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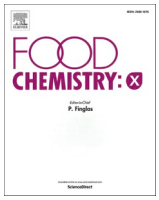

Characterization of the starch molecular structure of wheat varying in the content of resistant starch

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ABSTRACT

Resistant starch (RS) is the total amount of starch that is incompletely or not digested and absorbed in the small intestine. It plays a role similar to dietary fibre with beneficial effects for human health. In this study, the RS content of 129 wheat accessions was determined, and the relationship between the several starch physical properties and resistant starch content were analyzed. By comparing the total starch content, amylose starch content, starch chain length distribution, starch crystallization type, starch branching degree, and starch granule morphology between the high RS and low RS content wheat accessions, it was found that the amylose content and RS content were significantly positively correlated. However, in the range of chain length fb 3 (DP $\geq$ 37), there was a significant negative correlation between amylopectin content and RS content. The surface of starch granules became increasingly smooth as the content of RS increased.

Introduction

Wheat is the most important cereal crop and energy reserve consumed by large human population worldwide. It is the major source of the carbohydrates, proteins, fats, vitamins, and minerals that contribute to healthy human diet. Since starch as a main component of wheat endosperm, its nutritional composition affects the quality of food, and has potential to deliver health benefits to human being. Starch can be divided into three groups based on the digestion time in the human digestive system: rapidly digested starch, slow digesting starch, and resistant starch (RS). RS has a greater water-binding capacity but lower water-holding capacity than more digestible forms of starch; the physiological functions of RS are similar to dietary fiber, and thought to be a kind of functional food in preventing and improving non-communicable diseases,such as obesity, diabetes, colon cancer, cardiovascular diseases and chronic kidney disease. In addition, it also was used as food additive which could improve the food taste and flavor (Bello-Perez et al., 2018; Englyst et al., 1992; Higgins & Brown, 2013).

There are five types of RS in food: RS_1 is physically trapped starch in grains or seeds; RS_2 mainly includes its granules which do not allow different amylase to hydrolyze them in raw food material; RS_3 is regenerated starch found when food products are cooked and cooled. RS_4 is chemically modified starch, and amylose–lipid complexes are defined as RS_5 (Tian et al., 2020). The main RS types present in raw grains are RS_1 and RS_2 (Tian and Sun, 2020). It is clearly that different crops contain distinct RS contents. For example, compared with RS rich in beans, ranged 32% to 36 %, there is only 0.1 % to 3.2 % in cereal crops (Ragaee et al., 2006; Ambigaipalan et al., 2011).

Much previous studies have reported that few factors affecting the content of RS in raw grains include intrinsic properties of starch, such as the starch guanule structure, starch crystal structure, the amylose content and external factors, including processing conditions, treatment processes and environmental factors (Lockyer et al., 2017; Cheng et al., 2007). It was reported that the content of amylose was positively correlated with the RS content in maize and rice grains (Li et al., 2008; Ramadoss et al., 2015). The chain length distribution (CLD) is also a key factor affecting the properties of starch. Starch can be divided into four groups based on the CLD: fraction A chain (fa) (degree of polymerization (DP) $\leq$ 12), fraction B1 chain (fb 1) (13 $\leq$ DP $\leq$ 24), fraction B2 chain (fb 2) (25 $\leq$ DP $\leq$ 36), and fraction B3 chain (fb 3) (DP $\geq$ 37). Rice seeds with a high RS content have a high proportion of fa and low proportions of fb 1, fb 2, and fb 3 (Shu et al., 2007). The enzymatic properties of starch are affected by variations in the degree of crystallinity. Class V starch and class A starch show the highest and lowest resistance to hydrolysis, respectively. The morphology of starch granules also affects the content of RS (Yang et al., 2005). It was found that the fine structure of starch deficient in branching enzymes was altered, and this mutation resulted in significant changes in the digestive properties of starch

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(Satoh et al., 2003). The relationship between starch grain size distribution and amylose content has also been studied in grains such as barley, and it is currently believed that high-amylose barley contains more small particles than waxy or ordinary barley starch (Morrison et al., 1986).

At present, the research on resistant starch was mostly performed in diploid crops, such as rice, mazie and barley, which focused on the effect of physical–chemical modification on RS content in starch production. Few studies were conducted in ployploid crop wheat and there is a lack of systematic analysis on starch composition and molecular structure related to RS content in wheat grains. In this study, we investigated the RS content of 129 wheat accessions. We also studied molecular structures of high RS and low RS content accessions, including the total starch content, amylose starch content, starch chain length distribution, starch crystallization type, starch branching degree, and starch granule morphology. The result provides deep insight of intrinsic molecular structures in differential RS content wheat accessions, which is also beneficial for high RS wheat breeding.

Materials and methods

Experimental materials

The wheat accessions in this study were collected from the main wheat-producing areas in China (Schedule 1). Each germplasm was cultivated in Yunnan, grown under field conditions, harvested, and subjected to detailed quantitative analysis. After removing the wheat husk from the wheat kernel and balancing the water for a week, the powder is ground with the grinder (Model: MBI-48) at 55 HZ frequency for 400–500 s. The ground powder will be used as the initial sample for the following projects.

