Study on physicochemical properties of purple waxy wheat starch

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ABSTRACT
Morphological features and physicochemical properties of purple waxy wheat 168 (PWW168) starch were compared with those of normal wheat and maize starches. The morphologies of PWW168 were found to be similar for normal wheat starch. All three crop starches exhibited typical A-type crystallinity. However, PWW168 had lower apparent amylose content and higher degree of crystallinity than normal wheat and maize starches. These differences resulted in a higher light transmittance, swelling power and solution, freeze-thaw stability, peak viscosity, breakdown viscosity, lower retrogradation percentage, and final viscosity and setback viscosity in PWW168 starch. These information suggested that PWW168 should have greater resistance to retrogradation under cooling conditions. Highlighting the differences in physicochemical characteristics of PWW168 starch and normal wheat and maize starches could provide effective application of PWW168 starch in food industry.

Abbreviations: AAC, apparent amylose content; SP, swelling power; FTC, freeze-thaw cycles; PV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SB, setback viscosity; PT, pasting temperature.

Introduction
As one of the indispensable food crops in the world, wheat plays an important role in human health and nutrition. The major ingredient of wheat endosperm is starch (65 ~ 70% of the dry weight), which mainly consists of amylose, amylopectin, and a small amount of proteins and lipids. The ratio of amylose to amylopectin and their structures determine physicochemical properties of starch, and hence have an impact on the properties of wheat flour and the quality of end-use products.\(^1\) Granule size also can make a difference to physicochemical properties of starch. Wheat starch granules usually divide into large, lenticular A-type (diameter\(\geq10 \mu m\)) and small, irregular B-type granules (diameter\(\leq10 \mu m\)). The former account for more than 70% of the total starch weight, but less than 10% of the total number, while B-type granules account for more than 90% of the total number, but less than 30% of the total weight in the wheat endosperm.\(^2\)

Waxy wheat lacks an enzyme responsible for synthesis of amylose, called granule-bound starch synthase I (GBSSI, known as a waxy protein).\(^3\) The absence of GBSSI in starch of mutants (called waxy starch) results in the formation of starch granules without amylose molecular and presents. Therefore, the development of waxy wheat has provided a possibility to alter the unique structures and specific properties of starch used in food and other industry applications. The physicochemical properties of starch are related to each other, including granules morphology and size, amylose content, relative crystallinity, swelling, paste transparency, thermal and gelatinization properties and retrogradation.\(^4\) As a relatively new waxy starch...
compared to waxy maize and rice starches, waxy wheat starch has attracted increasing attention, and is superior to normal wheat starch in water retention capacity, swelling properties, resistance to retrogradation and viscosity.\cite{5} Amylose may be concentrated in the central region of the starch granule.\cite{6} It is difficult for amloose to escape when heated with water, which affected the swelling prosperity of starch. Previous studies have been reported that a lower amylose content is responsible for higher value for peak viscosity and breakdown and lower value for final viscosity and pasting temperature.\cite{7} Chao et al.\cite{8} have found the amylose content is highly negatively correlated with transparency, peak viscosity, and breakdown ($p < .05$) and is positively correlated with retrogradation percentage ($p < .01$). It is known that during the retrogradation process, amylose molecules retrograde at a much faster rate and form the skeleton of the starch hydrogel network, imbedded in which the amyllopectin molecules are retrograded at a much slower rate.\cite{9} Zhang et al.\cite{10} have found waxy wheat starch with predominant amyllopectin requires higher energy for gelatinization because of its higher crystallinity when compared with normal wheat starches.

Purple waxy wheat 168 (PWW168), which integrates purple, whole waxy property and high yield into group, has a broad prospect for development. However, to the best of our knowledge, little information is available on the morphological structures and physicochemical properties of starch in PWW168 as a whole new wheat variety. The morphological properties, crystalline structures as well as pasting characteristics of PWW168 were compared with normal wheat and maize starches in order to evaluate the potential of PWW168 as food raw material and provide necessary theoretical basis for the research and development of related products.

**Materials and methods**

**Materials**

Purple waxy wheat 168 was provided by Chengdu Institute of Biology, Chinese Academy of Science (Chengdu, China) and normal wheat (Jimai 22) were purchased from a supermarket in Chengdu, Sichuan, China. Maize (Lianguy 99) starch was purchased by Hongrun Baoshun Technology Co., LTD, Beijing, China.

