Diverse effects of nitrogen fertilizer on the structural, pasting, and thermal properties of common buckwheat starch

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Abstract

At present, the yield of common buckwheat, which is mainly grown in northern Shaanxi of China, is low and the grain quality is poor. Nitrogen is an important nutrient for the growth of common buckwheat, and appropriate nitrogen application can improve the grain quality. Nitrogen fertilizer could alter the starch granule morphology shapes and the granule size distribution. With increasing nitrogen levels, branch number, flower clusters number, grain number per plant, contents of protein and fat, size distribution of “C” granules, and percentages of light transmittance significantly increased, whereas amylase content and retrogradation decreased. All the samples displayed typical A-type X-ray diffraction patterns. Starch showed higher pasting temperature and gelatinization enthalpy but lower trough and final viscosities under high nitrogen levels. These results suggested N2 treatment was more suitable for common buckwheat growth, principal components and correlation analysis revealed that nitrogen fertilizer significantly affected the physicochemical properties of common buckwheat starches.

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1. Introduction

Common buckwheat (Fagopyrum esculentum M.) belongs to the Fagopyrum of Polygonaceae, originating from China [1], which is a very popular traditional crop and is widely grown in Asia, Europe, and America [2], and this crop has been extensively studied due to its high nutritional contents, such as starch, protein, lipid, and dietary fiber. In addition, it is receiving increasing attention as a potential material for enhancing marketing opportunities [4], and the seeds are used as additives to improve the quality of bread as enriching in zinc, copper and manganese.

Genetic background, environmental conditions, and agricultural treatments are reported to be responsible for the variation in composition, internal structure, and physicochemical properties of the starch [5]. Nitrogen is an important nutrient for the crop growth, and appropriate nitrogen can maintain and improve the crop quality [6], whereas the qualities of grain and cooking can decrease under high nitrogen levels [7]. Nowotna et al. [8] find that nitrogen fertilizer has a significant effect on the protein content and grain yield, and the protein content is significantly positively correlated with nitrogen fertilizer. Many studies also suggest that nitrogen fertilizer affects the functional characteristics of starches. Gu et al. [7] have found that the peak viscosity (PV), cool paste viscosity, and breakdown (BD) viscosity of rice starch can significantly decrease with increasing nitrogen levels. Wenhao et al. [9] believe that the starch granule accumulation during grain filling can be influenced by applying nitrogen fertilizer. Therefore, it is essential for improving the quality traits and yield of common buckwheat to understand the important quality traits and select appropriate nitrogen levels.

To date, few of the researches have studied the effects of various nitrogen levels on the agronomic traits and starch physicochemical properties of common buckwheat. Therefore, the objective of this study was to investigate the agronomic traits and starch physicochemical properties of common buckwheat affected by various nitrogen levels. The research was of critical importance for its potential use in modern common buckwheat production systems.

2. Materials and methods

2.1. Plant material and experimental design

The variety of Xinong 9976, bred by the Northwest A&F University, was selected in this study. Field experiment was conducted in Yulin Academy of Agricultural Science, Shaanxi, China (38°22’ N, 109°44’...
E) at common buckwheat growing seasons in 2017 and 2018. The soil in the test site was a typical sandy loam with organic matter 23 g/kg, total nitrogen 15 g/kg, total phosphorus 28 g/kg, total potassium 41 g/kg, available nitrogen 19.29 mg/kg, available phosphorus 1.82 mg/kg, available potassium 21.65 mg/kg, and the soil pH value was 8.76. The former crop was peas to common buckwheat.

The experimental design was a randomized block design with three replications in each year. The plots were assigned to four nitrogen fertilizer levels: N_0 (0 kg/hm^2), N_1 (90 kg/hm^2), N_2 (180 kg/hm^2) and N_3 (270 kg/hm^2). Plot size was 5 m long × 2 m wide. Sowing were performed on 10 June 2017 and 16 June 2018, and harvests were both performed on 5 October.

2.2. Measurement of agronomic traits

Main agronomic traits of common buckwheat were determined following the method of Fang et al. [10] when the seeds were mature. Five plants were randomly selected from each plot to investigate the plant height, section number, branch number, flower clusters number and grain number per plant during harvesting.

