International Journal of Biological Macromolecules 179 (2021) 542–549

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

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

Licheng Gao [a], Wenming Bai [a], Meijuan Xia [a], Chenxi Wan [a], Meng Wang [b], Pengke Wang [a], Xiaoli Gao [a], Jinfeng Gao [a],*

[a] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, Shaanxi Province 712100, China
[b] Yu'lin Institute of Agricultural Sciences, Yulin, Shaanxi Province 719000, China

ARTICLE INFO

Article history:
Received 17 January 2021
Received in revised form 6 March 2021
Accepted 9 March 2021
Available online 11 March 2021

Keywords:
Common buckwheat
Starches
Nitrogen fertilizer

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 amylose 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 N_2 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.

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 the development and production of functional foods and in combination with other health-promoting ingredients [3]. Common buckwheat starch, occupying 70% of the whole grain, its internal structure and physicochemical properties play an important role in the qualities of cooking and eating. In recent years, the nutritive value of common buckwheat is widely valued, the flour has been utilized to make products for enhancing marketing opportunities [4], and the seeds are used as additives to improve the quality of bread as riching 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′

---

* Corresponding author.
E-mail addresses: gao2123@nwsuaf.edu.cn (X. Gao), gaojf7604@126.com (J. Gao).

https://doi.org/10.1016/j.ijbiomac.2021.03.045
0141-8130/© 2021 Elsevier B.V. All rights reserved.

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. Urea (Inner Mongolia Boda Field Chemical Co., Ltd.) with a total nitrogen content of ≥46.0% was used as nitrogen fertilizer, and topdressing during the flowering period at the ratio of base fertilizer: topdressing = 1:1. Potassium, as potassium sulfate, and phosphorus, as calcium superphosphate, were applied for each plot as basal fertilizer at rates of 37.5 kg/hm^2 K_2O and 37.5 kg/hm^2 P_2O_5, respectively. 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 it 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 sampleto. 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+/PC, 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 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 μL 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 ($\triangle H$) 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/hm^2 to 270 kg/hm^2, 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 N_2 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 N_2 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 N_2 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 N_2 (180 kg/hm^2) 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 N_3 and N_2 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 the maximum values were appeared at N_3 treatment in both years, whereas starch content showed a trend of increasing initially and subsequently decreasing. Competition between ATP and carbon matrix usually occurs during high protein synthesis rates [19]. In our study, the contents of protein obviously increased by applying nitrogen fertilizer, which indicated that nitrogen fertilizer could effectively promote the accumulation of protein in common buckwheat, which in turn consumed more energy and led to lower starch content. Zhu et al. [19] found when more proteins were synthesized, lower starch content was observed of rice grains, which was consistent with our results.

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 smother 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]. Besides, obvious difference was observed that the proportion of starch granules at higher nitrogen levels was higher, which was consistent with the result in banana starch [21].

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 N_1 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

Table 1
Agronomic traits and relative crystallinity of common buckwheat at different nitrogen levels.[a]

Years	Treatments	Plant height (cm)	Section number	Branch number	Flower clusters number	Grainnumber per plant	1000-grain weight (g)	Grain yield (kg/hm^2)	Relative crystallinity (%)
2017	N_0	82.4 ± 4.00b	14.7 ± 0.30a	4.5 ± 0.25bc	24.6 ± 0.74c	66.0 ± 9.24c	35.4 ± 0.02c	900 ± 26.46c	26.46 ± 0.08d
	N_1	82.3 ± 3.70b	14.1 ± 0.45ab	4.3 ± 0.31c	26.4 ± 6.66bc	83.7 ± 9.18bc	36.0 ± 0.01b	1150 ± 10.00b	27.26 ± 0.29c
	N_2	99.0 ± 2.20a	13.1 ± 0.98ab	5.7 ± 0.67a	40.9 ± 2.53a	130.2 ± 18.48a	36.5 ± 0.03a	1243 ± 41.60a	28.95 ± 1.12a
	N_3	95.3 ± 3.58a	12.9 ± 0.80b	5.4 ± 0.30ab	36.1 ± 2.55ab	108.9 ± 4.85ab	36.3 ± 0.02a	1260 ± 20.00a	28.53 ± 0.37b
	Average	89.8	13.7	5	32	97.2	36.1	1138	27.80
2018	N_0	146.7 ± 8.44b	17.2 ± 0.16a	5.4 ± 0.25a	67.2 ± 11.19c	165.9 ± 22.96 cd	35.4 ± 1.50c	1160 ± 27.23c	26.38 ± 0.15c
	N_1	147.5 ± 11.57b	16.7 ± 0.74b	5.5 ± 0.19a	87.9 ± 7.74b	188.1 ± 22.86c	36.6 ± 0.81a	1253 ± 22.40b	27.45 ± 0.27b
	N_2	160.3 ± 10.31a	16.6 ± 0.71b	5.6 ± 0.28a	115.3 ± 5.15a	372.1 ± 50.76a	36.8 ± 0.84a	1417 ± 24.05a	28.91 ± 0.42a
	N_3	147.0 ± 3.25b	15.4 ± 0.86c	5.5 ± 0.25a	89.3 ± 12.34b	214.8 ± 38.25b	36.3 ± 0.89ab	1283 ± 19.00b	27.53 ± 0.24b
	Average	150.4	16.5	5.5	89.9	235.2	36.3	1278	27.57

