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Effects of Reduced Nitrogen Fertilization and Irrigation on Structure and Physicochemical Properties of Starch in Two Bread Wheat Cultivars

Jingyang Tong ¹, Shujun Wang ², Zhonghu He ^{1,3} and Yan Zhang ^{1,*}

¹ National Wheat Improvement Center, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China; tongjingyang03@gmail.com (J.T.); hezhonghu02@caas.cn (Z.H.)

² State Key Laboratory of Food Nutrition and Safety, Tianjin University of Science & Technology, Tianjin 300457, China; sjwang@tust.edu.cn

³ International Maize and Wheat Improvement Center (CIMMYT) China Office, Beijing 100081, China

* Correspondence: zhangyan07@caas.cn; Tel./Fax: +86-010-82108741

Abstract: Nitrogen (N) fertilization and irrigation are significant agronomic factors affecting wheat production, but little information is available on the effects of reduced N fertilization and irrigation on internal starch structure and physicochemical properties associated with the quality of wheat-based foods. In this study, reduced N fertilization and irrigation were separately applied to investigate their effects on composition and morphological changes, crystalline and external region structure features, swelling power, and gelatinization characteristics of starch granules in bread wheat, with a high N-use-efficiency and water-saving wheat cultivar Zhongmai 175 and a widely grown cultivar Jingdong 17. Compared with a non-N control, reduced N fertilization did not change the crystallinity type and short-range ordered degree of starch; however, it significantly increased relative crystallinity, swelling power and gelatinization enthalpy, whereas amylose content and transition temperatures were decreased. Under reduced irrigation, more small starch granules with compact arrangements appeared in comparison with non-water control. Relative crystallinity, swelling power and gelatinization enthalpy of starch were increased, whereas short-range ordered degree and transition temperatures were decreased. Moreover, the starch of the two cultivars appeared to differ in response to both the N and water treatments. The findings indicated that reduced N fertilization or irrigation markedly influenced the structure and physicochemical characteristics of wheat starch, providing important information for developing elite cultivars with high N and water use efficiency and outstanding starch quality.

Keywords: starch structure; wheat starch; wheat quality



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1. Introduction

Bread wheat (*Triticum aestivum* L.) is the most widely planted cereal crop and wheat-based foods are major constituents of the human diet worldwide [1]. The most plentiful component of wheat grain is starch, which accounts for about 70% of the endosperm as well as 75% of flour dry weight. It not only provides calories for energy but also plays an essential role in industry [2]. Wheat starch granules are natural semicrystalline aggregates primarily composed of two types of glucose homopolymers: basically, linear amylose and highly branched amylopectin. Amylose mainly constitutes amorphous regions, whereas crystalline/ordered domains mainly consist of amylopectin [3]. The relative contents of amylose and amylopectin are a key factor influencing morphological, internal structure and physicochemical properties of starch granules, including relative crystallinity, swelling power, and thermal and gelatinization properties [4–6]. The structure and physicochemical characteristics of starch are responsible for noodle-making quality [7]. Recent studies reported starch properties might also affect dough and bread behaviors [8,9]. Among them,

amylose content, relative crystallinity and short-range ordered degree of starch granules can affect molecule interactions in wheat dough and are further suggested to be indicators for better evaluation of starch and quality [8]. Amylopectin content, the number of small starch granules, swelling power and gelatinization temperature are reported to positively influence the total scores of noodle and bread quality [9].

Wheat grain quality is determined by both cultivar genotype and environmental factors, including cultural management. Fertilization and irrigation are fundamental practices greatly affecting grain yield and protein quantity and quality [10,11]. Nitrogen (N) is the basic component of living organisms and N fertilizer is essential for optimum grain yield and protein content [12]. N fertilizers influence the activity of amylolytic and starch biosynthesis enzymes in seeds and affect the accumulation and size distribution of starch granules [13–16]. Li et al. [17] found high N and sulfur (S) fertilization promoted the development of small starch granules during grain filling and increased the proportion of small starch granules in mature grains. High levels of N fertilization significantly changed the physicochemical properties of resistant starch in wheat [18]. Numerous research studies were performed on effects of N fertilization, especially high N, on grain yield, protein quality and accumulation characteristics and size distributions of starch granules. However, little information is available concerning the effects of reduced N fertilization on internal structure and physicochemical properties of starch granules in wheat, including structural features of crystalline and external regions related to gelatinization characteristics and pasting quality.

