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Brief Report

Elevated Ripening Temperature Mitigates the Eating Quality Deterioration of Rice in the Lower Grain Position Due to the Improvement of Starch Fine Structure and Properties

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Abstract: Elevated ripening temperature (ET) impacts rice grain quality. In this study, two rice varieties were investigated to evaluate the characterization of starch fine structure and grain eating quality under ET conditions. Rice exposure to ET increased the proportion of large-sized granules and starch granule average size, regardless of grain position. Compared to normal temperature (NT), the amylose content (AM) in the upper grain position (UP) exhibited a significant increase under ET, whereas the contrary results showed a decrease in the lower grain position (LP), and the proportion of shorter amylopectin chains increased under ET in UP or LP, whereas the proportion of long amylopectin chains decreased, resulting in a higher starch gelatinization temperature and enthalpy under ET. For grain position, compared to LP, UP had higher AM and a higher proportion of long amylopectin chains, leading to higher gelatinization enthalpy under ET. For eating quality, we found that ET deteriorated the eating quality of rice compared to NT, and UP had higher eating quality than LP under NT; however, there was a comparable eating quality between UP and LP under ET. Hence, elevated grain filling temperature mitigated the eating quality deterioration of rice grain in LP due to the lower AM and gelatinization enthalpy and the higher proportion of shorter amylopectin chains.

Keywords: rice; high temperature; eating quality; starch fine structure; gelatinization

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1. Introduction

Rice (*Oryza sativa* L.) is a basic cereal crop worldwide and is the primary staple food for over half of the world's population [1,2]. Therefore, it is of great importance to guarantee rice yield and grain quality, particularly eating quality, for rice consumers. Rice quality is influenced by the grain filling of spikelets, which is directly relevant to the spikelet's location within a panicle. In general, rice superior grains grow in the upper location of a panicle and have earlier flowering, a greater grain filling rate, and higher grain plumpness, whereas inferior grains in the lower location of a panicle have a relatively late flowering date, lighter grain, and poor grain filling traits [3–5]. Numerous previous studies have suggested that rice quality and grain filling have a considerable discrepancy in different grain positions [6–9]. Ma et al. (2017) reported that significant differences were found in protein content, amylose content, breakdown, setback, and pasting temperature in different rice grain positions, which were closely related to eating quality [6]. Chen et al. (2014) found that rice superior grains had higher gel consistency and better eating quality than inferior grains [7]. For starch quality, superior grains had higher amylose content, a longer chain of amylopectin content, a larger granule size, lower relative crystallinity, and a shorter chain of amylopectin content than inferior grains [8]. However, the difference between different grain positions in eating quality and starch fine structure and properties is unclear under different ripening temperatures for rice.

The temperature during the grain filling stage is the critical environmental factor affecting the formation of rice grain [9]. Previous studies have demonstrated that rice grain filling temperature impacts eating quality and starch structure and function [10–12]. Temperature stress during rice grain filling results in changes in eating quality, starch fine structure, relative crystallinity, and the proportion of amylose and amylopectin [13–16]. Lin et al. (2020) suggested that high temperatures (40 °C daily) during the primordial differentiation and pollen filling stages resulted in lower apparent amylose content and higher starch order degree and starch gelatinization temperatures [17]. Hu et al. (2021) reported that as rice grain filling temperature increased, the amylose content and the proportion of shorter amylopectin chains decreased, and the proportion of long amylopectin chains increased, leading to starch gelatinization temperature and enthalpy [18]. However, information about the effects of grain filling temperatures on the differences in eating quality and starch characteristics in different grain positions is limited. Thus, most previous studies adopted a constant high or low grain filling temperature controlled by a phytotron or greenhouse, and the time of temperature treatment was a part of the grain filling process [19,20]. Therefore, it is quite necessary to compare the differences in grain eating quality and starch fine structure in the different grain positions of rice induced to different dynamic temperatures during the whole grain filling stage, including elevated and normal temperatures, i.e., 26 °C (30/22 °C) and 22 °C (26/18 °C). Hence, we studied the effects of elevated ripening temperature during the whole grain filling stage on rice eating quality and starch fine structure in different grain positions. The findings of this study provide more insights into the applications of rice starch in the food industry.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