[a] Data are expressed as the mean ± standard deviation. Different letters within a column indicate significant difference among mean values at $p < 0.05$.

Table 2
Main chemical composition of common buckwheat seeds and granule sizes distribution of common buckwheat starches at different nitrogen levels.[a]

Years	Treatments	Main chemical composition					Distribution of starch granules (%)		
		Protein (%)	Fat (%)	Flavonoid (mg/g)	Amylose (%)	Starch (%)	C (<5 μm)	B (5–15 μm)	A (>15 μm)
2017	N_0	10.16 ± 0.21d	1.35 ± 0.03b	3.28 ± 0.16c	27.48 ± 0.09a	70.63 ± 0.11a	6.36 ± 0.05d	48.86 ± 0.13c	44.78 ± 0.22a
	N_1	10.43 ± 0.18c	1.43 ± 0.03a	3.56 ± 0.16bc	26.81 ± 0.12b	70.88 ± 0.60a	7.75 ± 0.15c	70.09 ± 0.24a	22.16 ± 0.16c
	N_2	11.80 ± 0.25b	1.44 ± 0.02a	4.22 ± 0.32a	25.67 ± 0.11c	70.80 ± 0.44a	8.40 ± 0.07b	59.26 ± 0.18b	32.34 ± 0.27b
	N_3	12.39 ± 0.31a	1.45 ± 0.01a	3.91 ± 0.27ab	25.13 ± 0.20d	70.60 ± 0.40a	9.52 ± 0.12a	68.91 ± 0.35a	21.58 ± 0.05c
	Average	11.2	1.42	3.74	26.27	70.73	8.01	61.78	30.22
2018	N_0	9.86 ± 0.43d	1.48 ± 0.03a	3.44 ± 0.45c	27.57 ± 0.22a	70.74 ± 0.88a	7.62 ± 0.04c	51.39 ± 0.27c	40.98 ± 0.28a
	N_1	10.21 ± 0.53c	1.48 ± 0.02a	3.56 ± 0.37c	26.93 ± 0.42b	71.19 ± 0.60a	7.98 ± 0.07c	67.17 ± 0.18a	24.85 ± 0.25c
	N_2	11.54 ± 0.25b	1.49 ± 0.02a	4.06 ± 0.51a	26.71 ± 0.26b	70.80 ± 0.56a	9.48 ± 0.02b	60.08 ± 0.14b	30.44 ± 0.15b
	N_3	11.98 ± 0.18a	1.50 ± 0.03a	3.88 ± 0.25b	25.89 ± 0.19c	70.60 ± 1.54a	12.21 ± 0.13a	59.40 ± 0.19b	28.39 ± 0.10b
	Average	10.9	1.49	3.74	26.78	70.83	9.32	59.51	31.17

[a] 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 the Table 1 that the relative crystallinity first increased and then decreased with increasing nitrogen levels, and had the largest value under N_2 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 N_0 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 strach were shown in Fig. 2E and F. The average retrogradation percentages of common buckwheat starch under N_0, N_1, N_2, and N_3 treatments of two years were 82.0%, 75.5%, 78.0%, and 73.5%, respectively. The retrogradation rates of starch samples at N_0 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 N_3 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