2.3. Chemical composition of seeds

Common buckwheat seeds under different treatments were shelled, and then pulverized with a high-speed universal crusher (FW100, Taisite LTD, Tianjin, China) and passed through the 100-mesh sieve to prepare common buckwheat flour. The contents of protein, fat and starch were obtained followed the method of Yang et al. [11]. The flavonoid content was measured according to Yu et al. [12] and the amylose content was measured following the previous report by Gao et al. [13].

2.4. Starch isolation

Common buckwheat starches were isolated through the method of Gao et al. [13]. 500 g flour was mixed with 0.3% NaOH solution and kept at 25 °C for 24 h to obtain a starch suspension. The suspension was sifted through a 200-mesh sieve and centrifuged (4000 rpm, 10 min) for 3 times. Then scraped the gray material off the top until only the white material was left. The precipitate was mixed with distilled water and neutralized with 0.1 mol L^-1 HCl to pH 7.0. Then the sediment was transferred to a beaker, dried at 40 °C for 24 h, and screened with a 100-mesh.

2.5. Scanning electron microscopy (SEM)

Starch granule morphology was observed using scanning electron microscopy (JSM-6360LV, Jeol, Japan). The starch samples were mounted on an aluminum stub and then sputter coated with gold. The working voltage and accelerating voltage were 100 V and 15 kV, respectively.

2.6. Granule size analysis

The granule size distribution of common buckwheat starch was measured using a laser diffraction particle size analyzer (Malvern Instruments Ltd, Worcestershire, UK) following the method of Gao et al. [14]. The specific analysis conditions were as follows: Weighed 50 mg starch, suspended it in distilled water and dispersed it with ultrasonic wave, then fed it into the samplete. Ultrapure water was used as the solvent, the shading coefficient was 1.3330, and the starch shading coefficient was 1.50; the shading range was 12%–17%, and the instrument was cleaned and calibrated before each sample was measured.

2.7. X-ray diffraction (XRD)

The crystalline structures of common buckwheat starches were analyzed with an X-ray diffractometer (D/Max 2550 VB+, Rigaku Corporation, Rigaku, Japan) following the procedure described by Chao et al. [15]. Measurements were collected at 40 kV and 40 mA with a scanning rate of 10°/min and a diffraction angle range from 5° to 50° (2θ).

2.8. Water solubility

Water solubility was measured following the method of Wang et al. [16]. 50 mg dry starch and 25 mL distilled water were added into the 45 mL centrifuge tubes, and heated in a 60 °C, 70 °C, 80 °C and 90 °C water bath for 30 min, every 5 min during the period of oscillation, after cooling to room temperature, out of the water bath pot with large capacity, centrifuged at 3800 r/min for 20 min. Poured the supernatant into a container with known mass, and baked it to constant weight at 105 °C and weighed it to calculate the solubility of buckwheat starch. Solubility = the weight of baking the supernatant × 4.

2.9. Retrogradation

Percentage of retrogradation was determined following the method of Karim et al. [17]. 20 mL of 1% starch paste was prepared and placed it in a graduated tube with a stopper. Then stored at room temperature for 24 h, and measured the supernatant volume of the tube every hour. Ploted the change curve of the volume percentage of supernatant with time.

2.10. Light transmittance

Weighed 1.0 g starch and prepared starch emulsion with mass concentration of 1.0%. Boil water bath for 15 min and made it gelatinize completely. After water bath, cooled it to 25 °C and added distilled water to the original scale. The light transmittance was measured with a spectrophotometer at 620 nm, and the reference solution was distilled water.

2.11. Pasting properties

Pasting properties of common buckwheat starch st were performed by rapid viscosity analyzer (Perten, TechMastet, Sweden). Briefly, starch suspension (8.0% solid content) was subjected to a heating (50 °C–95 °C) and cooling (95 °C–50 °C) program as described by Zhang et al. [18]. Peak viscosity (PV), trough viscosity (TV), breakdown (BD), final viscosity (FV), setback (SB) and pasting temperature (PT) were obtained.