Reduced irrigation has become a common practice in northern China in order to meet the challenge of increasing water shortage. Previous reports indicated that overall irrigation significantly increased the activity of enzymes involved in grain starch synthesis during the later stage of grain filling, thereby accelerating starch accumulation [19,20]. These studies suggested that water availability similarly affected enzyme activity. No irrigation applied during the whole growing season affected the size distributions of starch granules relative to irrigated controls [21]. Irrigation diminished the volume and percentage surface area of small starch granules [22]. Although the influence of water treatments on wheat starch accumulation is well documented, studies on changes in internal structure and physicochemical properties of starch in response to water treatment, especially reduced irrigation, are not available.

N fertilization and irrigation are both essential agronomic measures for wheat production. However, no or reduced N fertilization and irrigation have been recommended in the North China Plain as measures to reduce production costs and protect the environment. High N-use-efficiency (NUE) and water-saving wheat cultivars will help to achieve this objective. In the current study, no and reduced N fertilization (80 kg·ha⁻² N as the base fertilizer and 120 kg·ha⁻² N at the jointing stage), no and reduced irrigation (only twice at the jointing and anthesis stage, with 75 mm of rainfall equivalent each time) were separately conducted to investigate their effects on composition and morphological changes, crystalline and external region structure features using two wheat cultivars, Zhongmai 175 with high NUE and high water-use efficiency (WUE) and Jingdong 17, a widely grown cultivar. The objective of the research was to obtain information for developing cultivars with high N and water use efficiency and superior starch quality.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

Zhongmai 175 and Jingdong 17 have similar maturity. Zhongmai 175 is the most widely grown cultivar in the northern winter wheat region of China and is also widely cultivated in Huang-Huai dry land areas [23].

Experiments were carried out at Shunyi Experiment Station in Beijing (116°35' E, 40°16' N) during the 2014–2015 growing season. The soil type was a loam and the depth of ground water was more than 7 m. Before sowing, the organic matter, total N, hydrolyzed N, available phosphorous (P) and available potassium (K) of the soil at 0–20 cm were

determined to be $11.6 \text{ g}\cdot\text{kg}^{-1}$, $0.96 \text{ g}\cdot\text{kg}^{-1}$, $89.8 \text{ mg}\cdot\text{kg}^{-1}$, $29.1 \text{ mg}\cdot\text{kg}^{-1}$ and $163.6 \text{ mg}\cdot\text{kg}^{-1}$, respectively. The precipitation of the wheat growing season in 2014–2015 was 127.0 mm, which was not sufficient for high yield. For the N fertilizer treatment, Trial I had three replications in complete randomized blocks. The treatment plots received $80 \text{ kg}\cdot\text{ha}^{-2}$ N as the base fertilizer and $120 \text{ kg}\cdot\text{ha}^{-2}$ N at jointing. The control plots received nil N during the whole growing season. Irrigation for both control and treatment plots was conducted at the jointing stage and anthesis stage. Trial II relating to irrigation treatment also involved three replications in complete randomized blocks. The treatment plots were irrigated once at jointing and once anthesis stages, with 75 mm of rainfall equivalent each time. Both control and treatment plots received $80 \text{ kg}\cdot\text{ha}^{-2}$ of N as base fertilizer and $40 \text{ kg}\cdot\text{ha}^{-2}$ N at jointing. Trials I and II were sown September 28, 2014, with plot areas of 29.7 m^2 , row spacing of 24 cm, and targeted seedling numbers of 3×10^6 /ha. There were 4 m intervals between plots. All other field management practices, including weeding and pest control, were uniform and followed local recommendations.

Plots were harvested at maturity; sun-dried and seeds were stored for subsequent analysis. Grain hardness was determined on 300-kernel samples with a Perten Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Springfield, IL, USA). Grain samples were tempered overnight to achieve 14.5% moisture before grinding using Brabender Quadrumat Junior mill (Brabender Inc., Duisberg, Germany). Flour samples were sifted by a sieve and then saved as backup.

2.2. Isolation of Starch and Determination of Amylose Content

Wheat starch was isolated according to Zhang et al. [24]. Flour samples (6 g) and distilled water (4 g) were mixed to generate dough. After 10 min, the dough samples were washed with 60 mL distilled water to remove glutes. In order to ensure the whole starch was completely separated, the gluten was washed twice with 20 mL water. The homogenated starch was filtered through a nylon cloth (75 μm mesh). The resultant starch samples were centrifuged at $2500 \times g$ for 15 min, and the supernatant was discarded. The upper gray-colored tailings of precipitate were shifted to new tubes. Water was added to the lower light-colored precipitate and the homogenates were centrifuged again. To remove the gray-colored tailings completely, these steps were repeated. The collected tailings from each repeat were resuspended and centrifuged again with the top layer discarded as described above. The pelleted starch from the above steps was collected and freeze-dried until used. The starch was ground lightly with a mortar and finally collected with a 100-mesh sieve. An automated chemistry analyzer (FS3100, OI Analytical, College Station, TX, USA) was used to determine amylose content following Zhang et al. [24].