Content of RS assay

The content of RS was determined by enzyme digestion method with AOAC Official Method 2002.02 Resistant Starch in Starch and Plant Materials. Digestible starch was removed using 4 mL of alpha-amylase per 100 mg of pretreated wheat starch; the grains were then incubated with shaking at 37 °C for 16 h. Add 4 mL anhydrous ethanol, swirl and mix well, centrifuge at 12000 rpm (13200 × *g*) for 10 min, discard the supernatant. Add 2 mL 50 % ethanol, swirl well, add 6 mL 50 % ethanol, centrifuge at 12000 rpm (13200 × *g*) for 10 min, discard the supernatant, and repeat twice. (Note: After the supernatant is abandoned, the remaining ethanol in the tube is about 0.2 mL.) Add 2 mL 2 M KOH, mix well, and shake on ice for 20 min. Add 8 mL 1.2 M sodium acetate buffer solution, vortex evenly. Immediately add 0.1 mL amyloglucosidase and incubate at 50 °C for 30 min, during which the vortex is uniform. Hydrolysis was initiated by adding of 0.1 mL of starch glucosidase; the solution was then incubated at 50 °C for 30 min with shaking. The above liquid (0.1 mL) was mixed with 3 mL of the glucose oxidase/peroxidase (GOPOD) reagent, vortexed, and incubated at 50 °C for 20 min. The control contained 0.1 mL of glucose standard solution (1 mg/mL) and 3 mL of GOPOD reagent. The reagent blank solution comprised 0.1 mL of 0.1 M sodium acetate buffer and 3 mL of GOPOD reagent. The absorbance at 510 nm and the absorbance of glucose at a known concentration were determined using a colorimetric method, and the content of RS was calculated. Quantitative analyses were conducted using a multifunctional microplate analyzer (Thermo Fisher Scientific, USA). Resistant starch content <10 %: Resistant starch (%) = (ΔA × F × 9.27)/W; ΔA: Absorbance (reaction) read against the ragent blank. F = [100 (μg of D-glucose)]/absorbance for 100 μg of glucose.

Starch content assay

The starch content was determined by the method of AOAC (2021). Pretreated wheat starch (100 mg) was mixed with 80 % ethanol and placed in an incubator at 70 °C for 2 h. After the mixture was centrifuged at 12000 rpm (13200 × *g*) for 10 min, the supernatant was removed, and this procedure was repeated three times. Next, KOH was added to the mixture in a test tube in an ice bath and mixed well; 8 mL of 1.2 M sodium acetate buffer was then added and mixed well, and this was followed immediately by the addition of 0.1 mL of starch glucosidase. The mixture was incubated at 50 °C for 30 min with shaking. Distilled water was added to adjust the volume of the flour solution to 100 mL. Next, 3 mL of the GOPOD reagent was added to 0.1 mL of the flour solution; the solution was then vortexed and incubated at 50 °C for 20 min. The glucose control included 0.1 mL of glucose standard solution (1 mg/mL) and 3 mL of GOPOD reagent; the reagent blank solution comprised 0.1 mL of water and 3 mL of GOPOD reagent. A colorimetric method was used to measure the absorbance at 510 nm, and the content of starch was calculated. Quantitative analyses were conducted using a multifunctional microplate analyzer (Thermo Fisher Scientific, USA). Starch content (%) = (ΔA × F × FV × 0.9)/W; ΔA: Absorbance (reaction) read against the reagent blank. F = (100 (μg of D-glucose)) / (absorbance for 100 μg of glucose); FV = Final volume.

Amylose content assay

The amylose content was determined by the method of GB/T 15683–2008; the iodine reagent method was used to calculate the apparent AC of wheat grains. 10 mg of pretreated wheat starch was mixed with 100 μL of alcohol and 900 μL of NaOH solution; the solution was then boiled for 10 min, cooled, and precipitated. Next, 0.1 mL of acetic acid and 0.2 mL of potassium iodide solution were added to 0.5 mL of the supernatant; after the volume was adjusted to 10 mL, the solution was left to stand for 10 min at room temperature. The absorbance was measured at 720 nm. The apparent AC was calculated using standard curves of mixtures with different proportions of amylose and amylopectin. Quantitative analyses were conducted using a multifunctional microplate analyzer (Thermo Fisher Scientific, USA). The AC can be calculated according to the standard curve : Amylose content (%) = (C/M) × 10C: calculated according to the standard curve; M: the actual weighing mass (generally 10 mg); 10: the correction coefficient.

Starch branching degree

The branching degree of amylopectin was determined following the methods described in several previous studies (Wang et al., 2022; Xu et al., 2021). The wheat grains were removed from the husk and the water was balanced for a week, then crushed with a grinder at a grinding frequency of 55 Hz and the grinding time was 400–500 s (Model: MBI-48). 10 g of pretreated wheat starch was added to 1 mL of d6-D6-DMSO and heated overnight at 80 °C. After the mixture was centrifuged at 12000 rpm (13200 × *g*) for 10 min, the supernatant was added to a nuclear magnetic tube, and proton nuclear magnetic resonance (1H NMR) was used to determine the degree of branching of amylopectin. The data were analyzed using MestReNova software, and the peak range was determined based on the peaks of the flour solution. Calculations were conducted using the following formulas: DB (%) = A/(A + B) × 100 %; DB: Branching degree; A: Peak area of the α-1,6 bond; B: Peak area of the α-1,4 bond.