**Isolation of starch**

Isolation of the starch in waxy wheat and non-waxy wheat were following the alkaline steeping method in early reported study\cite{8} with modifications as described and stored in a desiccator for further analysis. Briefly, wheat seeds of the different varieties were separately taken, ground with multifunction high-speed pulverizer (DFY-500, Dingli LTD, Wenzhou, China). Samples were steeped in 0.1% aqueous NaOH solution with the solid–liquid ratio of 1:4 for 20 h at room temperature and stirred three times during this period. After steeping, these samples were sieved with 100 mesh and centrifuged at 3000×g for 15 min. Then, their supernatant and the top darker layer were discarded. The lower part of the centrifuge tube were starch samples. The starch samples were resuspended in distilled water, stirred, centrifuged as described above. The white starch samples were dried at 40°C for 15 h and ground to powder, and finally passed through a 100 mesh sieve.

**Apparent amylose content of starches**

The apparent amylose content (AAC) of the starch was determined according to ISO standard 6647-1-2007.\cite{11} The absorbance of the solution of starch was measured with UV756 spectrophotometer (Yoke Instrument CO., Ltd, Shanghai, China) at the wavelength of 620 nm. Depending on the standard curve of amylose and amyllopectin (Yuanye Biological Technology Co., Ltd., Shanghai, China) blends, the amylose content of starch was calculated by the measured absorbance.
Morphological properties

The granule morphologies of the starch samples were observed in a scanning electron microscope (SEM, JSM-7500, Jeol Ltd., Japan). Starch samples were suspended in ethanol to obtain 1g/100g suspension. The starch samples were fixed onto an aluminum stub with conductive double-sided adhesive tape, coated with gold/palladium (60:40). An accelerating potential of 10 kV was used to get magnifications.

Crystalline structures determined by X-ray diffraction (XRD)

The crystalline structures were analyzed using X-ray diffractometer (EMPYREAN, Panaco Co., Netherlands). In short, the dried samples were equilibrated at 44% relative humidity for 24 h in a sealed desiccator where the saturated potassium carbonate solution was placed at the bottom. Each sample was scanned by an X-ray beam generated with the following conditions: scanning speed, 2°/min; diffraction angle (2θ) 5°–40°; voltage 45 kV and current 40 mA. Relative crystallinity (%) was calculated by the ratio of the peak areas under diffraction curves to the total diffractogram area following the method of Komiya et al. [12]

Paste clarity

The paste clarity was determined according to modified method of Jing. [13] The starch sample was suspended in distilled water at 1% (w/w) and the suspensions were heated in a boiling water bath heating for 30 min with stirring constantly during the incubation, then cooled down to room temperature. The light transmittance T (%) of starches was measured at 620 nm with UV756 spectrophotometer (Yoke Instrument CO., Ltd, Shanghai, China) using water as their control.

Swelling power and solubility of starch

The swelling power and solubility at different temperature were measured following described by Bashir’s method with some modifications. [14] The starch suspensions (2%, w/w) with distilled water as suspension medium were kept at 50°C, 60°C, 70°C, 80°C, and 90°C for 30 min with stirring constantly. The suspension was centrifuged at 4000 × g for 15 min and then the supernatant was discarded. The supernatant was dried to constant weight (W₁) in an air oven at 100°C. The material attached to the wall of the centrifuge tube was weighed (W₂) as sediment. The solubility (S,%) and the swelling power (SP) were calculated with the equation as following,

\[ S(\%) = \frac{W_1}{W_0} \times 100 \]
\[ SP(g/g) = \frac{W_2}{W_0 \times (100 - S)} \]

Where W₁ was solubility of starch (%); W₀, dry weight of starch samples (g).

Freeze-thaw stability of starch pastes

The starch suspensions (5%, w/w) were cooked at 95°C in a water bath for 30 min under continuous stirring and cooled down to the room temperature. The starch pastes were subjected to two times freeze-thaw cycles (FTC) by freezing the sample at −18°C for 16–18 h and subsequently thawing at room temperature for 6 h. These samples were centrifuged at 4000×g for 15 min then the supernatants were discarded. The degree of syneresis was used as a direct measure of the freeze-thaw stability of the starch [15]. The weight of sediment on the wall of the centrifuge tube was measured and the water release was calculated by,

\[ W(\%) = \frac{(M-m)}{M} \]

where W, water release (%); M, weight of starch paste (g); and m, weight of sediment (g).
Retrogradation of starches

Starch pastes (1%) of the samples were prepared and placed into 25 mL test tubes, and stored at room temperature for 48 h. At the same time, the volume of the starch pastes was recorded. During this storage period, the volumes of supernatant or sediments below of the tubes were counted every 4 h. The sedimentation of the pastes was expressed by the change of the percentage of supernatant in the total volume of the pastes over time.