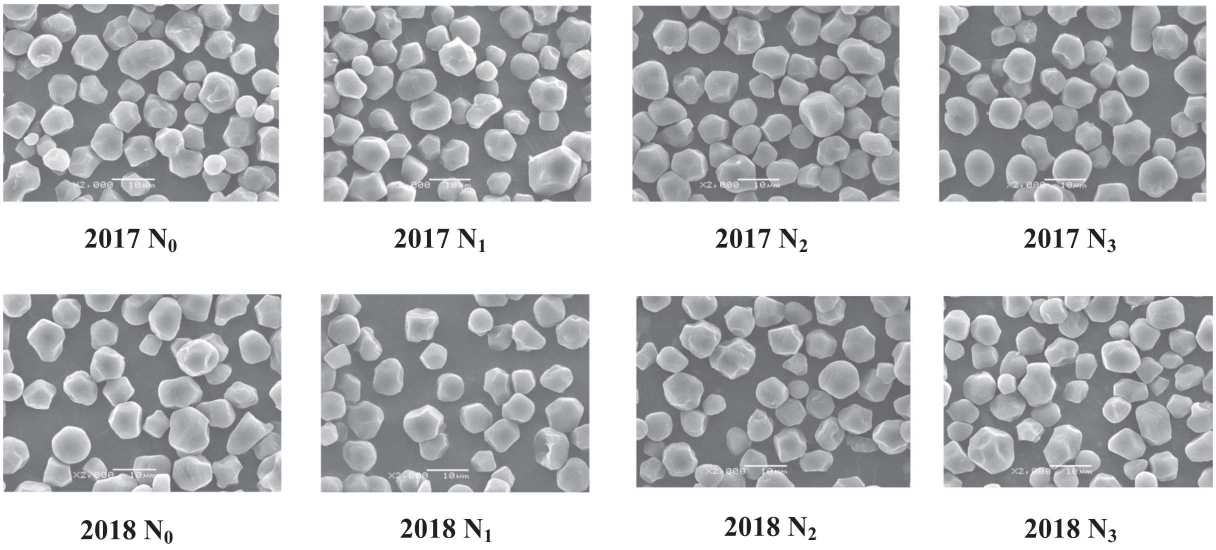
2017 N_0 2017 N_1 2017 N_2 2017 N_3
2018 N_0 2018 N_1 2018 N_2 2018 N_3

Fig. 1. Common buckwheat starch granule morphology treated with different nitrogen levels.

