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Effect of Nitrogen and Sulfur Fertilization on Accumulation Characteristics and Physicochemical Properties of A- and B-Wheat Starch

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ABSTRACT: The effects of nitrogen (N) fertilization at the rates of 0 and 230 kg hm^{-2} combined with selected sulfur (S) fertilization rates of 0, 46, 56, 77, and 115 kg hm^{-2} on A- and B-wheat starch granule accumulation, composition, and thermal and pasting properties were investigated. Scanning electron micrographs revealed that A- and B-starch granules accumulation during grain filling can be influenced significantly by N and S fertilization, and B-granules tend to be more sensitive to S fertilization than A-starch granules. The doses of nitrogen and sulfur fertilization on starch properties were not positively correlated; higher concentration of fertilizers failed to cause a higher effects. $N_{230}S_{46}$ and $N_{230}S_{56}$ treatment resulted in the higher peak viscosity, trough viscosity, breakdown viscosity, final and setback viscosity, and gelatinization temperature (T_o, T_p, T_c) according to differential scanning calorimeter (DSC) and rapid visco analyzer (RVA), respectively.

KEYWORDS: *starch, fertilizer, pasting, thermal, grain filling*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops primarily as a staple food for most of the world's population. Wheat provides a staple source of carbohydrates, proteins, and other nutrients for humans and livestock. It is the main energy source for human beings in several developing countries with inclusions of up to 60% of the daily calorie intake.[1] Starch and gluten protein are the most abundant components of wheat grain, accounting respectively for about 65−75 and 10−15% of the dry weight of the mature wheat grain, and wheat starch is composed of 25−30% amylose and 70−75% amylopectin.[2] Essentially, wheat starches are classified into A- and B-type starch granules according to their shape and size.[3] The large, disk-shaped A-type (diameter > 10 μm) granules and the small, spherical B−-type granules (diameter < 10 μm) have different physiochemical, structural, and functional properties.[4,5]

A- and B-wheat starch granules have significant differences in swelling, gelatinization, and pasting properties, amylose and lipid contents, and ratios of amylose to lipid.[3−6] These characteristic differences of A- and B-wheat starch granules have led to different applications in food and nonfood industries. Soh et al.[6] found that an increase in the large B-type granule content could increase farinograph water absorption and improve pasta quality. Park et al.[7] concluded that the characteristics of B-type granules showed many great correlations with wheat properties and flour quality after study using 98 hard red winter wheat varieties and 99 hard red spring wheat varieties as experimental materials. Therefore, the granule size distribution of the wheat starch has a significant impact on the quality of many wheat products. It is widely acknowledged that the grain qualities of wheat were determined by genetic origin, environmental conditions, and crop cultivation management. Numerous studies have been conducted on size distribution and properties of starch granules in wheat grains in relation to cultivar, environment, and cultivation practices. Environment conditions such as temperature[8] and rainfall,[9] and cultivation practices, that is, irrigation[9,10] and fertilization,[11−13] have important effects on grain development and starch accumulation.

Fertilization of soil is one essential agrotechnical practice with a great effect on accumulation characteristics A- and B-wheat starch and their properties. Nowotna et al.[11] found that higher nitrogen fertilization of triticale leads to a greater amount of amyloplast and higher amylolytic activity and then increased the synthesis of B-type starch granules. Ni et al.[13] reported that phosphorus (P) fertilization resulted in a significant increase in the proportions of B-type starch granules but a reduction of A-type starch granules. Nitrogen (N) is the essential nutrient applied to increase the yield and improve the quantity and quality of storage proteins of cereals.[11] The nitrogen fertilizer addition in wheat increases the quantity of enzymes involved in cellular metabolism and optimizes processes during kernel development, such as starch biosynthesis.[14] Additionally, the increased amount of proteins in starch granules influence pasting properties both through binding water and through a network linked by disulfide bonds.[15] Sulfur (S) is another essential nutrient for crop growth, which is linked to nitrogen in many physiological functions.[16] Sulfur fertilization application could increase the efficiency of nitrogen use; without sufficient sulfur, crops cannot reach their full potential in terms of yield.[17]