2.3. Morphological Observation of Starch Granules

Starch samples were mounted on the stub using double-sided, carbon-coated adhesive tape before coating with gold. A scanning electron microscope (SU1510, Hitachi High-technologies Corporation, Tokyo, Japan) was used for imaging at an accelerating voltage of 10.0 kV.

2.4. X-ray Diffraction (XRD) Analysis

Starch crystallinity was determined with a Panalytical X'Pert Pro X-ray diffractometer (PANalytical, Almelo, The Netherlands) with a $\text{Co-K}\alpha$ source [$\lambda = 0.1789 \text{ nm}$]. Starch samples were scanned from 4° to 40° (2θ) at 45 kV and 35 mA. Relative crystallinity was calculated as the ratio of the crystalline area to the total area ranging from 4° to 40° (2θ) using Origin software (ver. 7.5, Microcal Inc., Northampton, MA, USA) [25].

2.5. Fourier Transformed Infrared (FT-IR) Analysis

Short-range order of starches was measured using a Tensor 27 FT-IR spectrometer (Bruker, Karlsruhe, Germany) equipped with a DLATGS detector. The sample preparation

and operating procedure were described by Wang et al. [26]. The ratio of absorbance at 1045/1022 cm^{-1} was calculated to determine the short-range order of starch.

2.6. Determination of Swelling Power

The swelling power of starch samples was determined according to Wang and Copeland [27] with slight modifications. Starch samples (90–100 mg) (m_1) were added to a 10 mL centrifuge tube; ultrapure water (3 mL) was added and the contents were evenly mixed. The tube was heated in a water bath at 90 °C for 1 h and centrifuged at $4000 \times g$ for 5 min. The supernatant was discarded and the tube containing the sediment sample was weighed (m_2). The swelling power was calculated as follows:

$$\text{Swelling power} = (m_2 - m_1)/m_1 \text{ (g/g)} \quad (1)$$

2.7. Determination of Thermal Properties

The thermal properties of starch were measured using a scanning calorimeter (DSC 200 F3, NETZSCH, Selb, Germany) as reported by Wang and Copeland [28]. Onset (T_o), peak (T_p), and concluding (T_c) temperatures, and enthalpy change (ΔH_{gel}) were calculated.

2.8. Statistical Analyses

Data are shown as means of three replications \pm standard deviation. Statistical analyses were conducted with SAS v9.1. (SAS Institute, Cary, NC, USA) ANOVA was applied, followed by Duncan's multiple range tests to investigate significant differences ($p < 0.05$) among measured parameters. Pearson's correlation analysis was conducted to determine relationships between investigated starch properties.

3. Results and Discussion

3.1. Effects of Reduced N Fertilization and Irrigation on Starch Composition

With reduced N the amylose content of Zhongmai 175 and Jingdong 17 decreased from 30.78% to 29.96% and from 29.97% to 29.48%, respectively (Table 1), indicating that N fertilization affected the composition of starch in both cultivars, similar to Li et al. [17] who conducted high N fertilization and both studies showed that low amylose content was associated with the applications of N fertilizers. Given that N applied at later growth stages could improve the activity of starch-branching enzymes in cereal grain [29,30], the decreased amylose content was attributed to enhanced starch-branching enzyme activity causing synthesis of high-amylopectin starch. Moreover, higher amounts of amyloplast mainly composed of protein were generated with N fertilization and could increase the total starch and amylopectin content, leading to decreased amylose content [15].

Table 1. Amylose content, relative crystallinity, short-range ordered degree (1045/1022) and swelling power of starch from Zhongmai 175 and Jingdong 17 at non-nitrogen (NNT), reduced nitrogen (NT), non-water (NWT), and reduced water (WT) treatments ^{a,b}.