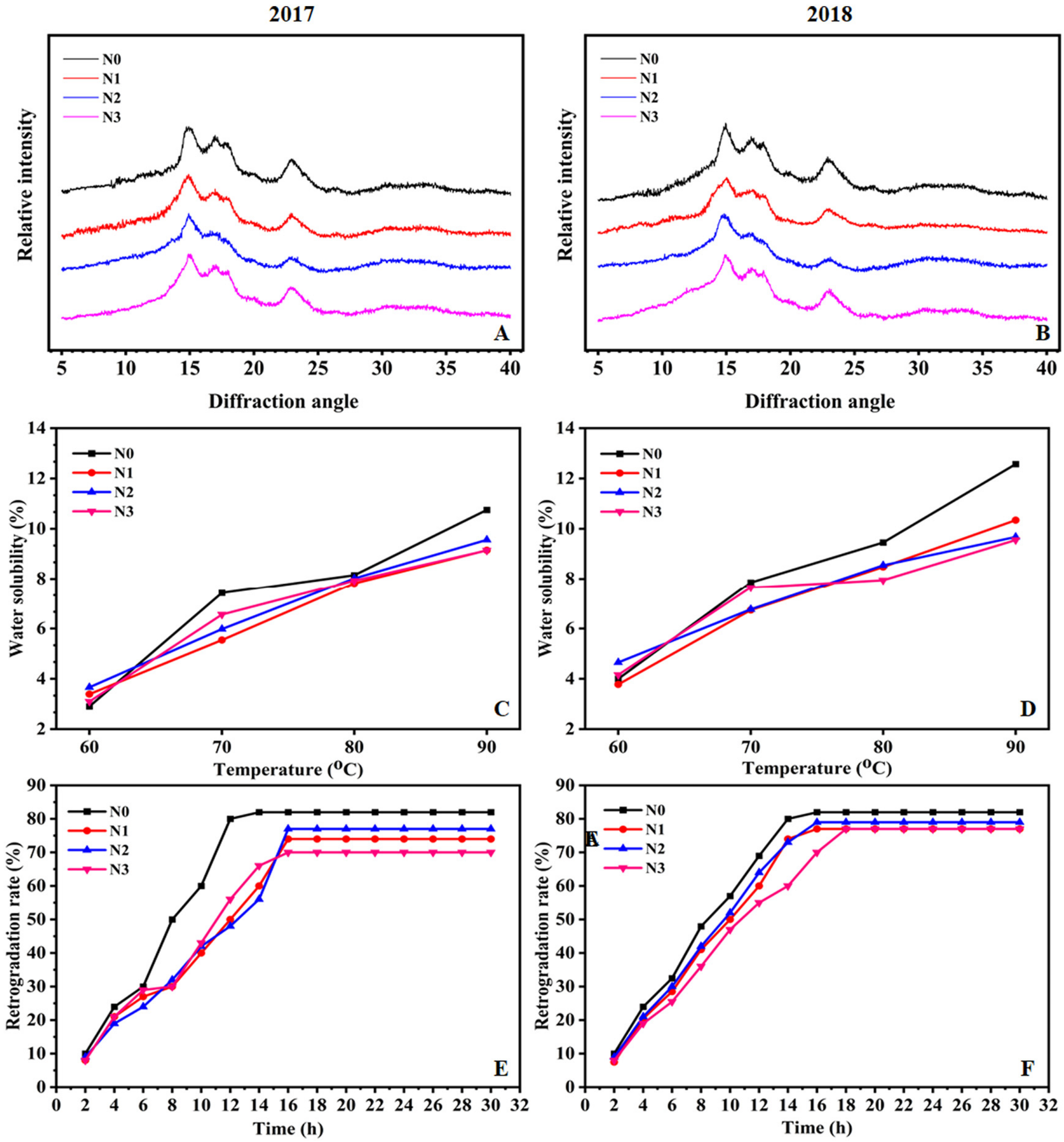

Fig. 2. (A, B), The X-ray diffraction patterns of common buckwheat starches at different nitrogen levels; (C, D),The water solubility of common buckwheat starch at different nitrogen levels; (E, F), The retrogradation of common buckwheat starch at different nitrogen levels.

light transmittance significantly increased with increasing nitrogen levels in both years. In 2018, the light transmittance under the N_0, N_1, N_2, and N_3 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/amylopectin 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

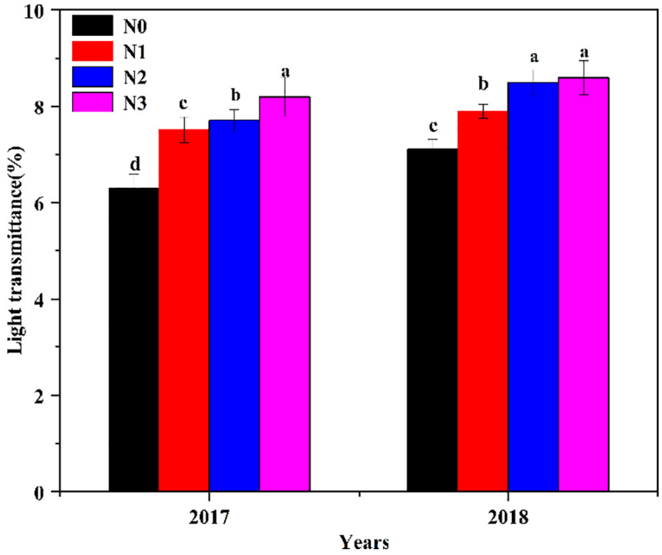

Fig. 3. The light transmittance of common buckwheat starch at different nitrogen levels.

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 ΔH 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.