X-Ray diffraction

XRD was conducted following previously described methods (Sandhu et al., 2021; Wang et al., 2022). The crystallinity of starch samples was analyzed using an X'Pert Pro X-ray diffractometer (PANalytical Netherlands).

The prepared starch after moisture balance was ground and dispersed. After the 100 mesh sample sieve, about 100 mg of starch was taken on the stage, pressed tightly, spread evenly, and tested on the machine. An X-ray diffractometer with Cu-Ka radiation (40 kV × 40 mA)

was used for XRD analysis. A NaI crystal scintillation counter was used to measure the intensity of X-rays; the scanning range was from 4° to 60°, the step length was 0.02°, and the scanning speed was 4°/min. Data were analyzed using MDI Jade 5.0 software.

SEM assay

SEM was conducted following previously described methods (Sandhu et al., 2021; Deng et al., 2021). The prepared wheat starch was screened with 100 mesh samples, 1 mL of 4 % SDS wash buffer was added to 100 mg of wheat starch. The mixture was then centrifuged at 10000 rpm (9166 × *g*) for 1 min and cleaned using deionized water. Finally, 1 mL of anhydrous ethanol was added, and the mixture was mixed well. A small amount of prepared starch suspension was then placed on a conductive adhesive on a copper table; after spreading the suspension as evenly as possible on the table, place it in an incubator at 37 °C overnight to dry the suspension. After samples were sprayed with gold using an ion sputterer (Cresstington, 108Auto), photographs of these samples were taken at a magnification of 1000–2000 × using a scanning electron microscope (Merlin Compact Zeiss, Germany).

Chain length distribution (CLD) of amylopectin

The CLD of amylopectin was determined following previously described methods (Zhou et al., 2018; Nishi et al., 2001). Weigh 5 mg each of DP 4-DP 7 of the oligosaccharide standard set and suspend in 5 mL double steaming water, boiling water bath for 60 min, and mix with intermittent vortices; 50 μL sodium acetate (0.6 M, PH 4.4), NaN3 (10 μL, 2 % w/v) and 10 μL isoamylase (1400 U) were added and incubated at 37 °C for 24 h. Add 0.5 % (w/v) sodium borohydride solution, vortically mix and leave for 20 h. Take 600 μL in a centrifugal tube and blow dry with nitrogen at room temperature. Dissolve in 30 μL 1 M NaOH for 60 min, then add 570 μL water to dilute, centrifuge at 12000 rpm (13200 × *g*) for 5 min, and take supernatant for sample.

5 mg of pretreated wheat starch was dissolved in 5 mL of water in a boiling water bath for 60 min. 10 μL of sodium azide solution (2 % w/v). Take 2.5 mL gelatinized sample, add 125 μL sodium acetate (0.1 M, PH6.0), 5 μL NaN3 (1 %) and 10 μL isoamylase (2000 μm /μL), and place at 38 °C for 24 h. Samples (600 μL) were dried in a vacuum at room temperature and dissolved in 30 μL of 1 M NaOH for 60 min. Next, the solution was diluted in 570 μL of distilled water. High-performance anion-exchange chromatography was used to analyze the sample extracts on a CarboPac PA-200 anion-exchange column (4.0 × 250 mm; Dionex) using a pulsed amperometric detector (Dionex ICS 5000 system, USA). The flow rate was 0.4 mL/min; the injection volume was 5 μL; and 0.2 M NaOH and 0.2 M NaAc were used as the solvent. The gradient program was as follows: 90:10 v/v at 0 min, 90:10 v/v at 10 min, 40:60 v/v at 30 min, 40:60 v/v at 50 min, 90:10 v/v at 50.1 min, and 90:10 v/v at 60 min. An ion chromatography system (ICS5000, Thermo Scientific, USA) was used to acquire data, and Chromeleon 7.2 CDS (Thermo Fisher Scientific, USA) was used to process the data. The ratio of starch with different chain lengths was determined by dividing the peak area and the total peak area of DP 6–DP 76. The DP values of the wheat flour were plotted against the percentage of each DP in the total peak area of DP 6–DP 76 to clarify the CLD of starch. A control dataset was obtained, and differences between the peak areas of DP 6–DP 76 between each sample and the control sample were determined.

Statistic analysis

IBM SPSS software was used to conduct one-way ANOVA, and the experimental data obtained were expressed as the mean "±" standard deviation of three repeated experiments, and the least significant difference (LSD) test was used to analyze the difference. The significance of this experiment is $P < 0.05$ significant.

Results

Content of RS

We detected RS content of 129 wheat accessions and found that the RS content was varying from 0.0705 % to 0.4731 %, with the average of 0.2994 % (Schedule 1). Among them, the RS content of 121 wheat accessions were greater than 0.15 % but less than 0.40 %, 4 accessions were greater than 0.05 % and less than 0.15 % and 4 accessions were greater than 0.40 % (Fig. 1A).