2.12. Thermal properties

The thermal properties of starches were measured using a differential scanning calorimeter (DSC) (Q2000, Perkin Elmer instruments, USA) according to the method of Gao et al. [13]. Mixed 3.0 mg of the starch sample with 6 mL distilled water into an aluminum pan, sealed the sample and put in refrigerator at 4 °C for 24 h. The sample was heated from 40 °C to 100 °C at 10 °C/min. An empty pan was used as reference. The onset (T_o), peak (T_p), conclusion (T_c) temperature and gelatinization enthalpy (ΔH) were recorded.
2.13. Statistical analysis

The measurements were done in triplicate. Analysis of variance and Duncan’s test were done with the SPSS software (Version 19.0, IBM Corporation, USA). Principal component analysis (PCA) was performed using the Origin software (version 2019, Microcal Inc., Northampton, MA, USA) to summarize differences and similarities among common buckwheat starches at different nitrogen levels.

3. Results and discussion

3.1. Agronomic traits and grain yield

Agronomic traits of common buckwheat were significantly affected by nitrogen fertilizer in both years (Table 1). As the nitrogen fertilizer rate increased from 0 kg/hm² to 270 kg/hm², the plant height increased from 82.4 cm to 99.0 cm in 2017 and from 146.7 cm to 160.3 cm in 2018, respectively. The maximum value of plant height was appeared at N3 treatment in both years. The section number significantly decreased in both years, while the branch number, flower clusters number, and grain number per plant all significantly increased and peaked at N2 treatment with increasing nitrogen levels. Nitrogen fertilizer also influenced the 1000-grain weight and grain yield, and these values all significantly increased with increasing nitrogen levels and showed the largest value at N2 treatment in both years. All the values of agronomic traits in 2017 were lower than those in 2018, which might be related to the difference of temperature and precipitation. These results in our study reflected that moderate nitrogen fertilizer application was beneficial to increase the common buckwheat yield, and the nitrogen level of N2 (180 kg/hm²) was the most suitable for common buckwheat growth in Yulin.

3.2. Chemical composition analysis

As shown in Table 2, the chemical composition of common buckwheat seeds under different nitrogen treatments showed significant variation. Fat content and flavonoid content gradually increased with increasing nitrogen levels, and peaked at N₁ and N₃ treatment, respectively. Increasing nitrogen levels results in a significant decrease in amylose content, which was consistent with the results in our previous report on Tartary buckwheat. Besides, increasing nitrogen levels resulted in a significant increase in protein content from 10.16% to 12.39% in 2017 and from 9.86% to 11.98% in 2018, respectively, and from 82.4 cm to 99.0 cm in 2017 and from 146.7 cm to 160.3 cm in 2018.

3.3. Starch granule morphology

SEM was used to observe the appearance and morphology of common buckwheat starches under different nitrogen treatments. All the common buckwheat starch particles exhibited irregular polygons (Fig. 1). The appearance and morphology of common buckwheat starches can be affected by different nitrogen levels. Common buckwheat starch granules had smoother surface at low nitrogen level, while the granule surface was uneven at high nitrogen levels. A similar result was also found in maize starch granules studied by Wang, White, Pollak, and Jane [20].

3.4. Granule size distribution

Common buckwheat starch granules under various nitrogen levels showed significant differences in size distributions (Table 2). Strach particle size distributions were divided into “A” (>15 μm), “B” (5–15 μm), and “C” (<5 μm) according to Bechtel, Zayas, Dempster, and Wilson [22]. Among the common buckwheat starches, “B” granules accounted for 48.86%–70.09%, followed by the “A” granules (21.58%–44.78%) and “C” granules (6.36%–12.21%). With increasing nitrogen levels, the proportion of “C” granules significantly increased while opposite trend was observed in “A” granules, “B” granules showed a trend of rising first and then falling and peaked at N₁ treatment in both years. Zhu et al. [6] reported that rice starch had higher medium-sized starch granules at higher nitrogen levels, the difference may be related to the genotype between the various crops. In the process of grain development, “A” granules developed earlier, while “B” granules and “C” granules appeared later [23]. The difference in grain development might be related to the effective improvement of common buckwheat growth by applying nitrogen fertilizer. Grain filling and starch synthesis can be promoted by appropriate nitrogen fertilizer. Higher nitrogen levels resulted in the common buckwheat starch had higher small granules but lower large granules. The difference of starch granule size in two years might be due to the differences in soil and climatic conditions.