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

Years	Treatments	Pasting properties						Thermal properties			
		PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
2017	N_0	3907 ± 27a	3529 ± 36a	478 ± 12a	5975 ± 53a	2474 ± 26a	71.8 ± 0.68c	63.67 ± 0.03b	67.46 ± 0.05b	70.45 ± 0.02c	4.56 ± 0.12c
	N_1	3897 ± 56a	3427 ± 45b	270 ± 6b	5903 ± 67a	2148 ± 34b	74.3 ± 0.96b	65.13 ± 0.07a	67.87 ± 0.04b	72.45 ± 0.04a	5.56 ± 0.22bc
	N_2	3279 ± 43b	3211 ± 46c	268 ± 9b	5350 ± 46b	2139 ± 27b	75.9 ± 0.66a	65.58 ± 0.09a	68.36 ± 0.24a	71.65 ± 0.07b	5.64 ± 0.14b
	N_3	3401 ± 46b	3190 ± 37d	211 ± 6c	5261 ± 49b	2071 ± 23c	75.1 ± 0.36ab	65.42 ± 0.14a	68.45 ± 0.13a	72.35 ± 0.13a	6.03 ± 0.14a
	Average	3621	3339	307	5622	2208	74.3	64.95	68.04	71.73	5.45
2018	N_0	3709 ± 79a	3339 ± 29a	370 ± 19a	5574 ± 56a	2235 ± 32a	72.7 ± 0.76c	64.56 ± 0.23c	68.54 ± 0.23c	70.45 ± 0.24b	4.64 ± 0.15b
	N_1	3344 ± 46b	3288 ± 36b	246 ± 24b	5692 ± 36a	1804 ± 19b	75.9 ± 0.64a	65.86 ± 0.13b	69.67 ± 0.14b	73.14 ± 0.23a	5.76 ± 0.24a
	N_2	3394 ± 32b	3271 ± 43b	223 ± 16bc	5193 ± 49b	1922 ± 19b	75.8 ± 0.99a	66.35 ± 0.12a	70.24 ± 0.08a	72.76 ± 0.21a	5.96 ± 0.14a
	N_3	3409 ± 29b	3204 ± 19c	205 ± 20 cd	5116 ± 36b	1912 ± 20b	74.4 ± 0.76a	66.56 ± 0.07a	70.15 ± 0.06a	72.45 ± 0.07a	5.88 ± 0.08a
	Average	3464	3276	261	5394	1968	74.7	65.83	69.65	72.20	5.56

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; ΔH, gelatinization enthalpy.