We identified 3 accessions with highest content of RS (CA12017, Gaoyuan 448 and JM18, ranged from 0.4209 % to 0.4731 %) and 3

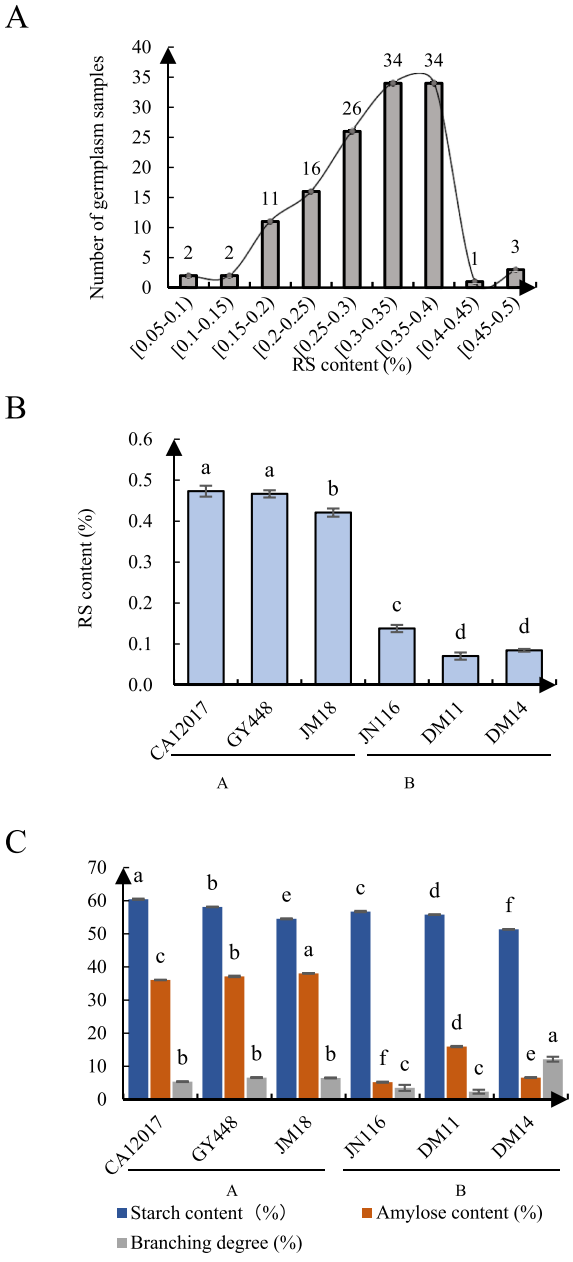

Fig. 1. (A) The distribution of the content of RS in 129 wheat accessions. (B) Two groups of wheat germplasm with different content of resistant starch. (C) Two groups of different resistant starch content of wheat grain starch content, amylose content, and branching degree. Different letters above bars indicate significant differences between wheat samples ($P < 0.05$). GY 448: Gaoyuan 448; JM 18: Jingmai 18; JN 116: Jinuo 116; DM 11: Dianmai 11; and DM 14: Dianmai 14.

accessions with lowest content of RS (Jinuo 116, DM11 and DM14, ranged from 0.0847 % to 0.1378 %) (Table 1).

The difference between these two groups (designated hereafter group A and group B) was significant (P value = 0.05) (Table 1, Fig. 1B). The content of RS was significantly (P value = 0.05) higher in CA12017 and GY448 than in JM18, and it was significantly (P value = 0.05) higher in JN116 than in DM11 and DM14. No significant (P value = 0.05) difference was observed between DM11 and DM14. These two groups were selected for further analysis on the total starch content, amylose content, starch CLD, starch branching degree, starch grain structure, and starch crystallinity.

Starch content

We further investigated and compared of total starch content in the high RS and low RS wheat accessions, the total starch of these two groups was ranged from 51.3487 % to 60.4475 % (Table 1). The total starch content of high RS wheat accessions CA12017, GY448 and JM18 were 60.4475 %, 58.1176 % and 54.5229 %. The total starch content of low RS wheat accessions Jinuo 116, DM11 and DM14 were 56.7304 %, 55.7926 % and 51.3487 %. No significant correlation was observed between the content of RS and the total starch content (Fig. 1C).

Amylose content

The amylose content (AC) of these six wheat accessions ranged from 5.2061 % to 36.0691 % (Table 1). The AC was significantly higher in high RS wheat accessions CA12017, GY448, and JM18 than in low RS wheat accessions JN116, DM11, and DM14. The AC was significantly higher in JM18 than in GY448 and CA12017. The AC was significantly higher in DM11 than in JN116 and DM14. Combining the two groups with significant difference in RS content, it can be found that the amylose content of wheat germplasm in group A with significantly higher RS content is extremely significantly higher than that of three wheat germplasm with low RS content in group B. It is concluded that the amylose content of wheat germplasm is positively correlated with RS content (Fig. 1C, Table 1).

Branching degree

The starch branching degree of the six wheat accessions ranged from 2.3323 % to 12.1468 % (Table 1). The RS content was the lowest and the starch branching degree was the highest in DM14. The starch branching degree was significantly higher in CA12017, GY448, and JM18 than in JN116 and DM11. The starch branching degree of DM14 was significantly higher than that of CA12017, GY448, JN116, DM11, and JM18. The starch branching degree of CA12017, GY448, and JM18 was significantly higher than that of JN116 and DM11. Therefore, according to the data analysis, there was no significant correlation between starch branching degree and RS content (Fig. 1C, Table 1).