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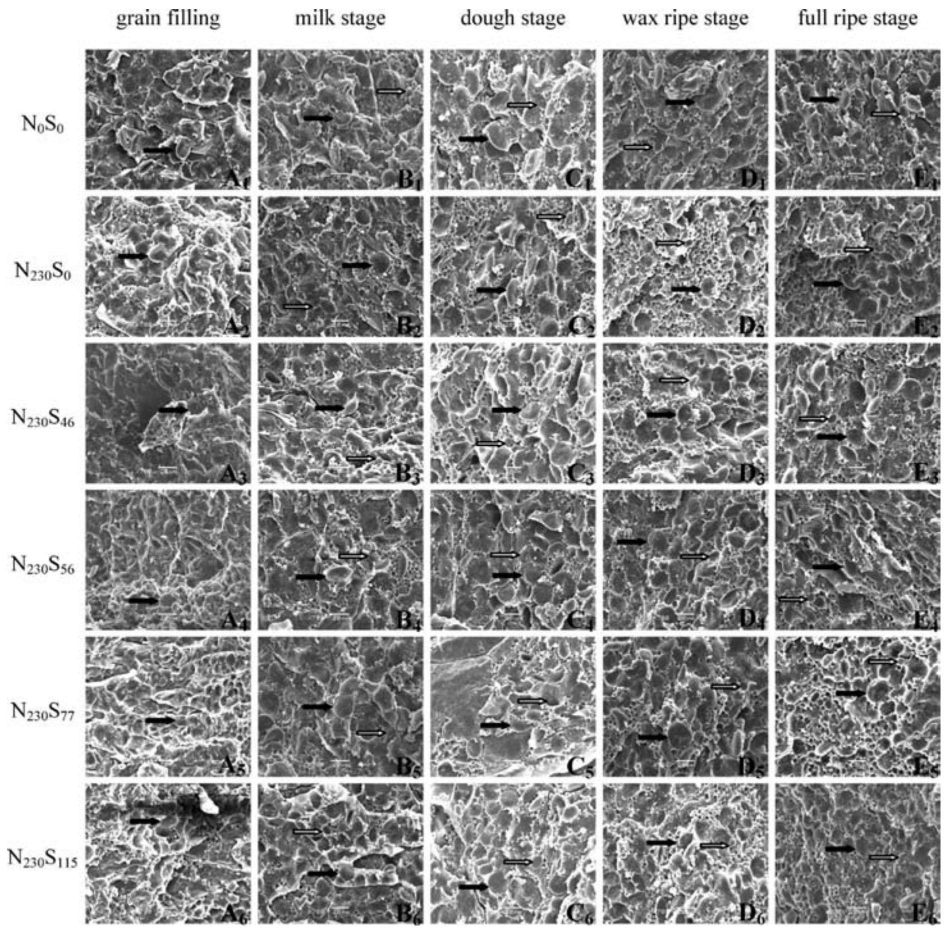

Figure 1. Influence of S and N fertilization on morphological characterization of A- and B-starch granules during wheat seed development observed under SEM. Bars represent 20 μm. The white arrows indicate the B-starch granule, whereas the black arrows indicate the A-starch granule.

Studies have revealed that the A- and B-granules are formed because of the different times of biosynthesis during grain filling. Synthesis of A-granules begins 4 days after anthesis, with granule growth and development continuing over the next 20 days, whereas initiation of B-granule synthesis occurs 10 days after anthesis, with significant granule growth beginning 20 days after anthesis.[11] Therefore, to develop strategies for manipulating starch composition and properties, it is imperative that the regulation of starch composition should be studied at different stages of wheat grain development. However, information about the influence of different doses of nitrogen and sulfur fertilization application on accumulation characteristics and physicochemical properties of A- and B-starch granules during wheat endosperm development is limited. Therefore, the objective of the present study was to investigate the effects of varied doses of nitrogen and sulfur application on morphological changes and accumulation characteristics of A- and B-starch granules at different stages of grain filling and physicochemical properties of starches under different fertilization levels.

■ MATERIALS AND METHODS

Field Experiment. The field experiment was performed at an experimental field of Northwest A&F University (Yangling, China) located at an altitude of 560 m, latitude 34° 16′ N, and longitude 108° 4′ E. The climate is temperate and semi-humid with a mean annual air temperature of 13 °C and a mean annual rainfall of 632 mm, respectively. The soil at the experimental site is calcareous clay loam soil. The wheat variety Xinong 978, bred by Northwest A&F University, was used in this study. Wheat was sown in early October 2009 and harvested on June 15, 2010. The experimental design was conducted in a randomized complete block design with split plot scheme with three replications. Plots constituted by two N (0 and 230 kg hm^{-2}) and five S (0, 46, 56, 77, and 115 kg hm^{-2}) levels and six fertilization combinations including N_0S_0, $N_{230}S_0$, $N_{230}S_{46}$, $N_{230}S_{56}$, $N_{230}S_{77}$, and $N_{230}S_{115}$ were assigned to subplots. All of the experimental units were fertilized with 76.7 kg P hm^{-2} as triple superphosphate, and no K fertilizer was applied because the potassium contents were sufficient. Nitrogen was applied as urea, and S was applied as sulfur powder. All of the fertilizers were applied manually to the plots 1 week before sowing and incorporated by mixing into the first 20 cm of the soil.

Preparation of Samples from Plant. Whole wheat plants were selected at random from three replicated plots of each experimental unit at the stages of grain filling (9 days postanthesis (DPA)), milk development (16 DPA), dough development (22 DPA), wax ripe (29 DPA), and full ripe (36 DPA), respectively. The plants were subsequently dried at 60 °C. Then, grains of wheat were taken under the median part of the ear after separation into glumes, lemmas, and paleas. A portion of the grains of each treatment was milled by using a laboratory milling (mill type Perten 3100, Swedish Perten Co., Sweden) to obtain a whole-wheat meal. Part of the grains was used for morphologic observation. Samples were stored at 4 °C and used for the study.