This study was conducted on the potted experiment farm of Yangzhou University in 2020. Two *japonica* rice varieties with low amylose content (AM), Nanjing 46 (NJ 46) and Suxiangjing 100 (SXJ 100), were selected for this study. The soil was taken from the loamy clay soil from 0 to 20 cm in the paddy, and the physicochemical properties were as follows: organic matter 23.7 g kg^{-1}, total N 1.58 g kg^{-1}, Olsen-P 14.40 mg kg^{-1}, and available K 130.56 mg kg^{-1}. Approximately 30-day-old seedlings were transplanted on June 19 into plastic pots. The diameter of the pot was 30 cm, and the height of the pot was 26 cm. A total of 15 seedlings were transplanted in circles in each pot. As basal fertilizers, 2 g of urea (nitrogen content 46%) and 1 g of monopotassium phosphate (KH_2PO_4 content $\geq$ 99.5%) were used in each pot before transplantation. Approximately 0.5 g of urea was applied to each pot 7 days after transplantation. A total of 0.5 g of urea and 0.5 g of monopotassium phosphate were applied to each pot in the rice panicle initiation stage. The pots were placed on the paddy ground under natural light and temperature conditions (mean daily temperature of 27 °C from transplantation to heading). Water management on the plots was conducted separately through leather pipes. Pest and disease control were carried out in accordance with conventional cultivation. After anthesis, the pots were transferred to two artificial climate chambers (the temperature and humidity can be accurately adjusted) with transparent glass until maturity.

Two ripening dynamic temperatures, elevated temperature (ET) and normal temperature (NT), were set in the two artificial climate chambers. The ET and NT regimes for daily mean temperature (daily high temperature/daily low temperature) were 26 °C (30/22 °C) and 22 °C (26/18 °C), respectively. The ripening dynamic temperature for the ET and NT regimes is summarized in Figure 1. Natural light was used as the light source in the chambers, and the relative humidity was maintained at 75 ± 5% with a wind speed of 0.5 m s^{-1}. At maturity, rice grains in the panicle upper position (UP, top three primary branches) and lower position (LP, bottom three primary branches) were collected and harvested, respectively. And the rice grains were air-dried to 14.5% moisture content and stored at room temperature for 2 months. Afterwards, the grains were milled to investigate rice eating quality and starch physicochemical properties.