3.5. XRD

Generally, natural starches can be divided into the A-, B- and C-type based on their X-ray diffraction patterns [24]. As shown in Fig. 2A and B, the XRD patterns under different treatments were not changed with increasing nitrogen levels. All the common buckwheat starches displayed the typical A-type patterns with strong peaks at about 15° and 23° and an unresolved doublet at around 17° and 18°, which was consistent with

Table 1

<table>
<thead>
<tr>
<th>Years</th>
<th>Treatments (kg/hm²)</th>
<th>Plant height (cm)</th>
<th>Section number</th>
<th>Branch number</th>
<th>Flower clusters number</th>
<th>Grains per plant (g)</th>
<th>1000-grain weight (g)</th>
<th>Grain yield (kg/hm²)</th>
<th>Relative crystallinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>N₀</td>
<td>82.4 ± 4.00b</td>
<td>14.7 ± 0.30a</td>
<td>4.5 ± 0.25bc</td>
<td>24.6 ± 0.74c</td>
<td>66.0 ± 9.24c</td>
<td>35.4 ± 0.02c</td>
<td>900 ± 26.46c</td>
<td>26.46 ± 0.08d</td>
</tr>
<tr>
<td></td>
<td>N₁</td>
<td>82.3 ± 3.70b</td>
<td>14.1 ± 0.45ab</td>
<td>4.3 ± 0.31c</td>
<td>26.4 ± 6.66bc</td>
<td>81.7 ± 9.18c</td>
<td>36.0 ± 0.01b</td>
<td>1150 ± 10.00</td>
<td>27.26 ± 0.29c</td>
</tr>
<tr>
<td></td>
<td>N₂</td>
<td>99.0 ± 2.20a</td>
<td>13.1 ± 0.98ab</td>
<td>5.7 ± 0.67a</td>
<td>40.9 ± 2.53a</td>
<td>130.2 ± 18.48a</td>
<td>36.5 ± 0.03a</td>
<td>1243 ± 41.60a</td>
<td>28.95 ± 1.12a</td>
</tr>
<tr>
<td></td>
<td>N₃</td>
<td>95.3 ± 3.58a</td>
<td>12.9 ± 0.80b</td>
<td>5.4 ± 0.30ab</td>
<td>36.1 ± 2.53ab</td>
<td>108.9 ± 4.85b</td>
<td>36.3 ± 0.02a</td>
<td>1260 ± 20.00a</td>
<td>28.53 ± 0.37b</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>89.8 ± 13.7</td>
<td>5 ± 32</td>
<td>32</td>
<td>67.2 ± 11.19c</td>
<td>165.9 ± 22.96</td>
<td>35.4 ± 1.50c</td>
<td>1114 ± 13.8</td>
<td>27.80</td>
</tr>
<tr>
<td>2018</td>
<td>N₀</td>
<td>146.7 ± 8.44b</td>
<td>17.2 ± 0.16a</td>
<td>5.4 ± 0.25a</td>
<td>67.2 ± 11.19c</td>
<td>165.9 ± 22.96</td>
<td>35.4 ± 1.50c</td>
<td>1160 ± 27.23c</td>
<td>26.38 ± 0.15c</td>
</tr>
<tr>
<td></td>
<td>N₁</td>
<td>147.5 ± 11.57b</td>
<td>16.7 ± 0.74b</td>
<td>5.5 ± 0.19a</td>
<td>87.9 ± 7.74b</td>
<td>188.1 ± 22.86</td>
<td>36.6 ± 0.81a</td>
<td>1253 ± 22.40</td>
<td>27.45 ± 0.27b</td>
</tr>
<tr>
<td></td>
<td>N₂</td>
<td>160.3 ± 10.31a</td>
<td>16.6 ± 0.71b</td>
<td>5.6 ± 0.28a</td>
<td>115.3 ± 5.15a</td>
<td>372.1 ± 50.76a</td>
<td>36.8 ± 0.84a</td>
<td>1417 ± 24.05a</td>
<td>28.91 ± 0.42a</td>
</tr>
<tr>
<td></td>
<td>N₃</td>
<td>147.0 ± 3.25b</td>
<td>15.4 ± 0.86c</td>
<td>5.5 ± 0.25a</td>
<td>89.3 ± 12.34b</td>
<td>214.8 ± 38.25b</td>
<td>36.3 ± 0.85ab</td>
<td>1287 ± 19.00b</td>
<td>27.53 ± 0.24b</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>150.4 ± 16.5</td>
<td>5.5</td>
<td>89.0</td>
<td>235.2</td>
<td>36.3</td>
<td>1271</td>
<td>27.57</td>
<td></td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± standard deviation. Different letters within a column indicate significant difference among mean values at p < 0.05.
with the characteristics of rice starches [6]. The peak positions under different treatments were basically unchanged, while the application of nitrogen fertilizer could change the relative crystallinity of common buckwheat starch. It can be seen from Table 1 that the relative crystallinity first increased and then decreased with increasing nitrogen levels, and had the largest value under N2 treatment in both years. These results indicated that the stability of common buckwheat starch crystalline can be influenced by nitrogen application, which was consistent with the results of rice starch [25]. Amylose is widely known as the predominant crystalline component, which could weaken the crystalline structure of amylopectin [26]. In our study, amylose contents were lower at higher nitrogen levels, which indicated that the effects of amylose on the crystalline structure was weak under high nitrogen levels.