Pasting properties of starches

The pasting properties of different starch samples were measured with Rapid Viscosity Analyzer (RVA 4500, Pertem, Australia). The sample starch (2 g, dry basis) and distilled water (20 mL) were added to the sample groove of the RVA following a previously described method with slightly modified. The sample suspension was equilibrated at 50°C for 1 min, heated to 95°C at 7.5°C/min, maintained at 95°C for 5 min, cooled to 50°C in 7.5 min, and then maintained at 50°C for 1 min. All tests were carried out at a constant frequency of 200 rpm for the whole experiments.

Statistical analysis

The data reported were the means of triplicate measurements and the data obtained were statistically analyzed by SPSS 17.0 software. Significant differences among the different starch samples were tested at p< .05 (LSD method).

Results and discussion

Apparent amylose content and starch granule morphology

Table 1 shows that AAC ranged from 33.32% (maize) to 21.3% (normal wheat) and were significantly higher than waxy wheat starch (1.21%) (p<0.05). The AAC of starch varied with the plant source of starch and was influenced by climate and soil conditions during the growing period and harvest time. It has been known that amylose content is an important index of starch application and affects the functional and physicochemical properties of starch, including its gelatinization, retrogradation, swelling, pasting properties, transparency, and in vitro enzyme digestibility.

The morphology of starch granules from PWW168 seed endosperm was compared to starch granules from the normal wheat and maize in figure 1. Similar feature was found in terms of morphologies between PWW168 and normal wheat. Compared to PWW168 and normal wheat starch with oval and spherical large granules (A-type) and irregular and polyhedral small granules (B-type), maize granules were distinguished by their small size with irregular and round shapes. This result was similar with the observations reported by Hsieh et al. Compared with B-type granules, A-type granules have lower gelatinization temperatures, higher relative crystallinities, transition enthalpy, and higher amylose content. Further, wheat starch granules were observed a compact structure and few intergranular cracks and indentations. The granule shape had much to do with biological sources of isolated starches, which may have an effect on different morphology between wheat and maize

| Variety       | AAC (%) | Paste clarity (%) | Relative crystallinity (%) |
|---------------|---------|-------------------|----------------------------|
| PWW168        | 1.21 ± 0.09 c  | 22.57 ± 1.35 a     | 43.21 ± 1.12 b             |
| Normal wheat  | 25.89 ± 0.22 a  | 11.64 ± 0.57 b     | 21.3 ± 1.24 c              |
| Maize         | 23.26 ± 0.45 b  | 8.81 ± 0.3 c       | 33.32 ± 1.05 b             |

AAC: apparent amylose content. Different lower-case letters in the same column indicate significant difference (p< 0.05).
Besides a small number of structure of honeycombs were captured on the surface of starch granules from three varieties, which was a consequence of alkali extraction treatment.

**Crystalline structure**

A peak characteristic was present in crystalline region, while a dispersion characteristic was shown in amorphous region. PWW168 showed a higher intensity of diffraction at 15°, 17°, 18°, and 23° 2θ than maize and normal wheat starch (figure 2) and gave all typical A-type crystalline structure, which were consistent with the typical characteristics of ordinary cereal starches. A minor peak at 20° 2θ was observed in maize and normal wheat starch, corresponding to the presence of amylose-lipid complex, which was not obvious in PWW168 starch.[10] The relative crystallinity of PWW168, normal wheat and maize starch was 43.21%, 21.3%, and 33.32%, respectively (table 1). Starch crystallinity has been shown to be influenced by amylopectin content, average amylopectin chain length, amylose content, and other factors. The relative crystallinity presented a negative correlation with amylose content and
a positive correlation with amyllopectin long chain content.\textsuperscript{[21]} The higher relative crystallinity of PWW168 starch could be attributed to its higher amyllopectin content and average amyllopectin chain length. The significant difference of starch granules between normal wheat and maize could be explained by the different botanical origin and starch compositions.\textsuperscript{[22]}