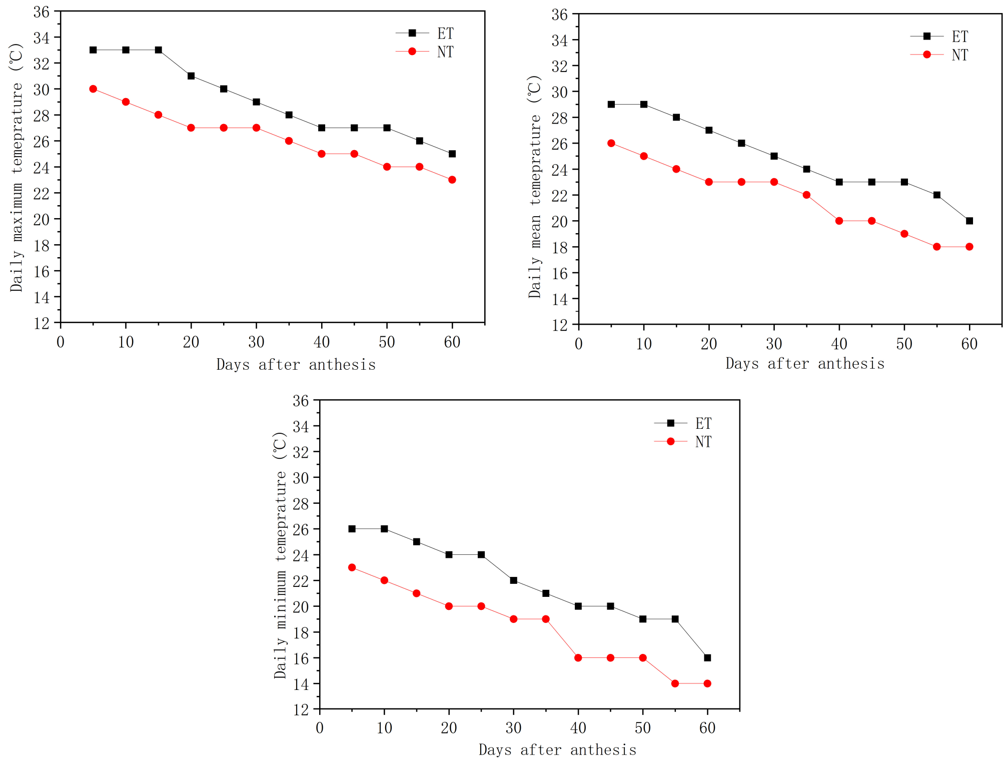

Figure 1. Daily mean temperature, daily high temperature, and daily low temperature were measured for ET and NT conditions during grain filling.

2.2. Flour and Starch Isolation

The rice grains were polished and then ground into flour using a mill (FOSS 1093 Cyclotec Sample Mill, Tecator, Hoganas, Sweden) with a 0.5 mm screen. Starch was isolated from the rice grains according to the previously published method [21].

2.3. Starch Fine Structure

Starch was debranched and analyzed using the size-exclusion chromatography (SEC) system according to the previously published method [22] (Wu et al., 2014). The SEC, a type of gel-permeation chromatography (GPC), system was an Agilent 1100 series (Waters, Wyatt) equipped with a differential refractive index detector.

2.4. Starch Granule Size Distribution

A laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instrument Co., Ltd., Malvern, UK) was used to determine the starch granule size distribution. The starch samples were placed in absolute ethanol and stirred at 671× g. The starch granule size was recorded in terms of volume distribution.

2.5. Gelatinization Properties

The starch gelatinization properties were assessed using a differential scanning calorimetry (DSC) approach (200-F3, Netzsch, Selbu, Germany). Specifically, 5 mg of starch was mixed with 15 µL of water in an aluminum pan, and the samples were chilled at 4 °C for 12 h. Following an incubation step at room temperature for 1 h, the samples were heated to 130 °C at a rate of 10 °C min^{-1}. The samples were assayed in triplicate.

2.6. Taste Value and Cooking Properties

The taste value of cooked rice was measured by an STA-1A rice sensory analyzer (Satake, Osaka, Japan). Approximately 30 g of milled rice was washed in a stainless-steel container and transferred into a 50 mL aluminum box containing 40 mL of water. The milled rice was cooked in an electric rice cooker (Z06YA3-S2, Supor, Hangzhou, China). After cooking, the sensory properties (palatability, hardness, stickiness, etc.) of the cooked rice were determined by a rice sensory analyzer.

2.7. Statistical Analysis

Microsoft Excel 2013 and SPSS 17.0 (SPSS, Chicago, IL, USA) were used to perform a statistical analysis of the data. A *t*-test was used to compare the means at a significance level of $p < 0.05$. The figures were prepared using Origin 8.5 (OriginLab, Hampton, MA, USA).

3. Results

3.1. Starch Granule Size Distribution

Starch granules obtained from rice grown under different grain-filling temperatures showed significant differences in size and distribution between UP and LP (Table 1). Compared to NT, ET showed a reduction trend in the proportions of small granules (≤2 μm) and medium-sized (2–5 μm) granules but exhibited a higher proportion of large-sized granules (≥5 μm) for both varieties. With regard to granule size, a high grain-filling temperature increased the average diameter of the starch granules by volume and surface area. For different grain positions, the proportion of large-sized granules (≥5 μm) and the average diameter of starch granules by volume and surface area were significantly greater in UP than those in LP under different dynamic temperatures. ET+LP treatment significantly increased the proportion of small granules (≤2 μm) compared to NT+LP treatment.

Table 1. Starch granule size and its distribution in different grain positions of rice under different grain-filling temperatures.