Trial	Cultivar	Amylose Content (%)	Relative Crystallinity (%)	1045/1022 cm^{-1}	Swelling Power ($\text{g} \cdot \text{g}^{-1}$)
I	Zhongmai175-NNT	30.78 \pm 0.03 ^a	19.40 \pm 0.00 ^a	0.69 \pm 0.00 ^a	7.30 \pm 0.40 ^a
	Zhongmai175-NT	29.96 \pm 0.09 ^b	18.67 \pm 0.12 ^a	0.68 \pm 0.00 ^a	7.20 \pm 0.50 ^a
	ANOVA (<i>p</i> -value)	<0.010 **	0.067 ns	0.116 ns	0.338 ns
	Jingdong17-NNT	29.97 \pm 0.10 ^a	19.93 \pm 0.47 ^b	0.67 \pm 0.00 ^a	7.50 \pm 0.10 ^b
	Jingdong17-NT	29.48 \pm 0.04 ^b	21.40 \pm 0.42 ^a	0.66 \pm 0.00 ^a	8.30 \pm 0.20 ^a
	ANOVA (<i>p</i> -value)	<0.010 **	<0.010 **	0.137 ns	<0.010 **

Table 1. Cont.

Trial	Cultivar	Amylose Content (%)	Relative Crystallinity (%)	1045/1022 cm^{-1}	Swelling Power ($\text{g} \cdot \text{g}^{-1}$)
II	Zhongmai175-NWT	29.69 \pm 0.27 ^a	18.77 \pm 0.54 ^b	0.68 \pm 0.00 ^a	6.80 \pm 0.10 ^b
	Zhongmai175-WT	29.75 \pm 0.07 ^a	19.70 \pm 0.42 ^a	0.65 \pm 0.01 ^b	8.60 \pm 0.10 ^a
	ANOVA (<i>p</i> -value)	0.213 ns	0.044 *	<0.010 **	<0.010 **
	Jingdong17-NWT	30.10 \pm 0.25 ^a	20.70 \pm 0.00 ^b	0.68 \pm 0.00 ^a	7.80 \pm 0.80 ^b
	Jingdong17-WT	30.31 \pm 0.28 ^a	21.77 \pm 0.18 ^a	0.67 \pm 0.00 ^a	8.60 \pm 0.10 ^a
	ANOVA (<i>p</i> -value)	0.435 ns	<0.010 **	0.126 ns	<0.010 **

^a Different letters show significant differences ($p < 0.05$). ^b ANOVA *p*-value is shown and asterisks mean significant effects: *, $p < 0.05$; **, $p < 0.01$; ns, not significant.

The different water treatments led to no marked change in amylose content in grain from either cultivar (Table 1). This result differed from those of Yu et al. [31] where irrigation level significantly affected amylose content in wheat grain. In this study, we conducted reduced irrigation and non-irrigation as control during the whole growing season in the field, whereas Yu et al. [31] performed sufficient watering and drought stress simulation during different wheat growth stages, indicating that the differing results were likely due to different water treatments and different genotypes.

3.2. Effects of Reduced N Fertilization and Irrigation on the Morphology of Starch Granules

Data for starch granule morphology of the two cultivars subjected to the various treatments are shown in Figure 1. There were some differences between the cultivars. Both Zhongmai 175-reduced nitrogen treatment (NT) and Jingdong 17-NT had irregular and elongated large granules compared with the regular and spherical starch granules in the non-nitrogen (NNT) treatments. According to Zhu et al. [6], the amylose content contributed to diverse morphologies of starch granules, the differences in the morphology of starch granules may be attributed to the altered content of amylose with small size in N-treated cultivars (Table 1). The amount of medium and small starch granules was higher in the NT than in the NNT treatments. This was consistent with previous studies showing that high N fertilization caused high amylolytic activity leading to synthesis of small starch granules, and eventually a higher proportion of small starch granules in the mature grains [15,17]. Irrigation changed the morphology of starch granules in both cultivars. The reduced water (WT) treatments led to higher proportions of more small starch granules exhibiting more ellipsoidal and irregular shape than the NWT treatment. In addition, greater numbers of micropores were present on the uneven, pitted surfaces of starch granules in the Jingdong 17-NT and Zhongmai 175-WT treatments. This could be explained by increased activity of α -amylase promoting hydrolysis of starch to form pores [32].

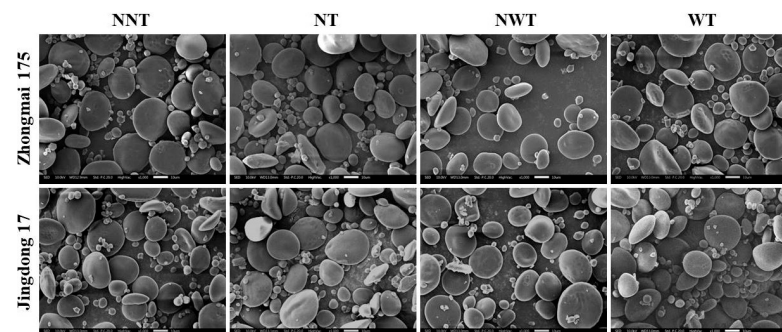


Figure 1. Scanning electron microscope images (1000 \times magnification) of starch granules isolated from Zhongmai 175 and Jingdong 17 subjected to non-nitrogen (NNT), reduced nitrogen (NT), non-water (NWT), and reduced water (WT) treatments.