**Paste clarity**

Starch paste clarity, as a critical factor limiting the applications of starch, can exert influence on their sensory qualities and appearance. PWW168 (22.57\%) had the highest paste clarity compared to normal wheat starch (11.64\%) and maize starch (8.81\%) (table 1). Clarity reflected swelling power and dispersion of starch granules in water. The higher clarity of PWW168 might be closely associated with paste of uniform dispersion and lack of gel beam, when the light through the paste will not scatter and reflect. Also, swelling power showed positively correlated to transmittance and negatively correlated to syneresis.\textsuperscript{[13]} Other factors that influenced the clarity were lipid content, amylose/amyllopectin ratio, and phosphate. The retrogradation of amylose will accelerate aggregation and aging process, which in turn decrease transparency of starch paste.\textsuperscript{[23]} Amylose-lipid complex may increase the opacity or turbidity of starch paste.\textsuperscript{[24]} Due to the lack of these ingredients, the swelling properties and transparency of PWW168 were much higher than the other starches.

**Swelling power (SP) and solubility of starch**

Swelling power (SP) and solubility of starch samples at different temperatures (50 ~ 90°C) are shown in figure 3 A and B. SP and solubility of starches from three examined starches ranged from 7.37 to 37.52 g/g and 6.83\% to 91.25\%, respectively. Up to 60°C, the SP and solubility of starches from different cultivars were similar, but major differences between PWW168 and two normal starches were observed at temperatures higher than 70°C. The solubility of two normal starches did not change at all 70°C, while that of PWW168 increased strongly at temperatures >60°C. PWW168 showed an initial swell stage and a rapid swell stage; hence, it belonged to typical two-stage-swell process. The starch used in this experiment was mostly composed of amyllopectin which could swell at lower temperature, confirming the swelling power of waxy starches showed higher values than those of normal starches.

Swelling and solution provided evidence of the degree of interaction between starch chains in amorphous and crystalline regions.\textsuperscript{[25]} Besides its lower clarity, the lower SP of normal wheat starch

![Figure 3](image_url)

*Figure 3. (A) Swelling power and (B) solubility of starch samples.*
was also affected by the presence of impurities (fat and protein). Amlose acted as an inhibitor of swelling by strengthening the internal network of the starch granules, especially in the presence of lipids, that could explain why PWW168 showed higher SP. The amylpectin is likely to swell due to the weakness of the intra- and inter-molecular coherence in starch, while as, amylose acts as an inhibitor of swelling. The difference in SP and solubility of starches may also be caused by the difference in morphological structure, various particle sizes, properties and intensity of the three-dimensional network of micelles in starch granule and chain length of starch granules.  

**Freeze-thaw stability**

The degree of syneresis of starch pastes subjected to two FTC presented in figure 4, and the appearances were also carefully observed. In general, the syneresis increased with the number of FTC and varied with botanical sources. Higher syneresis degree of maize and normal wheat starch reached to 38.52%~46.28% after the first FTC. Amounts of separated water from pastes were exuded and the pastes became opaque. And syneresis of these starches increased significantly after the second FTC, reaching 55.9-61.79%. The freeze-thaw stability of maize paste was better than that of normal wheat (p<0.05).

On the contrary, starch generated from PWW168 could maintain the translucent form accompanied with a relatively slow rising tendency in syneresis after treatment of FTC (20.56% ~33.44%). Compared to maize and normal wheat starches, PWW168 starch paste expressed a greater cold-storage stability that could contribute to remain water in gelatinized starch with slight separation of free water. Syneresis in a freeze-thawed gel was caused by an increase in molecular rearrangement and association between starch chain structure-especially retrogradation of amylose, which resulted in the expulsion of water from the gel structure. Amylopectin with its higher water holding capacity and anti-retrogradation ability was able to reduce the formation of spongy structure in freeze-thawed. It is known as that normal wheat starch contains more phospholipids than other starches. The limited swelling caused by phospholipids may accelerate the retrogenesis, contributing to the higher degree of syneresis of normal wheat starch. According to a previous reporter, the swelling property has a negative correlation with syneresis, which was consistent with the view of this article. Freeze-thaw stability is one of the

![Figure 4](image_url)

*Figure 4. Changes in degree of syneresis of starches subjected to freeze-thaw treatment.*
crucial determinants for their use as the clean-label ingredients in frozen food products.\textsuperscript{[19]} PWW168 has potentials to be used as a clean-label food ingredient with improved cold-storage stability.