Variety	Treatment	Grain Position	Small-Sized Granules ≤2 μm (%)	Medium-Sized Granules 2–5 μm (%)	Large-Sized Granules ≥5 μm (%)	D[3,2] (μm)	D[4,3] (μm)
NJ 46	ET	UP	4.55 ± 0.03 d	28.18 ± 0.08 c	67.27 ± 0.11 a	4.94 ± 0.01 a	5.89 ± 0.00 a
		LP	5.43 ± 0.03 b	27.48 ± 0.13 d	67.09 ± 0.14 b	4.82 ± 0.01 b	5.80 ± 0.01 b
	NT	UP	6.24 ± 0.01 a	29.00 ± 0.07 b	64.76 ± 0.07 c	4.71 ± 0.00 c	5.64 ± 0.00 c
		LP	5.30 ± 0.01 c	31.32 ± 0.14 a	63.38 ± 0.08 d	4.62 ± 0.01 d	5.66 ± 0.01 c
SXJ 100	ET	UP	5.61 ± 0.07 b	32.39 ± 0.11 c	62.00 ± 0.18 a	4.64 ± 0.00 a	5.55 ± 0.00 a
		LP	5.58 ± 0.01 b	36.42 ± 0.16 b	58.01 ± 0.14 b	4.54 ± 0.01 b	5.40 ± 0.00 b
	NT	UP	6.11 ± 0.03 a	31.11 ± 0.07 c	62.78 ± 0.03 a	4.59 ± 0.02 ab	5.57 ± 0.01 a
		LP	5.48 ± 0.07 c	41.54 ± 0.14 a	52.98 ± 0.01 c	4.40 ± 0.00 c	5.18 ± 0.00 c

Values in the same column with different letters are significantly different ($p < 0.05$). NJ 46, Nangjing 46; SXJ 100, Suxiangjing 100; ET, elevated temperature; NT, normal temperature; UP, upper grains; LP, lower grains.

3.2. Starch Fine Structure

Table 2 shows the structural parameters of starch determined via SEC. The AM in UP was significantly higher under ET than under NT, but the opposite result was found in LP. Compared with those under NT treatment, the AP1 to AP ratio and the AP1 to AP2 ratio showed a reduction under ET; on the contrary, the AP2 to AP ratio increased. For different grain positions, the AP1 to AP ratio and the AP1 to AP2 ratio were lower in UP than those in LP under ET and NT treatment, but the AP2 to AP ratio showed the opposite tendency.

Table 2. SEC determination of changes in chain length distribution of the whole range of debranched starch in different grain positions of rice under different grain-filling temperatures.

Variety	Treatment	Grain Position	Peak Area (%)			AP1/AP2
			AM/AM+AP	AP1/AP	AP2/AP	
NJ 46	ET	UP	7.68 ± 0.32 a	74.67 ± 0.22 c	25.33 ± 0.18 a	2.96 ± 0.03 c
		LP	7.16 ± 0.41 b	76.24 ± 0.15 bc	23.76 ± 0.35 ab	3.21 ± 0.08 b
	NT	UP	7.09 ± 0.71 b	77.09 ± 0.27 b	22.91 ± 0.24 bc	3.37 ± 0.15 b
		LP	7.51 ± 0.29 a	79.57 ± 0.11 a	20.43 ± 0.31 c	3.92 ± 0.09 a
SXJ 100	ET	UP	8.32 ± 0.43 a	74.89 ± 0.03 b	25.11 ± 0.01 a	2.98 ± 0.11 c
		LP	6.54 ± 0.35 c	77.27 ± 0.18 ab	22.73 ± 0.34 ab	3.40 ± 0.08 bc
	NT	UP	7.45 ± 0.51 b	77.96 ± 0.37 ab	22.04 ± 0.29 ab	3.54 ± 0.02 ab
		LP	7.25 ± 0.67 b	78.21 ± 0.14 a	21.79 ± 0.17 b	3.59 ± 0.06 a

Values in the same column with different letters are significantly different ($p < 0.05$). NJ 46, Nangjing 46; SXJ 100, Suxiangjing 100; ET, elevated temperature; NT, normal temperature; UP, upper grains; LP, lower grains.