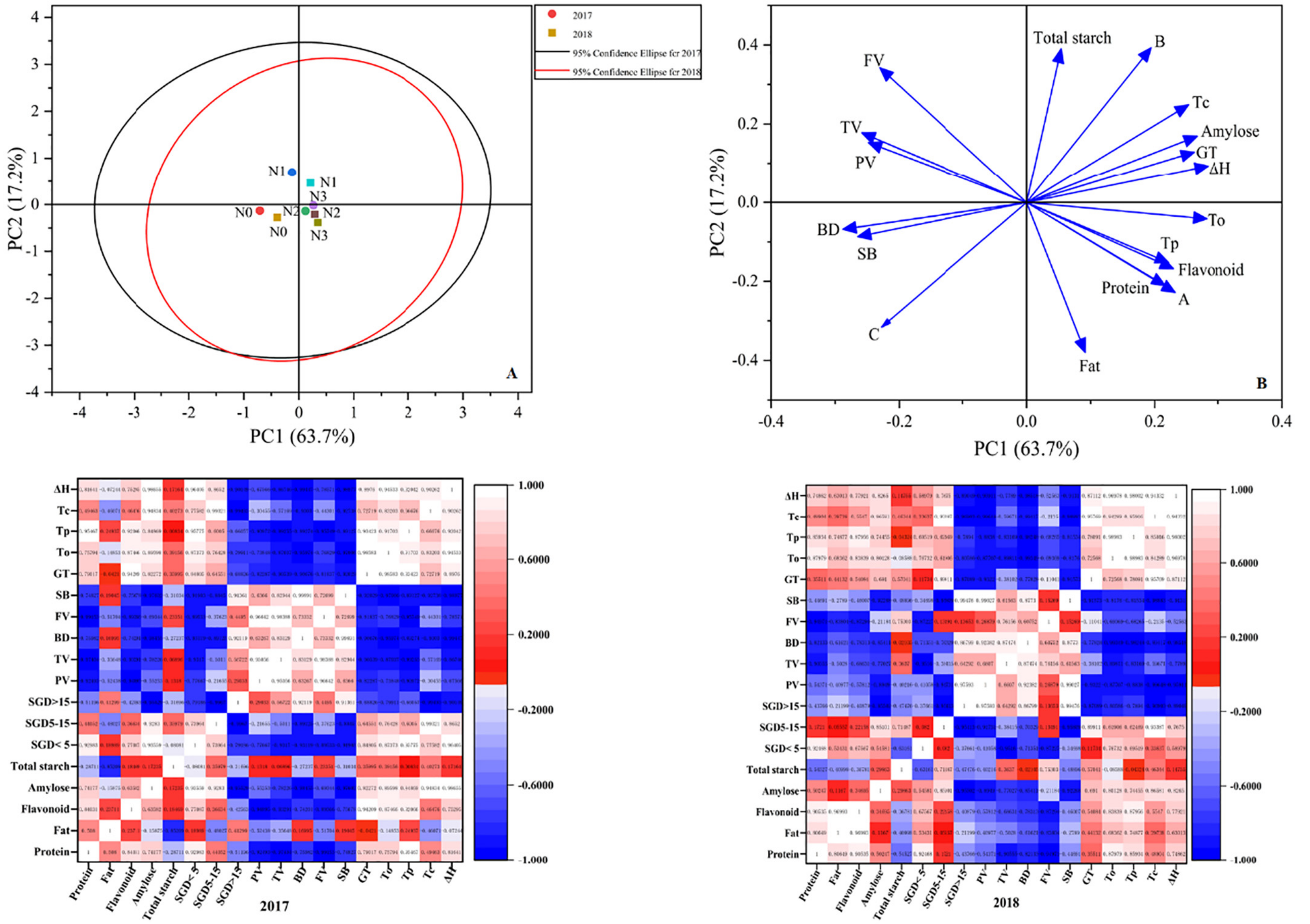

Fig. 4. PCA plot and correlations analysis of structural and physicochemical properties of common buckwheat starches. (A), Loading Plot; (B), Score Plot; Amylose, amylose content; SGD < 5, "A" granules; SGD 5–15, "B" granules; SGD >5, "C" granules; To, onset gelatinization temperature; Tp, peak gelatinization temperature; Tc, conclusion gelatinization temperature; ΔH, gelatinization enthalpy; GT, pasting temperature; PV, peak viscosity; TV, trough viscosity; FV, final viscosity; SB, setback; BD, breakdown.

The N_1 treatment in 2017 was located at the left of the score plot with negative and positive scores on PC1 and PC2, respectively, whereas N_1 treatment in 2018 was located positively on PC1 and PC2. The N_0 treatments of two years had negative scores on PC1 and PC2, and N_2 and N_3 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, T_o and T_p were loaded positively on PC1 and negatively on PC2, whereas T_c 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 N_2 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.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2021.03.045.

CRediT authorship contribution statement

Licheng Gao: Conceptualization, Data curation, Writing -original draft.

Wenming Bai: Conceptualization, Data curation, Software.

Meijuan Xia: Investigation.

Chenxi Wan: Formal analysis.

Meng Wang: Methodology.

Pengke Wang: Investigation, Methodology, Resources.

Xiaoli Gao: Validation, Visualization.

Jinfeng Gao: Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

This research was funded by the National Key R&D Program of China (2020YFD1000805), National Natural Science Foundation of China (31671631), Minor Grain Crops Research and Development System of Shaanxi Province (2016-2019), Focus on Research and Development of Science and Technology Plan Projects in Shaanxi Province (2018 TSCXL-NY-03-04).

References

[1] X. Yang, Z. Wu, H. Chen, J. Shao, Q. Wu, Karyotype and genetic relationship based on RAPD markers of six wild buckwheat species (Fagopyrum spp.) from southwest of China, Genet. Resour. Crop Evol. 57 (2010) 649–656.

[2] K. Tohgi, K. Kohno, H. Takahashi, H. Matsuo, S. Nakayama, E. Morita, Usability of Fag e 2 ImmunoCAP in the diagnosis of buckwheat allergy, Arch. Dermatol. Res. 303 (2011) 635–642.

[3] A. Juan, B Henryk .G,.Z., Buckwheat as a functional food and its effects on health, J. Agric. Food Chem. 63 (2015) 7896–7913.

[4] V. Skrabanja, I. Kreft, T. Golob, M. Modic, S. Ikeda, K. Ikeda, S. Kreft, G. Bonafaccia, M. Knapp, K. Kosmelj, Nutrient content in buckwheat milling fractions, Cereal Chem. 81 (2004) 172–176.