Scanning Electron Microscopy (SEM) Observation. The wheat grains were randomly collected from each treatment. The two ends of each individual seeds were cut away and the middle sections with a thickness of 2 mm to reveal the endosperm. The cut seed slices were mounted on an SEM stub with double-sided adhesive tape, coated

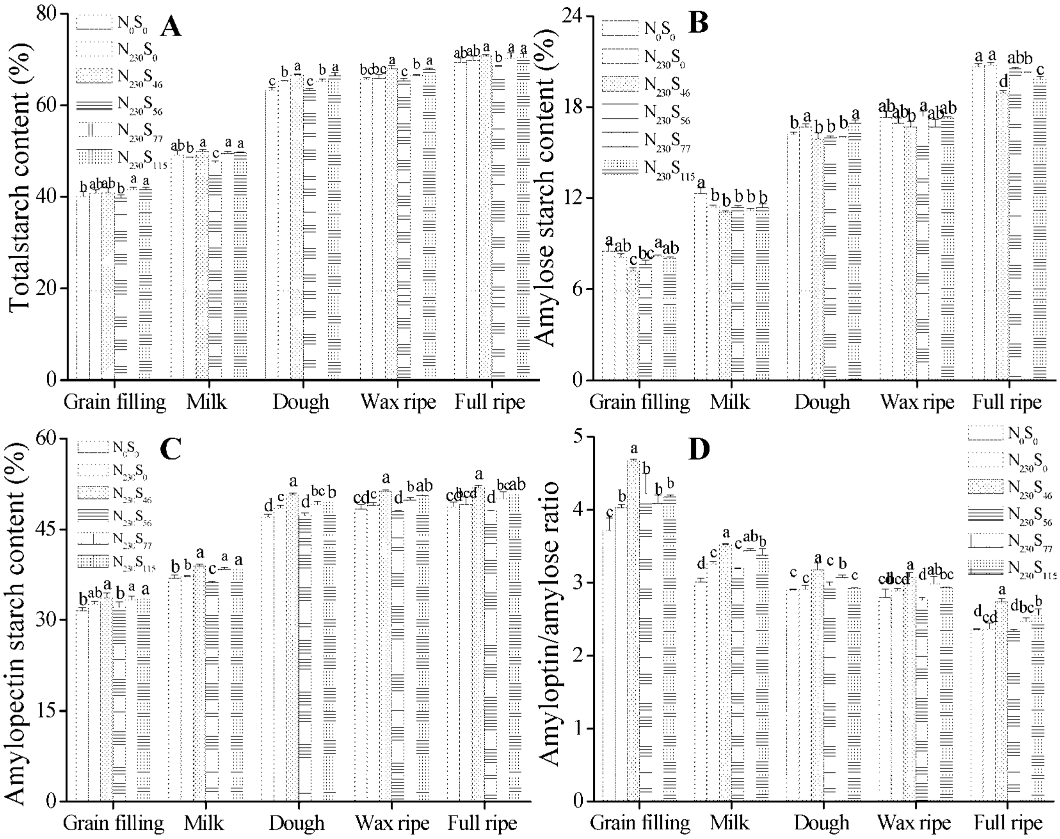

Figure 2. Influence of S and N fertilization on total starch content (A), amylose (B), and amylopectin (C) content and ratio of amylose/amylopectin (D) of wheat starch granules during different times of grain-filling stage. Bars bearing the same letter within a particular development stage are not significantly different ($p < 0.05$).

with gold, and imaged using a scanning electron microscopy (SEM) (JSM-6360LV, JEOL, Japan) at an accelerating voltage of 20 kV.

Determination of Total Starch, Amylose, and Amylopectin Content. Total starch, amylose, and amylopectin contents were determined using the colorimetric method reported by Jarvis and Walker.[18]

Isolation of Starch. The wheat starch granules were isolated from the flours following the method described by Singh et al.[19] Briefly, a stiff dough was prepared by mixing 100 g of flour and 50 mL of water in a pan, and the dough ball was subsequently kept at 30 °C for 1 h. The dough ball was then kneaded by hand in distilled water, and the starch slurry was collected. Starch was suspended in distilled water and passed through a 100 mesh nylon cloth twice to remove bran and endosperm cell wall impurities. The material retained on the cloth was discarded. Starch slurry was then centrifuged at 2500*g* for 10 min. The upper pigmented layer was carefully removed and decanted from any more starch, which had settled after 30 min. The starch fraction along with starch from the decanting step was purified by resuspension in distilled water and centrifugation before drying in an oven at 40 °C for 24 h.

Separation of the A- and B-Granule Starches. The A- and B-granules were separated according to the method described by Zeng et al.[20] The wheat starch suspension with a concentration of about 10% was sedimented using a 2 L graduated cylinder. The fraction of 1 h precipitate was collected as A-granules, and the fraction remaining in the supernatant after 1−15 h of sedimentation was collected as B-granules. The fractionation processes were repeated five times for the A-granules and three times for the B-granules. The separated A- and B-granule suspensions were centrifuged at 7000 rpm for 20 min and washed with 3 times the volumes of ethanol one time. These starches were recovered by using a filter and then dried in a convection oven at 40 °C for 24 h.

Determination of Swelling Power. Swelling power was determined following the modified method of Leach.[21] The starch suspension was stirred at 90 °C for 30 min, cooled, and centrifuged at 3000*g* for 15 min. The value for swelling power was the ratio in weight of the wet sediment to the initial weight of the dry starch.