Table 1
Total starch content, amylose content, branching degree of amylopectin, crystallinity of wheat grains with a high RS content (A) and wheat grains with a low RS content (B).

physicochemical properties	Group A	GrouB	F Value	P Value
RS content (%)	0.45 ± 0.03	0.098 ± 0.35	183.82**	0.00
Starch content (%)	57.70 ± 2.98	54.62 ± 2.87	1.65	0.27
Amylose content (%)	37.09 ± 0.99	9.26 ± 5.85	66.02**	0.00
Branching degree (%)	6.16 ± 0.68	5.99 ± 5.36	0.00	0.96

Note: Group A: CA12017; Gaoyuan 448; Jingmai 18. Group B: Jinuo 116; Dianmai 11; Dianmai 14. " *": significance at 5%. " **":means significance at 1%.

Crystal type and relative crystallinity of starch

Starch granules comprise two components: the crystalline phase and the amorphous phase. XRD analysis confirmed the presence of peaks in the crystalline phase. According to the XRD curve, the crystal shape of starch was divided into type A, type B, type C, and type V crystals. Peaks at 15.3°, 17.1°, 18.2°, and 23.5°; 17.2°, 22.2°, and 24.0°; 15.3°, 17.3°, and 18.3°; and 7.36°, 13.1°, and 20.1° were typically observed for type A, type B, type C, and type V crystals, respectively. As shown in Fig. 2, the X-Ray diffraction patterns of the two groups of wheat germplasm resources in this study were highly consistent, with four distinct peaks at 15.3°, 17.1°, 18.2° and 23.5°, respectively, indicating that the crystal types of the six wheat germplasm resources were the same, excluding the possibility that the six wheat germplasm resources were class B, C and V crystals. The starch crystal type of six wheat germplasm resources in groups A and B observed in the experiment is type A, so it is not certain whether the crystal type has a significant correlation with RS content according to the current data. Data analysis was performed using MDI Jade 5.0 software. The characteristic parameters of degree of crystallinity (%), crystal morphology and 2θ diffraction Angle were calculated respectively. (Diffraction peaks at 2θ value (angle)). Through software calculation, the relative crystallinity of 6 wheat starch grains was 31.83 % to 38.41 % (Fig. 2).

Morphology of starch granule

We used Scanning Electron Microscope (SEM) to study the starch granule of the six selected wheat accessions. According to the SEM images (Fig. 3), the starch granules were spherical in both group A and group B, which was consistent with previous studies (Lindeboom et al., 2004), and there was no significant difference in starch grain size between these two groups (the width of wheat starch grain flat surface of the two groups varied between 20 and 22 μm). The main difference was reflected by the starch grain surface morphology. We have conducted three pairwise studies: CA12017 versus DM14, JM18 versus JN116 and GY448 versus DM11. We observed that the starch particle surfaces of CA12017, JM18 and GY448 were smooth, while the surfaces of DM14, JN116 and GY448 either had obvious folds, uneven, or had holes and depressions. Based on the SEM results, we hypothesize that wheat with high RS content is associated with smooth starch granules while wheat with low RS content is associated with uneven starch particles that have more folds and holes on the surface.

Amylopectin CLD

According to the DP, the amylopectin chains can be divided into four fractions: fa (DP ≤ 12), fb 1 (13 ≤ DP ≤ 24), fb 2 (25 ≤ DP ≤ 36), and fb 3 (DP ≥ 37) (Fujita et al., 2007).

To reveal the detailed amylopectin branch structure of high RS and low RS wheat accessions, starch was debranched and analyzed by capillary electro-phoresis. Compared with the chain length distribution pattern, there was no significant difference in short chain, including fb 1 chain and fb 2 chain among the six wheat lines with different RS content. The long chain, fb 3 chain content of wheat germplasm with low RS wheat accessions was significantly higher than high RS wheat accessions. The result showed that there was significantly negative correlation between RS content and fb 3 content (Table 2, Fig. 4).

Discussion

Wheat is the most important crop among the big three cereal crops and is planted on a massive scale across the globe, serving as main source of daily nutrition for large population globally. The biochemical trait analysis considered a major option for better estimation of germplasms' intrinsic values and the corresponding response can further be utilized for better cultivar selections.