3.6. Water solubility

Water solubility of common buckwheat starches varied under different nitrogen levels in Fig. 2C and D. With increasing nitrogen levels, water solubility showed the trend of first increase and then decrease. Temperature also had significant effects on the common buckwheat starch, and water solubility exhibited steady increase with increasing temperatures. The starches under N0 treatment had lower water solubility at 60 °C but the largest values from 70 °C to 90 °C in both years. Higher solubility at high nitrogen levels was due to higher water affinity of small granules at high nitrogen level compared with that of the large ones [27]. Amylose can inhibit the further swelling of starch granules and maintain the structure of granule expansion, resulting in the increase of solubility [19]. The differences in two years may be relevant to granule size distributions affected by temperature during the filling stage [18].

3.7. Retrogradation

The effects of nitrogen fertilizer on the retrogradation of common buckwheat starch were shown in Fig. 2E and F. The average retrogradation percentages of common buckwheat starch under N0, N1, N2, and N3 treatments of two years were 82.0%, 75.5%, 78.0%, and 73.5%, respectively. The retrogradation rates of starch samples at N0 treatment rapidly increased within the first 14 h of placement and tended to stabilize after 14 h, while the retrogradation rates of starch under various nitrogen management conditions significantly increased before 16 h and the starch at N3 treatment showed the lowest value in two years. Nitrogen fertilizer significantly reduced the retrogradation rate of common buckwheat starch paste, which may be related to the starch granule size distribution under different nitrogen levels. Besides, starch structure and pasting temperature could also affect the starch retrogradation [28].

3.8. Light transmittance

As shown in Fig. 3, light transmittance of common buckwheat starch was significantly different under four nitrogen application levels. The

Table 2

<table>
<thead>
<tr>
<th>Years</th>
<th>Treatments</th>
<th>Main chemical composition</th>
<th>Distribution of starch granules (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein (%)</td>
<td>Fat (%)</td>
</tr>
<tr>
<td>2017</td>
<td>N0</td>
<td>10.16 ± 0.21d</td>
<td>1.35 ± 0.03b</td>
</tr>
<tr>
<td></td>
<td>N1</td>
<td>10.43 ± 0.18c</td>
<td>1.43 ± 0.03a</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>11.80 ± 0.25b</td>
<td>1.44 ± 0.02a</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>12.39 ± 0.31a</td>
<td>1.45 ± 0.01a</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>11.2</td>
<td>1.42</td>
</tr>
<tr>
<td>2018</td>
<td>N0</td>
<td>9.96 ± 0.43d</td>
<td>1.48 ± 0.03a</td>
</tr>
<tr>
<td></td>
<td>N1</td>
<td>10.21 ± 0.53c</td>
<td>1.48 ± 0.02a</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>11.54 ± 0.25b</td>
<td>1.49 ± 0.02a</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>11.98 ± 0.18a</td>
<td>1.50 ± 0.03a</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>10.9</td>
<td>1.49</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± standard deviation. Different letters within a column indicate significant difference among mean values at p < 0.05.
light transmittance significantly increased with increasing nitrogen levels in both years. In 2018, the light transmittance under the N0, N1, N2, and N3 treatments were 7.1%, 7.9%, 8.5%, and 8.6%, respectively, which were higher than those of corresponding treatments in 2017. It had been reported that starch paste with a higher proportion of large particles contains less particle residue, which enabled light to pass through, leading to higher light transmittance [29]. These results were similar with that in Table 2. The starch paste light transmittance was affected by amylose content, starch granule size, and amylose/amylpectin ratio [18]. Difference in two years may be due to the variation on the temperature and precipitation.