Determination of Thermal Properties. Thermal properties were measured by a differential scanning calorimeter (DSC2000, New Castle, DE, USA). Starch (3 mg) was directly measured into the aluminum DSC pan, and distilled water (12 μL) was added with a microsyringe, and an empty pan was used as reference for all measurements. The scanning temperature and the heating rates were 30−120 and 10 °C/min, respectively.

Determination of Pasting Properties. Pasting properties of starches were determined using a Rapid Visco-Analyzer (RVA) model Master (Newport Scientific, Pty Ltd., Australia). Deionized water (25.0 g) was added to starch (2.0 g, db) in the RVA canister to obtain a total constant sample weight of 27.0 g. The slurry was then manually homogenized using the plastic paddle to avoid lump formation before the RVA run. The starch slurry was heated from 50 to 95 °C at the rate of 12 °C/min, maintained at 95 °C for 2.5 min, and then cooled to 50 °C at the same rate.

Statistical Analysis. All of the experiments were performed at least in triplicate, and experimental data also were analyzed using analysis of variance (ANOVA) and expressed as the mean value ± standard deviation. Duncan's multiple-range test was conducted to assess significant differences among experimental mean values ($p < 0.05$). All statistical computations and analyses were conducted using SPSS version 13.0 for Windows.

■ RESULTS AND DISCUSSION

Development Process of A- and B-Granules of Wheat Starch during the Grain-Filling Stage. Starch granule accumulation in central endosperm tissue sections during the

Table 1. Swelling Power of A- and B-Wheat Starch Granules at Different Nitrogen−Sulfur Treatments[a]

treatment	unfractionated starch (%)	A-granule (%)	B-granule (%)
N_0S_0	8.23 ± 0.40bc	7.90 ± 0.02ab	9.15 ± 0.09cd
$N_{230}S_0$	8.38 ± 0.12b	7.19 ± 0.03d	8.69 ± 0.53e
$N_{230}S_{46}$	9.01 ± 0.00a	8.10 ± 0.32a	9.88 ± 0.21a
$N_{230}S_{56}$	7.98 ± 0.13d	7.22 ± 0.10d	9.09 ± 0.08d
$N_{230}S_{77}$	8.12 ± 0.39 cd	7.41 ± 0.10c	9.33 ± 0.11bc
$N_{230}S_{115}$	8.51 ± 0.18a	7.80 ± 0.29b	9.68 ± 0.10ab

[a]All values are means of triplicate determinations ± SD. Means within columns with different letters are significantly different ($p < 0.05$).

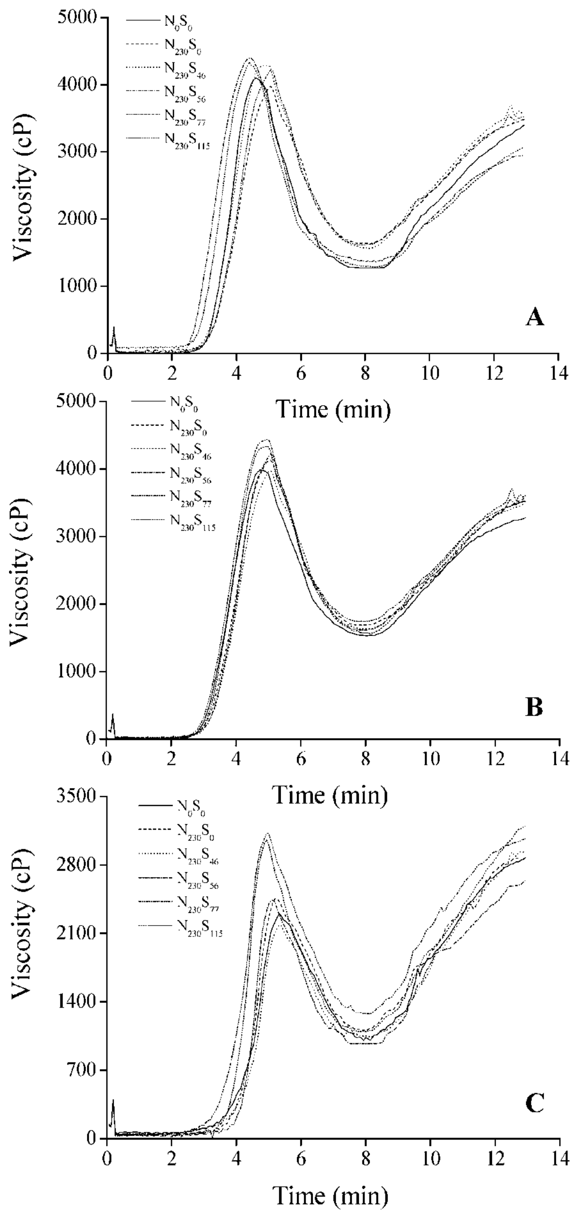

Figure 3. Pasting viscosity profiles of unfractionated starch (A), A-starch (B), and B-starch (C) granules at different nitrogen−sulfur level treatments.

grain-filling stage was examined using SEM (Figure 1). Starch granules in grains of all fertilization treatments were morphologically different from the granules of the control treatments at different grain-filling stages. At the early stage of grain filling (before 3 days after anthesis, Figure $1A_1$), no B-starch granules were formed in the wheat endosperm, and only a few A-granules and proteins deposited in the endosperm without application of nitrogen and sulfur fertilization. Nitrogen applications tended to increase A-starch granule accumulation, and the $N_{230}S_0$ treatment showed a higher amount of A-granules than N_0S_0 treatment (Figure $1A_2$). Sulfur fertilization tended to have a marked influence on the accumulation of B-starch granules and wheat proteins, and little difference was detected between levels of S fertilization (Figure $1A_2$−A_6).