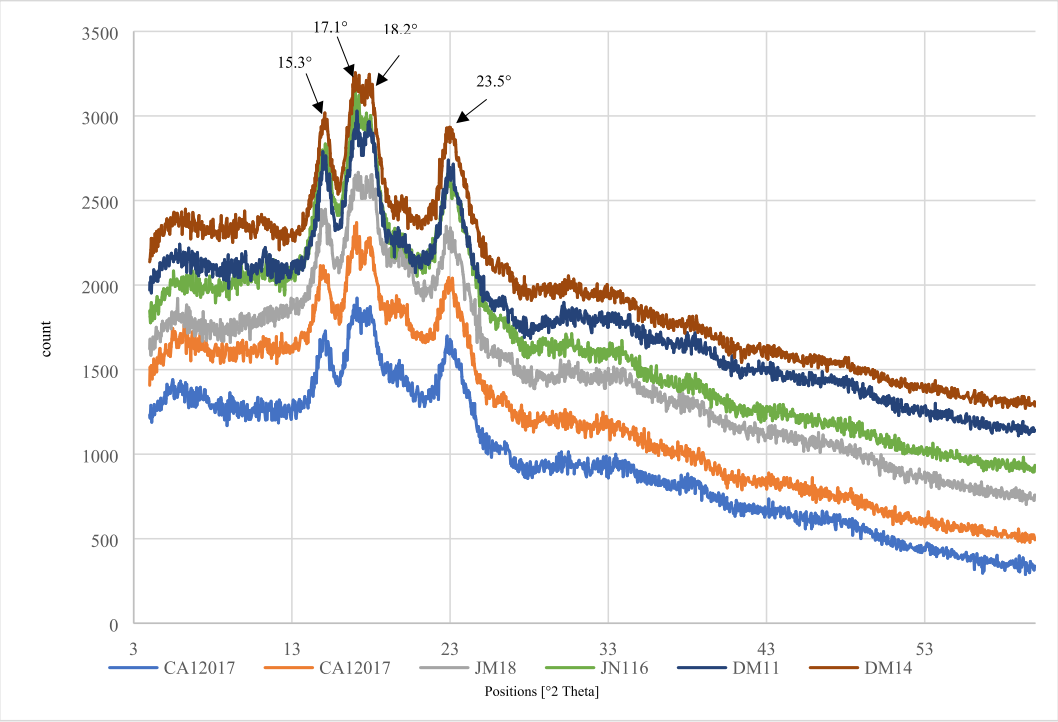

Fig. 2. XRD pattern of wheat accessions with a high RS content (A) and a low RS content (B). GY 448: Gaoyuan 448; JM 18: Jingmai 18; JN 116: Jinuo 116; DM 11: Dianmai 11; and DM 14: Dianmai 14.

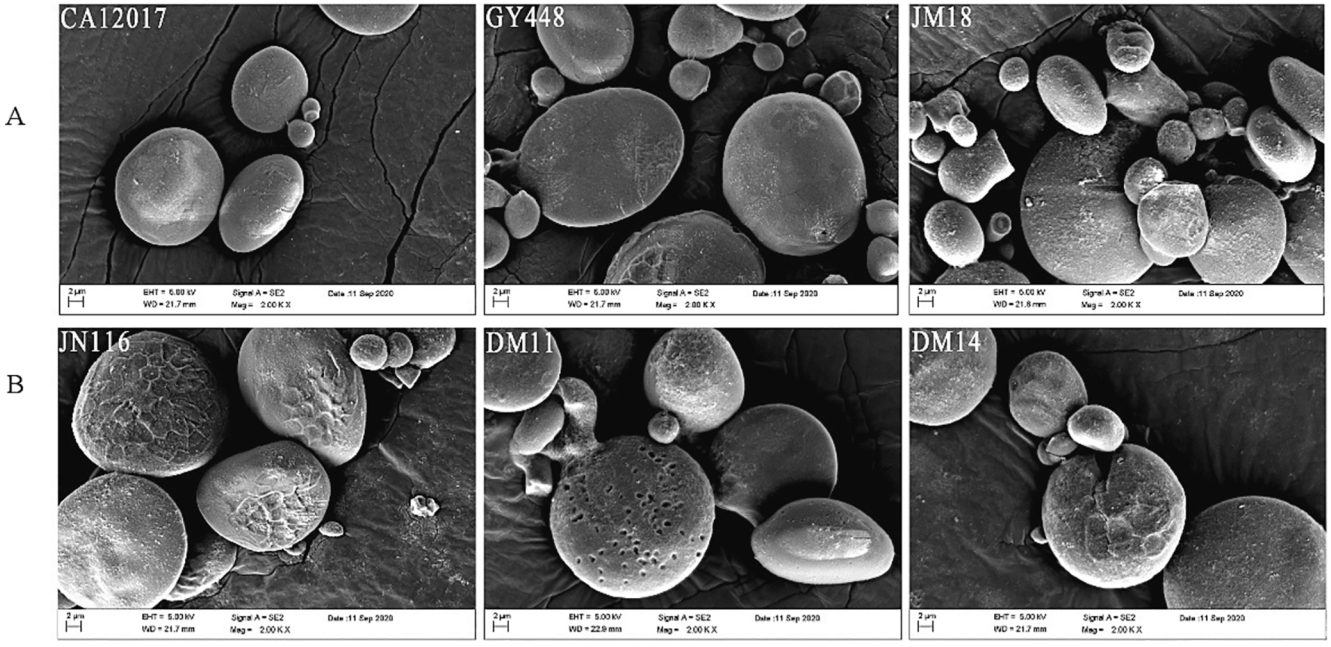

Fig. 3. Scanning electron microscopy (SEM) of the starch granules of wheat accessions with a high RS content (A) and a low RS content (B) (3000 ×). GY 448: Gaoyuan 448; JM 18: Jingmai 18; JN 116: Jinuo 116; DM 11: Dianmai 11; and DM 14: Dianmai 14.