3.9. Pasting properties

The pasting properties of common buckwheat starches significantly affected by nitrogen fertilizer (Table 3). Peak viscosity first decreased and then increased, trough viscosity, breakdown, final viscosity, setback significantly decreased, and pasting temperature obviously increased with increasing nitrogen levels. Peak viscosity was the maximum viscosity of gelatinized starch during heating in water and could reflect the expansion range of starch particles [30], the difference of peak viscosity was related to the water absorption rate of starch particles during the heating process [18]. In this study, the peak viscosity of common buckwheat starch was significantly reduced after the application of nitrogen fertilizer, indicating that nitrogen fertilizer can reduce the swelling rate of common buckwheat starch granules, causing the starch granules to absorb water slowly, thereby reducing the starch viscosity. Breakdown can reflect the ability to resist heating, the higher the breakdown viscosity, the lower the ability [25], indicating that common buckwheat starches had higher ability to resist heating under higher nitrogen levels. Setback was an index to measure the stability of starch paste after cooling, and a high setback viscosity indicated that the starch had a tendency to retrograde [31]. In our study, the setback viscosity of common buckwheat starches significantly decreased with increasing nitrogen levels, which indicated that common buckwheat starches at higher nitrogen levels were hard to retrograde, and the lower setback viscosity at higher nitrogen levels might be related to the molecular weight of amylose [32]. Zhu et al. [19] have reported that rice starch had higher final viscosity with increasing nitrogen levels, which was not consistent with our results, the difference might link to the
genotype. Pasting temperature was the temperature where the viscosity of starch paste began to rise [33]. Wang et al. [34] found that wheat starch had lower pasting temperature at higher nitrogen levels, while the pasting temperatures of common buckwheat starches became higher with increasing nitrogen levels, indicating that common buckwheat starches were hard to gelatinize at higher nitrogen levels. Pasting properties have been reported to be affected by amylose, amylopectin branching architecture, and granule size [35]. The whole change trend of pasting properties on common buckwheat starches affected by various nitrogen levels was similar in both years, while the average values in 2017 were higher than those in 2018, the differences could be due to the precipitation and climate.

3.10. Thermal properties

Nitrogen fertilizer had significant effects on the thermal properties of common buckwheat starch (Table 3). With increasing nitrogen levels, \( T_o \), \( T_p \), and \( \Delta H \) significantly increased, while \( T_c \) was first increased and then decreased. Molecular structure could influence the gelatinization parameters of the starch [36]. The gelatinization enthalpy was used to study the starch crystalline structure and relative crystallinity [37]. A high enthalpy was caused by the high relative crystallinity at high nitrogen levels [38]. The higher gelatinization temperature, the higher cooking temperature required and the longer cooking time consumed [13]. The difference in gelatinization temperatures at various nitrogen levels may be related to the starch granule size, amylose content, and amylopectin fine structure [19].

3.11. Principal components analysis

The principal components analysis (PCA) plot was analyzed to characterize the effects of nitrogen fertilizer on the physicochemical properties of common buckwheat starches. The score and loading plots for components 1 and 2 of PCA results were combined in Fig. 4A and B. To evaluate the relative contributions of components in the overall total data variability, only the values greater than one were considered. Thus, the first six principal components (PC) were found to be significant (Table S1). Fig. 4A presented the plot between the first two PC (PC1 and PC2). Observing two groups that were representative of the separation trend of different years based on the fertilization level was possible. The distance between the locations of any treatment on the plot indicated the degree of difference or similarity between them.