[5] F. Wu, H. Chen, Y. Na, J. Wang, D. Xiang, Z. Jin, X. Xu, Effect of germination time on physicochemical properties of brown rice flour and starch from different rice cultivars, J. Cereal Sci. 58 (2013) 263–271.

[6] D. Zhu, H. Zhang, B. Guo, K. Xu, Q. Dai, C. Wei, G. Zhou, Z. Huo, Effects of nitrogen level on structure and physicochemical properties of rice starch, Food Hydrocoll. 63 (2017) 525–532.

[7] J. Gu, C. Jing, C. Lu, Z. Wang, Z. Hao, J. Yang, Grain quality changes and responses to nitrogen fertilizer of japonica rice cultivars released in the Yangtze River Basin from the 1950s to 2000s, Crop J. 3 (2015) 285–297.

[8] A. Nowotna, H. Gambuś, G. Kratsch, J. Krawontka, F. Gambuś, R. Sabat, R. Ziobro, Effect of nitrogen fertilization on the physicochemical properties of starch isolated from German Triticale varieties, Starch-Stärke 59 (2010) 397–399.

[9] L. Wenhao, S. Yulin, X. Xinlong, Z. Jianmei, L. Qingui, O. Shaohui, Z. Guoquan, Effect of nitrogen and sulfur fertilization on accumulation characteristics and physicochemical properties of a- and B-wheat starch, J. Agric. Food Chem. 61 (2013) 2418–2425.

[10] X. Fang, Y. Li, J. Nie, C. Wang, K. Huang, Y. Zhang, Y. Zhang, H. She, X. Liu, R. Ruan, X. Yuan, Z. Yi, Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (Fagopyrum esculentum M.), Field Crops Res. 219 (2018) 160–168.

[11] Q. Yang, P. Zhang, Y. Qu, X. Gao, J. Liang, P. Yang, B. Feng, Comparison of physicochemical properties and cooking edibility of waxy and non-waxy proso millet (*Panicum miliaceum* L.), Food Chem. 257 (2018) 271–278.

[12] D. Yu, J. Chen, M. Jie, H. Sun, Y. Yuan, J. Qian, Y. Teng, Y. Mo, W. Li, K. Fujita, Effects of different milling methods on physicochemical properties of common buckwheat flour, LWT–Food Sci. Technol. 92 (2018) 220–226.

[13] J. Gao, I. Kreft, G. Chao, Y. Wang, X. Liu, L. Wang, P. Wang, X. Gao, B. Feng, Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) starch, a side product in functional food production, as a potential source of retrograded starch, Food Chem. 190 (2016) 552–558.

[14] L. Chao, M. Xia, Z. Li, M. Wang, P. Wang, P. Yang, X. Gao, J. Gao, Common buckwheat-resistant starch as a suitable raw material for food production: a structural and physicochemical investigation, Int.J.Biol. Macromol. 145 (2020) 145–153.

[15] G. Chao, J. Gao, L. Rui, W. Li, L. Cui, W. Ying, Q. Yang, B. Feng, Starch physicochemical properties of waxy proso millet (*Panicum miliaceum* L.), Starch-Stärke 66 (2015) 1005–1012.

[16] L. Wang, B. Xie, J. Shi, S. Xue, Q. Deng, Y. Wei, B. Tian, Physicochemical properties and structure of starches from Chinese rice cultivars, Food Hydrocoll. 24 (2010) 208–216.

[17] A. Karim, H. Norziah, C. Seow, Methods for the study of starch retrogradation, Food Chem. 71 (2009) 9–36.

[18] W. Zhang, Q. Yang, M. Xia, W. Bai, P. Wang, X. Gao, J. Li, B. Feng, J. Gao, Effects of nitrogen level on the physicochemical properties of Tartary buckwheat (Fagopyrum tataricum (L.) Gaertn.) starch, Int.J.Biol. Macromol. 129 (2019) 799–808.

[19] D. Zhu, H. Zhang, B. Guo, K. Xu, Q. Dai, C. Wei, H. Wei, H. Gao, Z. Huo, Y. Hu, Effect of nitrogen management on structure and physicochemical properties of rice starch, J. Agric. Food Chem. 64 (2016) 6–3173.