**Retrogradation**

The starch pastes retrogradation percentages of the samples are shown in figure 5. The retrogradation volumes of starch pastes had a direct reflection on reaggregating into gel at room temperature (25°C) after starch gelatinization. In contrast to gelatinization, sedimentation was the process that rearrangement of amylose and amylopectin molecules formed microcrystal strands connected by hydrogen bonds.\textsuperscript{[31]}

In this study, the retrogradation percentages of normal wheat starch and maize starch were faster during the initial 8 h standing and then increased slowly until the final retrogradation percentages. The starch paste of PWW168 starch was very stable and almost no coagulation occurred. After 48 h of resting, the average retrogradation percentages only reached 6.32%. The percentages of retrogradation of normal wheat starch and maize starch were obviously higher than that of PWW168 starch, which probably arose from the differences among their amylose contents. PWW168 starch with lower retrogradation percentage displayed strong water holding capacity. Easily retrograde starches could lead to the low digestibility and high hardness, which limited the application of starches in food industry. However, PWW168 had the characteristics of high transparency and low regeneration rate, and can be used as a good raw material resource for drinks and thickeners.

**Figure 5.** Retrogradation curves of the starch samples.

| Variety       | PV (cP)     | TV (cP)     | BD (cP)    | FV (cP)    | SB (cP)    | PT (°C)     |
|---------------|-------------|-------------|------------|------------|------------|-------------|
| PWW168        | 1899 ± 37   | 723 ± 23    | 1176 ± 32  | 760 ± 12   | 37 ± 2     | 71.5 ± 0.3  |
| Normal wheat  | 58 ± 4      | 43 ± 3      | 15 ± 2     | 1712 ± 27  | 1669 ± 24  | 89.9 ± 0.2  |
| Maize         | 340 ± 11    | 99 ± 8      | 241 ± 12   | 1680 ± 21  | 1581 ± 31  | 83.2 ± 0.2  |

PV = peak viscosity, TV = trough viscosity, BD = breakdown viscosity, FV = final viscosity, SB = setback viscosity, PT = pasting temperature. Values sharing a common letter within the same column are not significantly different (p< 0.05).
**Pasting properties of starches**

The pasting properties of the starch samples analyzed with RVA are summarized in table 2. The starch of PWW168 had higher PV (1899cP), TV (723 cP) and BD (1176cP), but lower FV (760cP), SB (37cP) and PT (71.5°C) than maize and normal wheat starches. With an absence of amylose-lipid complexes, frequent granules’ mutual frictions of PWW168 contributed to starch granule swelled quickly (lower PT) and notably (higher PV).\textsuperscript{32} The normal wheat starch with the highest PT (89.9°C) illustrated higher resistance to swelling and fracturing.\textsuperscript{33} The starch of PWW168 had the highest BD with the decreasing viscosity, which may be explained by the lower amylose content. Their granules could be easily dispersed and broken while absorbing water and swelling, resulting in faster shear thinning and higher BD.\textsuperscript{34} Previous studies have shown that waxy starch with low AAC also exhibited greater PV followed by a more noticeable BD and a lesser degree of SB during pasting which is in agreement with our present study.\textsuperscript{10, 19, 35} The more notable SB of maize starch (1581cP) and normal wheat starch (1669cP) accompanying with high amylose contents than PWW168 revealed that these starches weakened starch pastes thermo-stability in heat and mechanical shearing processes and enhanced retrogradation tendency of starches during the cooling. In other words, amylose acted as a major contributor to SB. After gelatinization, amylose molecules dissolved during the heating process could reaggregate more easily compared to amyllopectin, and act as a dominating contributor to remarkable FV and SB.

**Conclusion**

The morphology observations, structural features, and physicochemical characteristics of PWW168 starch were investigated and compared with those of normal wheat and maize starches. PWW168 starch exhibited morphology features and a crystal type that were similar to those of normal wheat starch. However, PWW168 starch contained a lower amylose and higher degree of crystallinity than normal wheat and maize starch. As a result, PWW168 had a higher light transmittance, swelling power and solubility, freeze-thaw stability, peak viscosity, breakdown viscosity, but a lower retrogradation percentage and final viscosity and setback viscosity. These results suggested that PWW168 starch could have better water retention capacity and resistance to retrogradation under cooling condition. These unique and specific properties were favorable for improving high-quality cold-storage stability. The study highlighted the differences in the physicochemical properties of PWW168 and normal wheat and maize starches, which will provide helpful information and be exploited as a new source for industrial utilization of PWW168 starch.

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**Disclosure statement**

*The authors have declared no conflict of interest.*

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