During the milk stage (Figure $1B_1$−B_6), the number and diameter of A- and B-starch granules in the wheat grain endosperm of all fertilization treatments increased rapidly. More and more B-granules appeared in the surrounding of the A-granules. In comparison of N_0S_0 and $N_{230}S_0$ treatments, addition of sulfur fertilization resulted in much larger increases of B-starch granules, whereas no micrographic difference could be detected between S fertilization treatments. At the dough stage (Figure $1C_1$−C_6), the size of A-granules for all fertilization treatments increased even more, whereas the number of A- and B-starch granules tended to be stable. A-granules of the $N_{230}S_{77}$ (Figure $1C_5$) treatment showed the largest granule size compared to other treatments, whereas the treatment of $N_{230}S_{46}$ (Figure $1C_3$) and $N_{230}S_{115}$ (Figure $1C_6$) exhibited the highest B-granule number and a relatively lower A-granule size. Until the wax ripe stage (Figure $1D_1$−D_6) and full ripe stage (Figure $1E_1$−E_6), the size of the A-starch granules continued to increase. Nitrogen and sulfur fertilization of wheat leads to accumulation of a great amount of proteins, including enzymes in amyloplast, which would increase the synthesis of B-type starch granules.[11] The results revealed that the protein content and proportions of A- and B-starch granules in wheat grain during grain filling can be influenced significantly by N and S fertilization, and B-starch granules tend to be more sensitive to S fertilization than A-starch granules.

Composition of Wheat Starch Granules during Different Times of Grain-Filling Stage. The variations of total starch, amylose, and amylopectin contents and ratio of amylose/amylopectin of wheat grain during different developmental phases under different fertilization levels are presented in Figure 2. The values of total starch, amylose, and amylopectin contents and amylose/amylopectin ratio during the grain-filling stage are strongly dependent on the fertilization treatment. $N_{230}S_{46}$, $N_{230}S_{77}$, and $N_{230}S_{115}$ treatments led to higher total starch content compared to the control treatment during the whole wheat grain development stage. There was no significant difference ($p < 0.05$) in total starch content between grains treated by $N_{230}S_{56}$ and that of the control during the whole grain-filling stage (Figure 2A). Starch is the major storage component of wheat grain, accounting for about 60−70% of its dry weight, and changes in starch concentration are indicators of a variety of plant developmental processes, both positive and negative.[22] Therefore, the amounts of A- and B-starch granules as well as the composition of starch determine the starch quality and hence the quality of the wheat grain.

The amylose and amylopectin contents and amylose/amylopectin ratio of wheat grain were significantly affected by N and S fertilizer treatment, the $N_{230}S_{56}$ treatment showing the lowest amylose content and the highest amylopectin content and lowest amylose/amylopectin ratio during the whole grain development stage (Figure 2B−D). In general, the nitrogen and sulfur treatments led to higher amylopectin content and lower

Table 2. Pasting Properties of A- and B-Granule Wheat Starch at Different Nitrogen−Sulfur Treatments[a]