In this study, most spring wheat accessions that collected from major wheat regions in China were planted in Yunnan, and the resistant starch content data of 129 wheat samples were obtained. The RS content in these raw wheat grains was under 1.0 %. Previous studies showed that the resistant starch content of commercially viable varieties of wheat was under 0.9 % (Alsamadany et al., 2022). Compared with other cereal crops, the RS content in wheat seems a bit low. Given the fact that wheat is the worldwide staple crop and main daily intake energy source, improving and increasing its RS content presents a greaty opportunity for potentially large-scale benefits in public health. Therefore, collecting and understanding the resistant starch content of wheat germplasm, which is significant for their usage in cultivating high resistant starch wheat varieties.

Here, the content of resistant starch in 129 wheat accessions varied from 0.07 to 0.47 %, which was consistent to the RS yield in previous report (Hazard et al., 2012). Ninty wheat accessions had higher content

Table 2
Comparison df chain length distribution of polymerization in starch.

Degree of polymerization	Group A	Group B	F Value	P Value
DP6-12 (fa)	58.83 ± 0.31	57.73 ± 1.13	2.59	0.18
DP13-24(fb1)	30.66 ± 0.13	30.35 ± 0.54	0.97	0.38
DP25-36(fb2)	7.66 ± 0.28	8.16 ± 0.21	6.12	0.07
DP ≥ 37 (fb3)	2.85 ± 0.08	3.76 ± 0.44	12.74*	0.02

Note: Group A: CA12017;Gaoyuan 448; Jingmai 18, Group B: Jinuo 116; Dianmai 11; Dianmai 14. " *" means significance at 5%.

of resistant more than Yunmai and Dianmai accessions. Among these 90 accessions, 33 % accessions were from Xinjiang winter and spring wheat sowing area, 10 % accessions were from Huang-Huai winter wheat area and 10 % accessions were from Northwest spring wheat area, China. It was confirmed in the previous studies that ecological factors in planting areas have a significant impact on resistant starch yield in cereal grains (Wang et al., 2021; Tian and Sun, 2020). Xinjiang winter-spring wheat area and the Northwest spring wheat area have higher altitudes and a large temperature difference between day and night, whereas Huanghuai winter wheat area have lower altitudes, which may beneifical for RS yield than other wheat production regions in China.

Starch can be divided into amylose and amylopectin according to the structure of starch chains, among which amylose generally accounts for 5–40 % of the total starch content, while the relative amylopectin accounts for 60–95 % of the total starch content (Buléon, Planchot, & Ball, 1998). In this study, amylose content varied from 5.21 to 38.06 %, and there was a very significant positive correlation between amylose content and resistant starch content, which was consistant to the previous stud (Li et al., 2021). In previous studies on cereals, amylose content is often regarded as a measure of starch digestibility. It has been confirmed in sorghum, corn, rice and beans that amylose content will affect the digestibility of starch, such as gelatinization characteristics and disintegration value (Wang et al., 2012). Given starch microstructure, amylose is mainly composed of glucose units connected by α-1, 4-glucoside bond to a single dextran chain or several long dextran chains (Vandeputte et al., 2003). On the other hand, amylopectin is a glucose backbone linked by the glucose main chain connected by the short intramolecular α-1, 4-glucoside bond and then connected by the α-1, 6-glucoside bond at the branch points, forming a highly branched bundle structure, which is less prone to aggregation and aging (Fu et al., 2013). The aging of starch is irreversible and cannot be restored to the pre-aging state by gelatinization again. After aging, the starch not only tastes worse, but also the digestion and absorption rate is reduced. The proportion of amylose content that is easy to age, which leads to the difficulty of digestion of starch. Therefore, amylose content is feasible parameter in high RS germplasm evaluation.

Starch particles are generally crystalline, and the lamellar structure of starch is usually composed of a crystal layer and an amorphous layer by alternating arrangement with each other. The crystal types of starch of different plants are also different. In general, the crystal morphology is the same in same crop, most cereal starch belongs to type A, and most rhizome starch belongs to type B. However, legume starch was mostly C-type (Zhang, Luo, Huang, Huang, & Guan, 2010). The size of starch particles has a significant impact on the quality and shape of separated starch, and is related to the digestibility and taste of starch products. The differences in diffraction spectra and crystallinity of starch particles are mainly related to the variety, extraction process, agronomic conditions, and cultivation conditions. The starch crystal type of the six wheat germplasm resources with high RS and low RS were all type A, and there was no significant difference in the relative crystallinity of the 6 wheat accessions here.

Starch exists in the form of granules in wheat endosperm, and the structure and distribution of starch granules play a decisive role in starch quality (Yan, Li, Li, & Wang, 2016). In this stduy, the wheat starch granules with different resistant starch contents in the two groups were spherical in shape, and the starch grain width varied between 20 and 22 μm, which was consistent with the reported research (Lindeboom et al., 2004). No significant difference was observed in grain size between the high RS and low RS groups, but it was found in the images amplified by electron microscope that the surface of wheat starch particles with higher resistant starch content was smoother, while those with lower resistant starch content had more holes and folds on the surface, which was similar to Ho & Wong's study in fruit (2020). The results were also consistent with morphology of starch granules in legumes (Li et al., 2011).