3.3. Effects of Reduced N Fertilization and Irrigation on the Crystalline Structure of Starch

XRD patterns of starch samples are presented in Figure 2A,B. Native starches are classified into A-, B- and C-types based on XRD pattern [33]. In the present study, all starches display the characteristics of A-type, with strong peaks at 15° and 23° and an unresolved doublet at 17° and 18°, indicating that reduced N treatment and irrigation has little influence on crystallinity type. Although no significant variation in crystal type was caused by different treatments, there were differences in relative crystallinity between the reduced and non-treatments (Table 1). The relative crystallinity of starch in Jingdong 17-NT (21.40%) was significantly higher than that in Jingdong-NNT (19.93%). Starch composition is the main factor affecting starch crystallinity; that is, the crystalline packaging of amylopectin chains is prone to suffer from amylose and be disrupted, and high amylose content can lead to low relative crystallinity [33]. The decreased amylose content caused by reduced N treatment in Jingdong 17-NT might be responsible for higher relative crystallinity compared with the control.

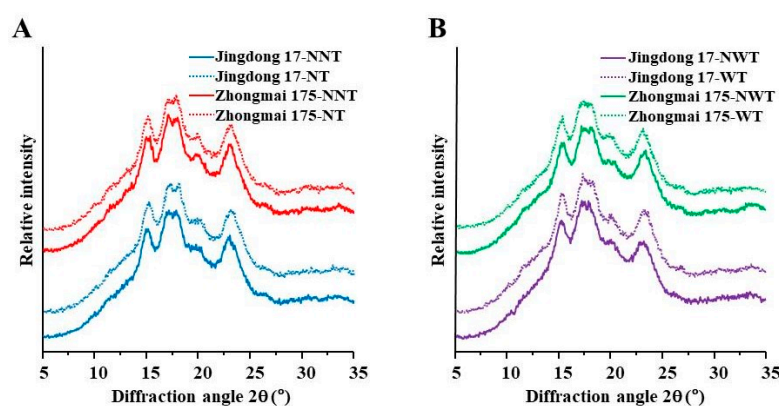


Figure 2. X-ray diffraction spectra of starch granules extracted from Zhongmai 175 and Jingdong 17 at non-nitrogen (NNT) and reduced nitrogen treatments (NT) (A), non-water (NWT) and reduced water (WT) treatments (B).

After reduced irrigation, the relative crystallinity of starch distinctly increased relative to non-irrigation in both cultivars (Table 1); this might be attributed to the increased proportion of small granules (Figure 1), leading to increased amylopectin with its branch-chains contributing to crystal structure [34]. The finding is in agreement with Zeng et al. [35] who found that small starch granules generally caused sharper intensity in XRD pattern and higher relative crystallinity than large granules. Thus, in the present study, reduced irrigation led to increased relative crystallinity through promoting formation of small starch granules.

3.4. Effects of Reduced N Fertilization and Irrigation on Short-Range Molecular Order of Starch

The order degree of starch at a short-range near the granule surface can be determined by ATR-FTIR and the spectrum of tested starch is presented in Figure 3A,B. The 1045/1022 cm^{-1} ratio is shown in Table 1. There was no significant difference between the N-treated cultivars and the control, indicating that reduced N fertilization hardly affected the short-range ordered degree of starch in wheat. However, a considerable decline is observed in 1045/1022 cm^{-1} ratio in Zhongmai 175-WT starch, illustrating that reduced irrigation decreased the order degree at the short-range scale of wheat starch. As a higher value of relative crystallinity has been suggested to generally associate with a lower 1045/1022 cm^{-1} ratio in rice starch [6], it can be implied that significantly increased relative crystallinity of starch after irrigation contributed to the decreased 1045/1022 cm^{-1} ratio. Besides, more small starch granules adhered to the surface of each other (Figure 1) tend to change the ratio of 1045/1022 cm^{-1} related to the structural order of the starch external region, in agreement with Cai et al. [36], in which regular maize starches with more small

granules appeared to have a higher 1045/1022 cm^{-1} ratio, suggesting that the degree of short-range order could be affected by granule size. Hence, with regard to short-range molecular order, we found that reduced N fertilization almost had no influence on this characteristic. In contrast, reduced irrigation decreased the degree of short-range order, affecting starch hydrolysis, digestion, and gelatinization properties [37,38]. Low ability of starch hydrolysis and digestion can improve the nutritional quality of wheat-based food products [39]. Given that gelatinization conclusion temperature is an indicator affecting noodle and bread scores, the changed short-range molecular order of starch could also have an impact on them [9].