3.3. Starch Thermal Properties

The starch thermal properties of rice exposed to different dynamic temperatures were analyzed through use of DSC. The DSC parameters are shown in Table 3. The gelatinization temperatures (T_o, T_p, and T_c) in UP increased significantly for NJ 46 under ET compared to NT. However, LP showed a significantly lower T*c* gelatinization temperature than UP for both varieties under ET conditions. Gelatinization enthalpy (ΔH_{gel}) in UP increased significantly under ET than that under NT, but there was no significant difference in LP between ET and NT. Hence, the gelatinization temperatures (T_o, T_p, and T_c) in UP were higher than those in LP under different treatment conditions. And UP had a higher gelatinization enthalpy (ΔH_{gel}) than LP.

Table 3. Thermal properties of starch in different grain positions of rice under different grain-filling temperatures.

Variety	Treatment	Grain Position	*To* (°C)	*Tp* (°C)	*Tc* (°C)	Δ*H*gel (J/g)
NJ 46	ET	UP	61.0 ± 1.08 a	68.6 ± 0.22 a	81.8 ± 1.21 a	9.93 ± 0.34 a
		LP	59.8 ± 2.14 ab	68.0 ± 0.35 a	77.8 ± 0.58 b	6.53 ± 0.54 b
	NT	UP	58.9 ± 0.18 b	66.1 ± 0.14 b	74.5 ± 2.11 c	7.44 ± 0.02 b
		LP	59.9 ± 1.22 ab	66.5 ± 0.06 b	73.1 ± 0.32 c	6.92 ± 0.41 b
SXJ 100	ET	UP	61.4 ± 1.23 a	69.4 ± 1.02 a	82.8 ± 0.25 a	7.09 ± 0.24 a
		LP	61.2 ± 2.51 a	69.6 ± 0.35 a	77.3 ± 0.12 b	5.52 ± 1.12 b
	NT	UP	61.9 ± 1.64 a	67.3 ± 0.07 b	78.7 ± 0.04 b	6.91 ± 0.26 ab
		LP	61.5 ± 0.55 a	66.9 ± 0.31 b	78.0 ± 0.12 b	4.51 ± 0.35 b

Values in the same column with different letters are significantly different ($p < 0.05$). NJ 46, Nangjing 46; SXJ 100, Suxiangjing 100; ET, elevated temperature; NT, normal temperature; UP, upper grains; LP, lower grains.

3.4. Grain Eating Quality

The evaluation parameter of rice grain eating quality was measured using the taste analyzer (Table 4). Compared to NT treatment, the palatability of cooked rice decreased significantly under ET treatment. Moreover, we observed higher hardness and lower appearance, stickiness, and balance in rice cultivated under ET conditions. For different grain positions, the palatability was higher in UP than in LP under NT treatment. However, there was no significant difference between UP and LP under ET treatment. Further analysis showed that UP had a higher appearance, stickiness, balance, and lower hardness than LP, except for ET treatment. Hence, a higher grain filling temperature could mitigate the eating quality deterioration of rice grain in LP.

Table 4. Grain eating quality in different grain positions of rice under different grain-filling temperatures.

Variety	Treatment	Grain Position	Appearance	Hardness	Stickiness	Balance	Palatability
NJ 46	ET	UP	6.30 ± 0.21 b	7.27 ± 0.07 a	5.70 ± 0.28 c	5.77 ± 0.21 b	64.67 ± 0.00 b
		LP	6.30 ± 0.28 b	7.03 ± 0.14 a	5.87 ± 0.07 c	5.77 ± 0.00 b	66.00 ± 0.71 b
	NT	UP	8.27 ± 0.07 a	5.05 ± 0.14 c	7.77 ± 0.00 a	8.20 ± 0.14 a	79.33 ± 1.41 a
		LP	7.63 ± 0.00 a	5.87 ± 0.00 b	7.07 ± 0.00 b	7.47 ± 0.42 a	70.33 ± 0.07 b
SXJ 100	ET	UP	5.60 ± 0.07 d	6.87 ± 0.42 a	5.00 ± 0.14 b	5.17 ± 0.00 c	63.33 ± 0.71 b
		LP	7.57 ± 0.14 b	6.43 ± 0.42 a	5.87 ± 0.42 b	6.37 ± 0.21 b	66.00 ± 1.41 b
	NT	UP	8.50 ± 0.00 a	5.40 ± 0.00 b	7.73 ± 0.00 a	8.30 ± 0.07 a	80.67 ± 0.07 a
		LP	6.50 ± 0.21 c	6.43 ± 0.07 a	5.40 ± 0.14 b	6.20 ± 0.28 b	65.00 ± 0.00 b