![Fig. 3. The light transmittance of common buckwheat starch at different nitrogen levels.](image)

**Table 3.** Pasting and thermal properties of common buckwheat starches at different nitrogen levels.

<table>
<thead>
<tr>
<th>Years</th>
<th>Treatments</th>
<th>Pasting properties</th>
<th>Thermal properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV (cP)</td>
<td>TV (cP)</td>
<td>BD (cP)</td>
</tr>
<tr>
<td>2017</td>
<td>N0</td>
<td>3907 ± 27a</td>
<td>3529 ± 36a</td>
</tr>
<tr>
<td></td>
<td>N1</td>
<td>3897 ± 56a</td>
<td>3427 ± 45b</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>3279 ± 43b</td>
<td>3211 ± 46b</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>3401 ± 46b</td>
<td>3190 ± 37d</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>3621</td>
<td>3339</td>
</tr>
<tr>
<td>2018</td>
<td>N0</td>
<td>3709 ± 79a</td>
<td>3339 ± 29a</td>
</tr>
<tr>
<td></td>
<td>N1</td>
<td>3344 ± 46b</td>
<td>3288 ± 43b</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>3374 ± 32b</td>
<td>3271 ± 40b</td>
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<td></td>
<td>N3</td>
<td>3404 ± 29b</td>
<td>3204 ± 19c</td>
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<td></td>
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</tbody>
</table>

PV, peak viscosity; TV, trough viscosity; BD, breakdown; FV, final viscosity; SB, setback; PT, pasting temperature; \( T_o \), onset gelatinization temperature; \( T_p \), peak gelatinization temperature; \( T_c \), conclusion gelatinization temperature; \( \Delta H \), gelatinization enthalpy.
The N₁ treatment in 2017 was located at the left of the score plot with negative and positive scores on PC1 and PC2, respectively, whereas N₁ treatment in 2018 was located positively on PC1 and PC2. The N₀ treatments of two years had negative scores on PC1 and PC2, and N₂ and N₃ treatments of two years had positive scores on PC1 and negative scores on PC2. Common buckwheat starches at different nitrogen levels exhibited high variability in both years. PC1 and PC2 accounted for 63.7% and 17.2%, respectively, of the total variability. The loading plot provided the information about the correlations among the physicochemical properties of common buckwheat starch (Fig. 4B). Among the main composition of common buckwheat seeds, protein, fat, and flavonoid contents were loaded positively on PC1 and negatively on PC2, whereas amylose and starch contents were loaded positively on PC1 and PC2. Among the granule size distribution, “A” granules (>15 μm) were loaded positively on PC1 but negatively on PC2, “B” granules (5–15 μm) were loaded positively on PC1 and PC2, whereas “C” granules (<5 μm) were loaded negatively on PC1 and PC2. Among the pasting properties, PV, TV, and FV were loaded positively on PC2 but negatively on PC1, and SB and BD were loaded negatively on PC1 and PC2. Among the thermal properties, To and Tp were loaded positively on PC1 and negatively on PC2, whereas Tc and ΔH were loaded positively on PC1 and PC2. From the loading plot shown in Fig. 4B, “B” granule was evidently more closely related to ΔH as opposed to the pasting properties. In the PCA plot, the relationship between nitrogen fertilizer application and the structure and physicochemical properties of common buckwheat starch could be determined.

3.12. Correlation analysis

Pearson correlation analysis was used to analyze the starch physicochemical properties on common buckwheat, and the results were shown in Fig. 4. Results reflected that amylose content and ΔH had significantly positive correlation with the “C” (<5 μm) and “B” (5–15 μm) granules but negative correlation with “A” (>15 μm). Meanwhile, amylose content had a significant negative correlation to PV, TV, and FV, a significant positive correlation to PT, and a significant negative correlation to “C” granules of the pasting properties in 2017. However, the “B” granules in 2018 had positive correlation with FV, which was in contrast to the results in 2017. There were some certain differences in correlation coefficient between 2017 and 2018, which may be related to the temperature, rainfall capacity, and other aspects in two years.

4. Conclusion

These results showed N₂ was more suitable for common buckwheat growth, and nitrogen fertilizer did not alter the starch granule morphology shape and the XRD patterns but changed granule size distribution and crystalline stability. With increasing nitrogen levels, branch number, flower clusters number, grain number per plant, content of protein and fat, size distribution of “C” granules, and percentages of light transmittance significantly increased, whereas amylose content and retrogradation decreased. Common buckwheat starches had higher pasting
temperature and gelatinization enthalpy but lower TV and FV at higher nitrogen levels. PCA and correlation analysis reflected that the nitrogen fertilizer and year had obvious effects on the starch physicochemical properties of common buckwheat.

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Licheng Gao: Conceptualization, Data curation, Writing -original draft.
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Xiaoli Gao: Validation, Visualization.
Jinfeng Gao: Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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