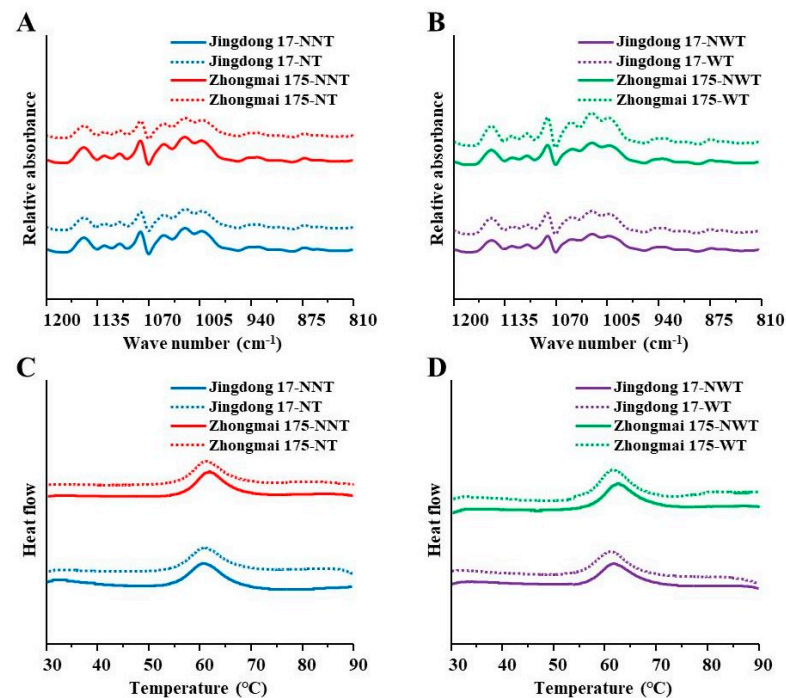


Figure 3. ATR-FTIR and DSC analyses of starch granules. Spectra of ATR-FTIR analysis of starch extracted from Zhongmai 175 and Jingdong 17 at non-nitrogen (NNT) and reduced nitrogen (NT) treatments (A), non-water (NWT) and reduced water (WT) treatments (B); DSC thermograms of gelatinization properties for starch extracted from Zhongmai 175 and Jingdong 17 at NNT and NT (C), NWT and WT (D).

3.5. Effects of Reduced N Fertilization and Irrigation on the Swelling Power of Starch

With reduced N treatment, Jingdong 17 had significantly higher starch swelling power ($8.30 \text{ g}\cdot\text{g}^{-1}$) than the nil treatment ($7.50 \text{ g}\cdot\text{g}^{-1}$) (Table 1). The main factor determining swelling power is amylose: amylopectin ratio. As the amylose and long amylopectin branch-chains tend to inhibit the swelling of starch granules and maintain the swollen structures, starch with low amylose content generally presents high swelling power [40]. This could be the reason why Jingdong 17-NT with changed amylose content has discrepant swelling power compared to Jingdong 17-NNT. Moreover, compared with large starch granules, small granules have much more short amylopectin branch-chains, which improves water absorbing capacity and swelling power [41]. Hence, the increased amount of small starch granules (Figure 1) might also contribute to the significantly higher swelling power in Jingdong 17-NT compared with Jingdong 17-NNT. Similarly, with water treatment, the obviously improved starch swelling power in both cultivars (Table 1) can be attributed to the presence of a higher proportion of small granules compared with the nil treatments (Figure 1). Our results are in conformity with Li et al. [17] who concluded that small starch granules have higher swelling power than large particles. Additionally, it is interesting to find that with irrigation both Zhongmai 175 and Jingdong 17 had significantly higher starch

swelling power, which is associated with improved quality of wheat-based food products such as noodle and bread [9,42,43]. The results indicate that reduced N fertilization and irrigation could affect noodle and bread qualities contributed by different starch functions between two cultivars.