starch	treatment	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (°C)
unfractionated	N_0S_0	4102 ± 68bc	1400 ± 41ab	2702 ± 46c	3276 ± 58e	1876 ± 11c	80.65 ± 0.17b
	$N_{230}S_0$	4012 ± 97c	1390 ± 25ab	2622 ± 63c	3723 ± 63ab	2333 ± 23a	86.40 ± 0.21a
	$N_{230}S_{46}$	4403 ± 101a	1237 ± 35c	3166 ± 77a	3408 ± 44d	2171 ± 30b	78.40 ± 0.00c
	$N_{230}S_{56}$	4208 ± 83b	1462 ± 34a	2746 ± 30c	3601 ± 41bc	2139 ± 39b	80.65 ± 0.03b
	$N_{230}S_{77}$	4425 ± 71a	1324 ± 20bc	3101 ± 26a	3503 ± 69 cd	2179 ± 33b	78.35 ± 0.12c
	$N_{230}S_{115}$	4317 ± 62ab	1365 ± 23b	2952 ± 19b	3780 ± 59a	2415 ± 39a	78.40 ± 0.07c
A-granule	N_0S_0	3981 ± 75d	1530 ± 17c	2451 ± 29c	3291 ± 81d	3981 ± 84c	69.30 ± 0.04a
	$N_{230}S_0$	4130 ± 66 cd	1688 ± 31ab	2442 ± 31c	3601 ± 97ab	4130 ± 113bc	69.70 ± 0.01a
	$N_{230}S_{46}$	4332 ± 63ab	1563 ± 38c	2769 ± 27a	3616 ± 44a	4332 ± 100a	61.95 ± 0.11c
	$N_{230}S_{56}$	4436 ± 38a	1742 ± 23a	2694 ± 31ab	3531 ± 93ab	4436 ± 91a	66.20 ± 0.23b
	$N_{230}S_{77}$	4221 ± 49bc	1614 ± 41b	2607 ± 27b	3504 ± 62bc	4221 ± 89ab	70.30 ± 0.41a
	$N_{230}S_{115}$	3979 ± 48d	1631 ± 34b	2348 ± 23d	3487 ± 73c	3979 ± 93c	70.25 ± 0.10a
B-granule	N_0S_0	2302 ± 42c	1005 ± 41bc	1297 ± 47c	2895 ± 63c	1890 ± 26bc	75.05 ± 0.48a
	$N_{230}S_0$	2455 ± 44b	1110 ± 16b	1345 ± 33c	2876 ± 39c	1766 ± 19d	73.50 ± 0.15b
	$N_{230}S_{46}$	3060 ± 51a	1093 ± 29b	1967 ± 39a	3216 ± 28a	2123 ± 32a	68.50 ± 0.13d
	$N_{230}S_{56}$	3127 ± 69a	1278 ± 23a	1849 ± 29a	3086 ± 43ab	1808 ± 45 cd	68.65 ± 0.09d
	$N_{230}S_{77}$	2443 ± 20b	972 ± 28c	1471 ± 37b	2643 ± 38d	1671 ± 25e	71.80 ± 0.22c
	$N_{230}S_{115}$	2189 ± 27d	1045 ± 31b	1144 ± 19d	2945 ± 29bc	1900 ± 43b	75.96 ± 0.35a

[a]All values are means of triplicate determinations ± SD. Means within columns for the same starch variety with different letters are significantly different ($p < 0.05$). PV, peak viscosity; TV, trough viscosity; BD, breakdown; FV, final viscosity; SB, setback; PT, pasting temperature.

Table 3. Thermal Properties of the A-Granule Wheat Starches at Different Nitrogen−Sulfur Treatments[a]

starch	treatment	T_o (°C)	T_p (°C)	T_c (°C)	ΔT_r (°C)	ΔH (J/g)
unfractionated	N_0S_0	56.44 ± 0.17b	60.89 ± 0.31ab	66.78 ± 0.38a	10.34 ± 0.03b	5.88 ± 0.03e
	$N_{230}S_0$	60.42 ± 0.32a	60.71 ± 0.20b	67.54 ± 0.27a	7.12 ± 0.01c	7.09 ± 0.03a
	$N_{230}S_{46}$	56.18 ± 0.14b	61.45 ± 0.24ab	67.39 ± 0.19a	11.21 ± 0.01a	6.71 ± 0.01b
	$N_{230}S_{56}$	56.11 ± 0.16b	61.20 ± 0.18ab	66.90 ± 0.16a	10.79 ± 0.00b	6.33 ± 0.07c
	$N_{230}S_{77}$	56.34 ± 0.27b	61.38 ± 0.18ab	66.86 ± 0.35a	10.52 ± 0.04b	6.36 ± 0.06c
	$N_{230}S_{115}$	56.21 ± 0.21b	61.64 ± 0.33a	67.46 ± 0.34a	11.25 ± 0.01a	6.61 ± 0.05d
A-granule	N_0S_0	56.53 ± 0.01ab	60.86 ± 0.07a	66.44 ± 0.48b	9.92 ± 0.48a	6.88 ± 0.01c
	$N_{230}S_0$	56.86 ± 0.25a	61.53 ± 0.47a	67.31 ± 0.17a	10.46 ± 0.41ab	6.72 ± 0.03c
	$N_{230}S_{46}$	56.42 ± 0.32ab	60.53 ± 0.51a	66.55 ± 0.32b	10.13 ± 0.19ab	7.31 ± 0.08b
	$N_{230}S_{56}$	56.49 ± 0.02ab	60.99 ± 0.02a	67.31 ± 0.26a	10.83 ± 0.22a	7.09 ± 0.04bc
	$N_{230}S_{77}$	56.06 ± 0.47b	60.50 ± 0.33a	67.31 ± 0.19a	11.25 ± 0.16a	8.58 ± 0.03a
	$N_{230}S_{115}$	56.52 ± 0.11ab	60.93 ± 0.18a	66.25 ± 0.33b	9.73 ± 0.21b	6.81 ± 0.04c
B-granule	N_0S_0	57.70 ± 0.04c	62.98 ± 0.36bc	68.15 ± 0.48c	10.46 ± 0.4ab	4.46 ± 0.38d
	$N_{230}S_0$	58.87 ± 0.35ab	63.79 ± 0.50ab	68.79 ± 0.16bc	9.92 ± 0.51b	4.65 ± 0.31 cd
	$N_{230}S_{46}$	58.37 ± 0.05b	64.05 ± 0.27a	69.32 ± 0.44a	10.96 ± 0.43a	5.27 ± 0.30bc
	$N_{230}S_{56}$	59.34 ± 0.78a	64.09 ± 0.36a	70.04 ± 0.28a	10.70 ± 0.41a	5.48 ± 0.39b
	$N_{230}S_{77}$	57.03 ± 0.15c	62.24 ± 0.04c	67.16 ± 0.53d	10.14 ± 0.28ab	6.18 ± 0.08a
	$N_{230}S_{115}$	58.39 ± 0.50b	63.04 ± 0.41b	68.35 ± 0.14b	9.97 ± 0.29b	4.80 ± 0.18 cd