Amylose has fewer branches, and CLD is generally divided into DP-100–500, DP500-5000, and DP5000-20000 three regions, which are much longer than amylose. However, there are more branches of amylopectin. According to the composition of starch chains, they can be classified into A chains, B chains, and C chains. Chain A is an outer chain without inner branches and is connected to other parts through α-D-1,6-

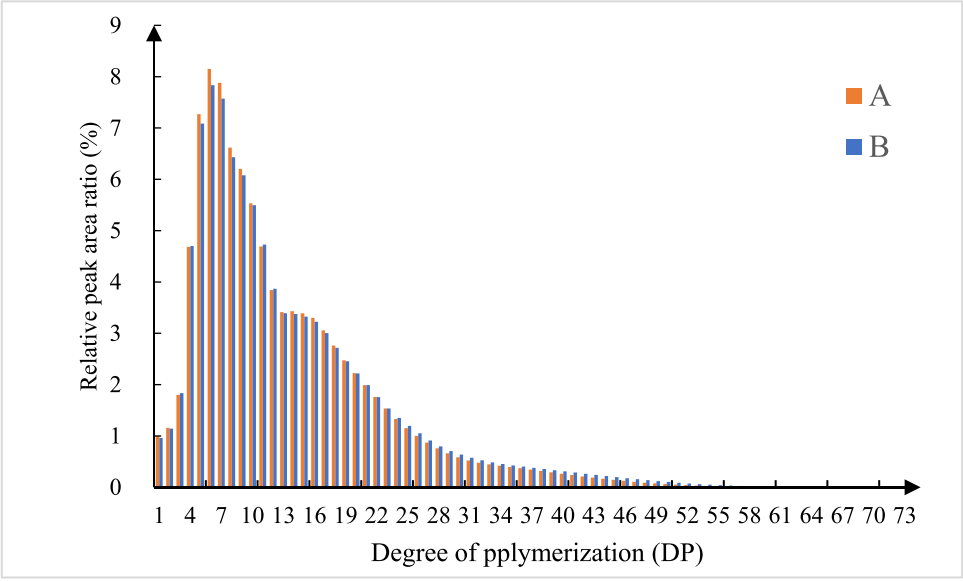

Fig. 4. The distribution of CLDs of wheat accessions with a high RS content (A) and a low RS content (B). JM 18 was used as a reference. GY 448: Gaoyuan 448; JM 18: Jingmai 18; JN 116: Jinuo 116; DM 11: Dianmai 11; and DM 14: Dianmai 14.

glucoside bonds. Chain B is connected to one or more other chains. Chain C is connected to the non-reducing end and each macromolecule contains only one C chain. However, this classification method does not provide a direct observation of the number of starch chains with different degrees of polymerization (DP). Based on Hizukuri et al.'s classification (1981), amylopectin was divided into fa: DP $\leq$ 12; fb 1:13 $\leq$ DP $\leq$ 24; fb 2:25 $\leq$ DP $\leq$ 36; fb 3:37 $\leq$ DP. The branched chain length of amylose was determined using the HPAEC-PAD method while excluding the influence of amylose on chain length distribution. Our research focused on analyzing the distribution of branch lengths in amylopectin. The results showed that there was no significant difference in the content of fa, fb 1, and fb 2 chains of the two groups of with different content of resistant starch wheat accessions, and the contents of the fb 3 chain in high RS wheat accessions were significantly lower than that in low RS accessions. Previou studies showed that the content of resistant starch in rice starch was negatively correlated with DP12-24 chain; the ratio of DP 6–12 was negatively correlated with RS_2 (Chung et al., 2011; Zhou et al., 2018). A significant increase in the proportion of long chains (DP $\geq$ 37) was found in rice mutants with very high RS_2 content (Nakamura et al., 2015), which is contrary to the results of this experiment. In the study on starch gelatinization, Hanashiro et al. (2002) proposed that fa did not change when the crystal melted, while fb1 did, indicating that the short chain was not easy to form a double helix structure, but interfered with the crystalline structure and caused defects in the crystalline layer of the particles. The aging of amylose at different temperatures showed that the amylose microcrystals obtained at higher temperature were composed of longer chain length. This is consistent with the effect of growth temperature on the length of branched chain of amylopectin. The amylopectin synthesized at higher temperature is composed of longer B chain. The mechanism of starch biosynthesis and the growth of aggregates need further study.

Conclusions

The RS content of 129 wheat accessions was detected in present study. The amylose content of these wheat germplasm with higher RS content was significantly higher. The starch granules of wheat accessions with high RS content were smoother, and there were no wrinkles and holes on the granule surface. The amount of fb 3 chains (DP $\geq$ 37) of starch was inversely proportional to the RS content. Our results provide fundamental information of RS content in spring wheat accessions and indepth insight of intrinsic molecular structures in high and low RS wheat accessions.

Conflict of interest

The authors declare no competing financial interest.

CRediT authorship contribution statement

Xingchen Liu: Conceptualization, Writing – review & editing, Writing – original draft. **Liang Qiao:** Data curation. **Yixi Kong:** Investigation. **Huiyutang Wang:** Investigation. **Baoju Yang:** Conceptualization, Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101103.

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