3.6. Effects of Reduced N Fertilization and Irrigation on the Thermal Properties of Starch

The DSC thermograms of tested starch samples are presented in Figure 3C,D and gelatinization transition temperatures (onset (T_o), peak (T_p) and concluding (T_c) temperatures) and gelatinization enthalpies (ΔH_{gel}) are given in Table 2. T_o , T_p and T_c of starch were significantly lower in Zhongmai 175-NT after N fertilization, whereas ΔH_{gel} was distinctly raised. Low T_o , T_p , and T_c indicate that the starch has a low pasting temperature and is prone to be gelatinized when cooked [2]. A higher ΔH_{gel} resulting from N treatment indicates more energy is required to disrupt the crystalline double helices and a longer pasting time. The main factor determining ΔH_{gel} is the degree of crystallinity; higher relative crystallinity provides more structural stability by enhancing the resistance of starch granules to gelatinization [44]. Moreover, as crystalline regions mainly consist of amylopectin, high amylopectin content helps to hinder gelatinization and increases the melting point of crystalline domains and ΔH_{gel} [45]. Thus, the higher ΔH_{gel} of starch in Zhongmai 175-NT may be related to the different amylose content adjusted by N treatment.

Table 2. Thermal properties of starch from Zhongmai 175 and Jingdong 17 at non-nitrogen (NNT), reduced nitrogen (NT), non-water (NWT), and reduced water (WT) treatments ^{a,b}.

Trial	Cultivar	T_o (°C) ^b	T_p (°C) ^b	T_c (°C) ^b	ΔH_{gel} (J/g) ^c
I	Zhongmai175-NNT	56.50 ± 0.08 ^a	61.27 ± 0.04 ^a	66.97 ± 0.04 ^a	9.70 ± 0.14 ^b
	Zhongmai175-NT	55.86 ± 0.16 ^b	60.73 ± 0.04 ^b	66.20 ± 0.00 ^b	10.30 ± 0.28 ^a
	ANOVA (<i>p</i> -value)	<0.010 **	<0.010 **	<0.010 **	0.031 *
	Jingdong17-NNT	55.20 ± 0.08 ^a	60.30 ± 0.24 ^a	66.50 ± 0.21 ^a	9.20 ± 0.53 ^a
II	Jingdong17-NT	55.43 ± 0.20 ^a	60.56 ± 0.23 ^a	66.56 ± 0.09 ^a	9.10 ± 0.14 ^a
	ANOVA (<i>p</i> -value)	0.210 ns	0.330 ns	0.710 ns	0.811 ns
	Zhongmai175-NWT	56.80 ± 0.14 ^a	61.50 ± 0.14 ^a	67.33 ± 0.18 ^a	9.00 ± 0.00 ^b
	Zhongmai175-WT	56.10 ± 0.28 ^b	60.93 ± 0.09 ^b	66.63 ± 0.18 ^b	9.67 ± 0.04 ^a
II	ANOVA (<i>p</i> -value)	0.016 *	<0.010 **	0.021 *	<0.010 **
	Jingdong17-NWT	55.80 ± 0.43 ^a	60.80 ± 3.32 ^a	66.97 ± 0.41 ^a	9.10 ± 0.08 ^a
	Jingdong17-WT	55.67 ± 0.30 ^a	60.67 ± 0.09 ^a	66.90 ± 0.21 ^a	9.63 ± 0.41 ^a
	ANOVA (<i>p</i> -value)	0.741 ns	0.609 ns	0.849 ns	0.152 ns

^a Different letters show significant differences ($p < 0.05$). ^b ANOVA *p*-value is shown and asterisks mean significant effects: *, $p < 0.05$; **, $p < 0.01$; ns, not significant. ^c T_o : onset temperature; T_p : peak of gelatinization temperature; T_c : conclusion temperature; ΔH_{gel} : gelatinization enthalpy.

Under reduced irrigation, T_o , T_p , and T_c of starch significantly decreased in Zhongmai 175-WT, whereas ΔH_{gel} distinctly increased. This result resembles that at reduced N treatment, which can be attributed to the improved relative crystallinity of starch caused by water treatment. As reported by Sasaki and Matsuki [46], high ΔH_{gel} was generally coupled with high swelling power, and this is also verified in the present study that Zhongmai 175-WT has high values for swelling power and ΔH_{gel} . Besides, it is notable to find that with either N or water treatment, the starch from Jingdong 17 had no significant change in the thermal properties, however, Zhongmai 175 performed different. The different responses between two cultivars are mainly due to the distinguished genetic backgrounds and performance, that is, Zhongmai 175 has high NUE and WUE, whereas Jingdong 17 shows average [23].