[20] Y. Wang, P. White, L. Pollak, J. Jane, Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background, Cereal Chem. 70 (1993) 171–179.

[21] C. Mesquita, E. Garcia, A. Batista, S. Leonel, C. Franco, M. Leonel, Phosphate fertilization changes the characteristics of 'Maca' banana starch, Int.J.Biol. Macromol. 112 (2018) 1138–1145.

[22] D. Bechtel, I. Zayas, R. Dempster, J. Wilson, Size-distribution of starch granules isolated from hard red winter and soft red winter wheats, Cereal Chem. 70 (1993) 238–240.

[23] R.N. Waduge, X. Song, E. Bertoft, K. Seetharaman, Exploring the surface morphology of developing wheat starch granules by using atomic force microscopy, Starch-Stärke 65 (2013) 398–409.

[24] N. Cheetham, H.W.L. Tao, Variation in crystalline type with amylose content in maize starch granules: an X-ray powder diffraction study, Carbohydr. Polym. 36 (1998) 277–284.

[25] T. Zhou, Q. Zhou, E. Li, L. Yuan, W. Wang, H. Zhang, L. Liu, Z. Wang, J. Yang, J. Gu, Effects of nitrogen fertilizer on structure and physicochemical properties of 'super' rice starch, Carbohydr. Polym. 239 (2020) 116237.

[26] Y. Kim, K. Woo, H. Chung, Starch characteristics of cowpea and mungbean cultivars grown in Korea, Food Chem. 263 (2018) 104–111.

[27] E. Chiotelli, M. Meste, Effect of small and large wheat starch granules on thermomechanical behavior of starch, Cereal Chem. 79 (2007) 286–293.

[28] S. Wang, C. Li, L. Copeland, Q. Niu, S. Wang, Starch retrogradation: a comprehensive review, Compr. Rev. Food Sci. Food Saf. 14 (2015) 568–585.

[29] J. Singh, O. Mccarthy, H. Singh, P. Moughan, L. Kaur, Morphological, thermal and rheological characterization of starch isolated from New Zealand Kamo (Cucurbita pepo) fruit – a novel source, Carbohydr. Polym. 67 (2007) 233–244.

[30] E. Shimelis, M. Meaza, S. Rakshit, Physico-chemical properties, pasting behavior and functional characteristics of flours and starches from improved bean (*Phaseolus vulgaris* L.) varieties grown in East Africa, Int. Comm. Agric. Eng. 8 (2006) 1–19.

[31] V. Uarrota, M. Maraschin, M.E. Amante, I. Demiate, Physicochemical, thermal, and pasting properties of flours and starches of eight Brazilian maize landraces (Zea mays L.), Food Hydrocoll. 30 (2013) 614–624.

[32] J. Loh, The effect of shear rate and strain on the pasting behavior of food starches, J. Food Eng. 16 (1992) 75–90.

[33] A. Kaur, N. Singh, R. Ezekiel, N. Sodhi, Properties of starches separated from potatoes stored under different conditions, Food Chem. 114 (2009) 1396–1404.

[34] Q. Wang, W. Zheng, G. Fan, Y. Tang, G. Huang, S. University, Effect of ecological conditions and nitrogen strategy on starch RVA profile characteristics of wheat in Sichuan, J. Triticeae Crops. 01 (2016) 86–92.

[35] S. Singh, N. Singh, N. Isono, T. Noda, Relationship of granule size distribution and amylopectin structure with pasting, thermal, and retrogradation properties in wheat starch, J. Agric. Food Chem. 58 (2010) 1180–1188.

[36] S. Lee, H. Ju, H. Chung, Impact of diverse cultivars on molecular and crystalline structures of rice starch for food processing, Carbohydr. Polym. 169 (2017) 33–40.

[37] Y. Ji, L. Pollak, S. Duvick, K. Seetharaman, P. Dixon, P. White, Gelatinization properties of starches from three successive generations of six exotic corn lines grown in two locations, Cereal Chem. 81 (2007) 59–64.

[38] H. Tang, H. Ando, K. Watanabe, Y. Takeda, T. Mitsunaga, Some physicochemical properties of small-, medium-, and large-granule starches in fractions of waxy barley grain, Cereal Chem. 77 (2000) 153–178.