[a]All values are means of triplicate determinations ± SD. Means within columns for the same starch type with different letters are significantly different ($p < 0.05$). T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔT_r, gelatinization temperature range ($\Delta T_r = T_c - T_o$); ΔH, enthalpy of gelatinization.

amylose/amylopectin ratio for wheat grain, but led to a lower amylose content compared to the control. Our result is consistent with the result obtained by Meredith.[23] Additionally, no significant changes were observed in amylose, and amylopectin contents and amylose/amylopectin ratio after treatment with N fertilizer compared to treatment with N combined with S fertilizer indicate that N combined with S application is a good strategy for improving wheat starch granule composition. These results can also be attributed to treatment with N combined with S fertilizer leading to an increase in the amount of protein in the amyloplast, which could increase amylopectin content.

Swelling Power. Swelling powers of large A-type and small B-type starch granules at different fertilization levels and the results of statistical analysis are presented in Table 1. Different nitrogen and sulfur fertilization levels affected the swelling power of wheat starch granules. The swelling power of unfractionated starch and A- and B-starch granules under different fertilization levels varied from 7.98 to 9.01%, from 7.19 to 8.10%, and from 8.69 to 9.88%, respectively. $N_{230}S_{46}$

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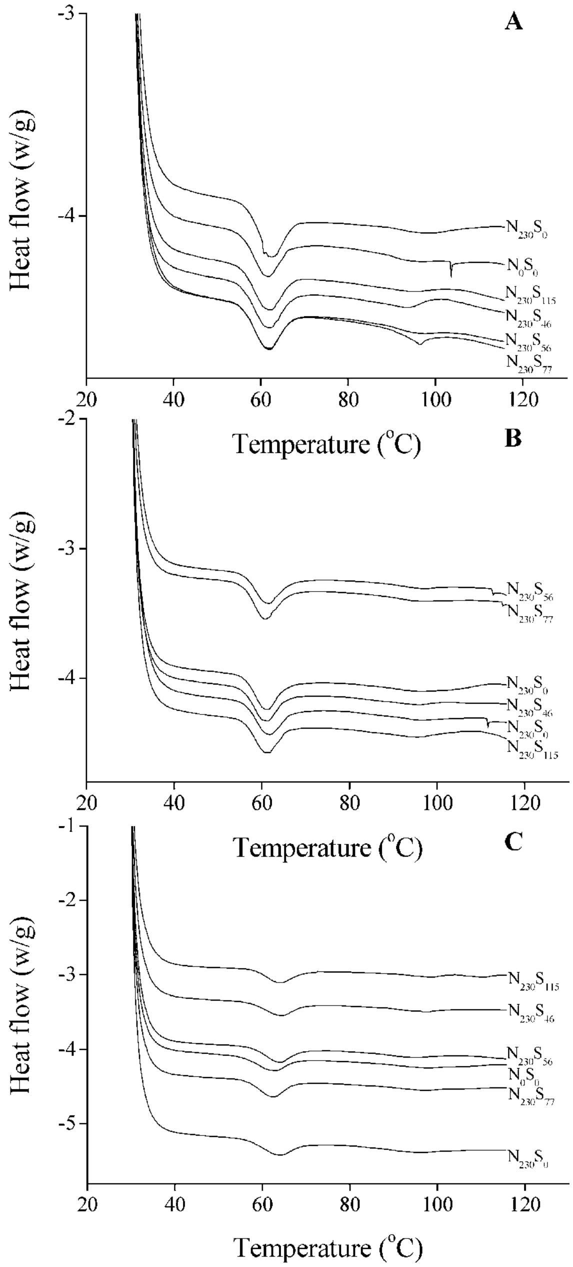

Figure 4. DSC thermograms of gelatinization properties for unfractionated starch (A), A-starch (B), and B-starch (C) granules at different nitrogen−sulfur level treatments.

treatment showed the highest swelling power value compared to other fertilization treatments, whereas $N_{230}S_{56}$ treatment showed the lowest value. There must exist a critical N and S fertilization concentration between the fertilization level of $N_{230}S_{46}$ and $N_{230}S_{56}$ at which the wheat grain produces the best quality of starch under a given time and field situation. The reason for these phenomena remains unclear. Additionally, the large starch granule exhibited a lower swelling power value than that of small starch granule at a certain fertilization level (Table 1). This could be attributed to the B-type starch granules being associated with a higher rate of water absorption, earlier hydration, and more swelling than were A-type granules.[24]

Pasting Properties. The pasting profiles of unfractionated starch and A- and B-starch granules under different N and S fertilization treatments are presented in Figure 3, and the parameters of starch peak viscosity, trough, breakdown, setback and final viscosities, and pasting temperature are summarized in Table 2. According to the RVA patterns, the pasting behaviors of starches were greatly affected by different N and S fertilization treatments. Statistical analyses indicated that single nitrogen applications ($N_{230}S_0$ treatment) increased peak, trough, and final and setback viscosities in both A- and B-starch granules compared to control treatment (N_0S_0 treatment), but a decreased pasting temperature of B-starch granules. At the same time, the final viscosity, setback viscosity, and pasting temperature were significantly decreased ($p < 0.05$) by $N_{230}S_0$ treatment. However, the effects of nitrogen combined with sulfur application on starch viscosity were not positively correlated. $N_{230}S_{46}$ and $N_{230}S_{56}$ treatments resulted in the highest peak viscosity, trough viscosity, breakdown viscosity, and final and setback viscosities compared to other treatments. Significant effects in starch viscosity properties were not brought about by excessive sulfur fertilization (Table 2).