3.7. Correlation Analyses between Starch Properties

The relationships between starch physicochemical characteristics are shown in Figure 4. Swelling power is positively correlated with relative crystallinity, but is negatively correlated with the ratio of 1045/1022 cm^{-1} . The results indicate that the increased relative

crystallinity caused by reduced N and irrigation leads to the improved ability of starch to bind with water, associated with improved quality of wheat-based foods, such as textural quality of noodles and shelf life of bread [9]. Amylose content is seen as not significantly correlated with other parameters of starch, which is different from the results that starch characteristics were significantly influenced by amylose content in rice [6]. One possible reason is that we used two different wheat genotypes with distinguished responses to N and water treatments, and different levels of N or irrigation could also affect the results [18]. Although there is no statistical difference, positive relationship between amylose content and short-range order is found ($r = 0.639$), and they could have an impact on noodle behaviors [9]. Taken together, reduced N fertilization decreased amylose content but increased relative crystallinity, swelling power and gelatinization enthalpy of starch, possibly leading to improved quality of wheat-based foods, such as textural quality of noodles and storage quality of bread. Reduced irrigation decreased the degree of short-range order and gelatinization temperatures of starch, while relative crystallinity and swelling power were increased; this was positively associated with superior sensory quality of noodles.

In the current study, we demonstrated that reduced N fertilization and irrigation affected the structure and physicochemical properties of wheat starch compared to no applied N or water. As recent studies have reported that in natural wheat flour, the functional characteristics of starch might be responsible for dough rheological properties, noodle and bread qualities [8,9], we propose that different N fertilization and irrigation management techniques had an impact on the quality of wheat flour-based food products. Considering a pivotal role of N fertilization and irrigation measures in wheat production, this study provided important information for breeding wheat cultivars with high NUE and WUE and outstanding starch quality. Further research could be conducted with diverse cultivars to explore the effects of different N fertilization and irrigation levels on wheat starch quality combined with yield and grain protein content during multiple seasons.

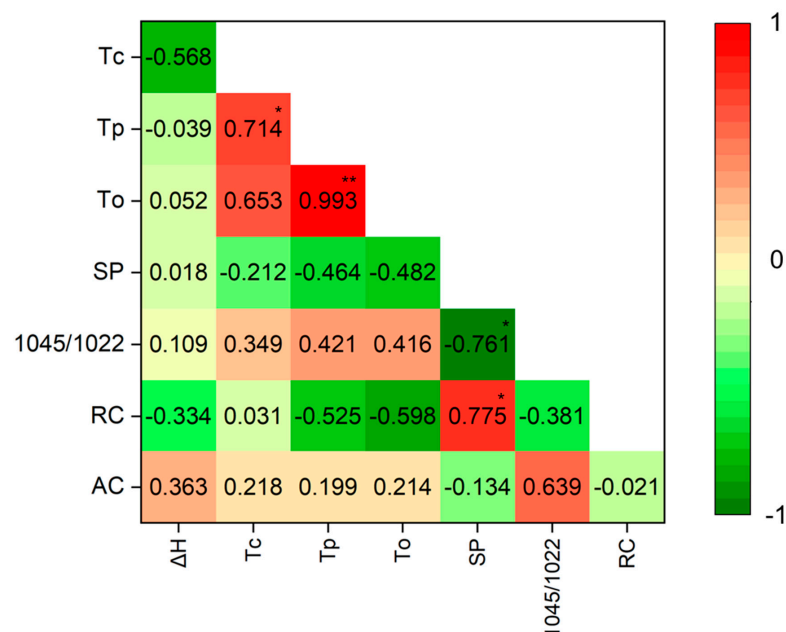


Figure 4. Heatmap plots of Pearson correlation coefficients between starch properties. r values are shown as a gradient from green to red, corresponding to from -1 to 1 . * and ** indicate significant difference at $p < 0.05$ and $p < 0.01$, respectively ($n = 8$). AC, amylose content; RC, relative crystallinity; 1045/1022, the ratio of absorbance $1045/1022 \text{ cm}^{-1}$; SP, swelling power; T_o , onset temperature; T_p : peak of gelatinization temperature; T_c : conclusion temperature; ΔH : gelatinization enthalpy.

4. Conclusions

More small starch granules with compact arrangements were observed in two wheat cultivars grown under conditions of reduced N treatment. Reduced N fertilization did not change the XRD pattern and short-range ordered degree but increased relative crystallinity, swelling power, and gelatinization enthalpy of starch, whereas amylose content and gelatinization transition temperatures were decreased. Reduced irrigation did not significantly affect the amylose content; however, relative crystallinity, swelling power, and gelatinization enthalpy of starch were obviously increased, whereas short-range ordered degree and gelatinization transition temperatures were decreased. In addition, starch from both cultivars appeared to have some differences in response to N and water treatments. For example, the thermal properties were significantly changed in Zhongmai 175 after N or water treatment, whereas Jingdong 17 was found to be different. Hence reduced N fertilization and irrigation could improve the quality of starch, especially in Zhongmai 175 with high NUE and WUE.

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