). In the present study, the T_o, T_p, T_c, and ΔH in gelatinization and the PAT in RVA were significantly higher in early maturing varieties with lower amylopectin short-chain content than late maturing varieties (Tables 2 and S1), probably due to the high proportion of AP2 and amount of amylopectin medium and long chains (DP 25-36) and amylopectin long chains (DP > 36) in early maturing soft rice (Figure 3, Table 4). The higher proportion of amylopectin long chains in early maturing soft rice may require higher temperatures to unravel, causing higher T_o, T_p, T_c, and PAT. The higher GT and PAT in early maturing varieties may be related to their lower eating quality.

4. Conclusions

The late maturing varieties had lower whiteness, chalkiness, hardness, and higher stickiness and eating quality values, indicating that the appearance and eating qualities of late maturing soft rice were significantly higher than early maturing soft rice. Compared with the late maturing soft rice, early maturing soft rice possessed significantly higher gelatinization and pasting properties. These results provide a theoretical basis for high-quality rice breeding.

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Authors Contributions

Conceptualization, S.W. and L.Z., methodology, K.W. and Y.W., investigation, L.Z., K.W., Y.Y., and Z.H., formal analysis, J.Y. and H.Y., data curation, M.L. and Y.C., writing—original draft, L.Z and K.W., writing—review & editing, S.W. and L.C. All authors read and approved the final manuscript. L.Z. and K.W. contributed equally to the study.

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Competing Interests

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Data Sharing Statement

No additional data are available.