The results are in accordance with the results of starch composition and swelling power analysis. As a result, the 46 and 56 kg hm^{-2} $N_{230}S_{46}$ sulfur fertilization combined with the 230 kg hm^{-2} nitrogen rate would be considered standard, whereas the 77 and 115 kg hm^{-2} sulfur fertilization levels would be considered excessive. The relatively higher pasting properties after different fertilization level treatments could be mainly due to the variation of composition for different starch granules. The various granule size distributions and protein contents may lead to different abilities to swell and rupture starch granules. These starch granule stabilities can account for the higher pasting properties during the heating cycle. Changes in starch viscosity during the heating period give an indication of the stability, and the change characteristics during cooling show the consistency of the product when consumed.[25] Therefore, the pasting characteristics play an important role in the selection of a variety for use in the food and other industries.[26] On the other hand, the variety differences observed in the pasting properties among the different fertilization level treated starch samples in the present study could be useful in selecting a proper sample for a particular industrial application.

Thermal Properties. The thermal characteristics (onset temperature, T_o; peak temperature, T_p; conclusion temperature, T_c; gelatinization temperature range, $\Delta T_r = T_c - T_o$, and enthalpy of gelatinization, ΔH) of unfractionated starch and A- and B-starch granules as affected by application of nitrogen−sulfur at different levels are shown in Table 3, and the DSC thermograms of starch samples at different fertilization levels are presented in Figure 4. The T_o, T_p, and T_c of the B-type starch granules under the same fertilization level treatment were higher than those of unfractionated and A-starch granules. A-granules from the same fertilization level treatment showed the highest ΔH value, whereas B-type starch granules showed the lowest (Table 3). These results are also clearly illustrated in Figure 4: the DSC curves of A-starch granules exhibited wider peaks than those of unfractionated and A-starch granules under the same fertilizer treatment. Our results are in close agreement with earlier findings.[4,20,24]

There was a large difference in the gelatinization temperature values (T_o, T_p, T_c, and ΔT_r) and gelatinization enthalpy (ΔH) of unfractionated starch and B-starches after different levels of nitrogen and sulfur fertilization application. The unfractionated starch granules of $N_{230}S_0$ treatment showed the highest T_o, T_c, and ΔH values and the lowest T_p and ΔT_r values compared to other fertilizer-treated samples. Additionally, the $N_{230}S_0$-treated

A-starch granules exhibited the highest T_o, T_c, T_p, and ΔH values and the lowest ΔT_r values among different A-starch samples. However, $N_{230}S_{56}$-treated B-starch granules hold the highest T_o, T_c, and T_p values, and $N_{230}S_0$- and N_0S_0-treated B-granules presented the lowest ΔT_r and ΔH values, respectively (Table 3). Higher levels of nitrogen and sulfur fertilization application seemed to have little or no impact on gelatinization properties determined by DSC. The variation in transition temperatures and gelatinization enthalpy in starches from different fertilization level treatment might be due to differences in amylose content, amylopectin content, and amylose/amylopectin ratio as discussed in a previous section and, consequently, result in the variation in crystallinity.

In summary, this is the first time that the accumulation characteristics and physicochemical properties of A- and B-wheat starches under different doses of nitrogen combined with sulfur fertilization have been investigated. N combined with S fertilization application is a good measure to improve A- and B-type starch granule accumulation in central endosperm tissue sections during the grain-filling stage. The A- and B-granule compositional properties including total starch, amylose, and amylopectin contents and amylose/amylopectin ratio during the grain-filling stage are also strongly affected by N and S fertilization treatments. N and S fertilization treatments tend to increase increased peak, trough, and final and setback viscosities in both A- and B-starch granules, but decreased pasting temperature of B-starch granules. Different levels of nitrogen and sulfur fertilization application seemed to have little or no impact on gelatinization properties. Here we observed that the levels of nitrogen combined with sulfur application on starch properties were not positively correlated; there must exist a critical N and S fertilization concentration between the fertilization level of $N_{230}S_{46}$ and $N_{230}S_{76}$, and at this fertilization level point, the wheat grain produces the best physical properties of starch. The present results also demonstrated that nitrogen combined with sulfur fertilization treatment has better effects on accumulation characteristics and physicochemical properties of A- and B-wheat starches compared to single nitrogen applications. The results obtained here provide a better understanding of the effects of N and S fertilizers on wheat starch granule accumulation and help in the selection of the proper variety for a particular industrial application.

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Notes

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