Values in the same column with different letters are significantly different (p < 0.05). NJ 46, Nangjing 46; SXJ 100, Suxiangjing 100; ET, elevated temperature; NT, normal temperature; UP, upper grains; LP, lower grains.

4. Discussion

Rice starch's fine structure and function are markedly influenced by ripening temperature [10]. Previous studies reported that high temperatures during grain filling decreased amylose content and amylopectin short-chain content but increased amylopectin long-chain content, which led to high gelatinization enthalpy and temperature [23,24]. In this study, we found that high grain-filling temperatures reduced amylose content, the AP1 to AP ratio, and the AP1 to AP2 ratio and increased the AP2 to AP ratio (Table 2). The starch gelatinization enthalpy and temperature were greater in ET than those in LT (Table 3). Hence, our results are consistent with previous studies in that rice high grain filling temperatures could reduce amylose content and amylopectin short-chain content but enhance amylopectin long-chain content, resulting in deteriorating starch thermal properties [23,24]. For different grain positions, there is a significant difference in starch granule size, structure, and function between superior grains and inferior grains [6–9]. In this study, UP had a significantly greater proportion of large-sized granules and average diameter of starch granules by volume and surface area than LP under different dynamic temperatures. Zhu et al. (2021) also reported that rice superior grains had a larger granule size than inferior grain, and higher amylose content and amylopectin long-chain content were found in superior grains [8]. In this study, compared with LP, UP had greater amylose content and higher amylopectin long-chain content under ET treatment, which led to higher gelatinization temperatures and gelatinization enthalpy. Therefore, high grain filling temperatures could improve the starch fine structure and gelatinization properties of rice grains in the lower panicle position.

Eating quality is the most important and core index in rice grain quality, which is related to starch structure and function. Previous reports reported that lower amylose content and gel temperature and higher amylopectin shorter chain content and breakdown were beneficial to improving eating quality in rice [25–27]. Rice eating quality is often evaluated by manual or machine evaluation, and high eating quality shows lower hardness and a higher appearance, stickiness, and palatability [28]. In this study, higher grain filling temperatures increased hardness and decreased the appearance, stickiness, and palatability of rice grain, indicating that ET treatment deteriorated the eating quality of rice grain compared with NT. For different grain positions, we found that UP had a higher eating quality than LP under NT treatment; however, there was a comparable eating quality between UP and LP under ET treatment, resulting in higher appearance, stickiness, balance, and lower hardness in LP under ET treatment. The high grain taste had lower amylose content and a higher proportion of shorter amylopectin chains, which reduced the starch gelatinization temperatures and enthalpy, decreased the hardness, and increased the adhesiveness of cooked rice [29]. In this study, we also found lower amylose content and a higher ratio of shorter to longer amylopectin chains in LP under ET treatment, leading to lower starch gelatinization temperatures and enthalpy. Hence, higher grain filling temperatures mitigated the eating quality deterioration of rice grain in LP due to the improvement of starch fine structure and properties.

5. Conclusions

ET decreased the proportion of shorter amylopectin chains and increased the proportion of long amylopectin chains compared with NT, resulting in a higher starch gelatinization temperature and enthalpy, which led to the deterioration of eating quality. For grain position, compared to LP, UP had higher amylose content and a higher proportion of long amylopectin chains, leading to higher gelatinization enthalpy under ET. UP had a higher eating quality than LP under NT; however, there was a comparable eating quality between UP and LP under ET. Hence, higher grain filling temperatures mitigated the eating quality deterioration of rice in LP.

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