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Exploration of unique starch physicochemical properties of novel buckwheat lines created by crossing Golden buckwheat and Tatary buckwheat

Dongao Huo[a,1], Xue Xiao[b,c,1], Xiao Zhang[a], Xuefeng Hao[a], Zhanyang Hao[a], Enpeng Li[b,c,*]

[a] College of Biological Sciences and Technology, Taiyuan Normal University, Taiyuan 030619, China
[b] Jiangsu Key Laboratory of Crop Genomics and Molecular Breeding/Key Laboratory of Plant Functional Genomics of the Ministry of Education/Jiangsu Key Laboratory of Crop Genetics and Physiology, Agricultural College of Yangzhou University, Yangzhou 225009, China
[c] Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China

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ABSTRACT

Buckwheat is considered as a healthy cereal food, and it is essential to cultivate new buckwheat lines with good starch physicochemical properties for both consumers and food producers. Six novel buckwheat (Duoku, Dk) were generated by crossing of Golden buckwheat and Tatary buckwheat, and their kernel appearance properties and starch physicochemical properties were analyzed together with one domestic line (Cimiqiao) and one wild line (Yeku). The results showed that Dk samples had better appearance properties than two control samples. The Dk samples showed lower amylose content, similar amylopectin molecular structure and chain length distributions, and larger starch granules compared with Cimiqiao. The digestion results showed that two Dk samples: Dk6 & Dk9 had high resistant starch content; while the other two Dk samples: Dk37 & Dk38 had a steady glucose releasing rate. The Dk samples also showed high gelatinization temperature, indicating they were good raw materials for producing glass noodle. This study proved that Dk buckwheat had unique starch physicochemical properties, and could be used as new food materials in the future.

1. Introductions

Buckwheat is a widely grown cereal crop around the world and it can be used as daily food, baked food, beer brewing, etc. Buckwheat is considered as a healthy cereal due to its low starch content and enrichment in bioflavonoids (rutin, quercetin, etc.), buckwheat glycols, polyphenols, and other active functional components. Buckwheat food can reduce blood glucose, blood lipids, and enhance human immune function, and has auxiliary curative effects on patients with diabetes, hypertension, hyperlipidemia, coronary heart disease and stroke (Jogawat, Yadav, Chhaya, Lakra, Singh, & Narayan, 2021; Zhu et al., 2019).

Starch is the major nutrient component of buckwheat grains, which counts 50 ~ 60 % total weight of the grains. Starch fine structure and functional properties are important for controlling the quality of cereals. The amylose content is widely believed to affect the characteristics and quality of rice starch. Many previous studies have proven that low amylose content starch has high pasting viscosity, low gelatinization temperatures, high stickiness, and low hardness, which makes the cereals have good eating quality and are more popular among consumers (Li, Wen, Wang, & Sun, 2018; Nakamura et al., 2016; Zhao, Henry, & Gilbert, 2021). Starches with high amylose content, or large whole molecular size of starch, or more long amylopectin chains and short amylose chains, all could decrease starch digestion rate (Li, Gong, Huang, & Yu, 2021; Li, Hu, Gu, & Gong, 2021; Syahariza, Sar, Tizzotti, Hasjim, & Gilbert, 2013; Zhu, Tao, Zhang, Gilbert, & Liu, 2021). Cereals with slow starch digestion rate are generally considered as healthy food, which can reduce postprandial blood sugar index and relieve stress on the human metabolic system.

Cultivated buckwheat can be grouped into two main species: Common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tataricum*). Tartary buckwheat plays an important role in people's life, especially in poor mountainous areas. It is not only a main food crop, but also one of the main sources of economy (Yang et al.,

* Corresponding author at: Yangzhou University, Hanjiang District, Jiangsu, China.
E-mail address: lep@yzu.edu.cn (E. Li).
[1] These two authors contributed equally for this study.

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2021). In a traditional agricultural production model, the establishment of annual crops has both economic and agronomic implications such as high seed and nutrient inputs, ploughing, and may involve numbers of sowings each year. Golden buckwheat (*Fagopyrum dibotrys* or *Fagopyrum cymosum*) is a wild relative species of tartary buckwheat, which has the characteristics of large grains and richer active components such as rutin, underground enlarged stems, perennial, long flowering time, drought tolerance and cold resistance. Golden buckwheat is a perennial crop which has an important property, that is, "plant once and harvest always", indicating a lower seeding cost and workload. Agriculture based on perennial crops may provide a model with high output and with lower inputs (Chen, Huang, Li, Yang, & Cui, 2018). Therefore, *Fagopyrum cymosum* is considered as an important distant hybrid material for genetic improvement of tartary buckwheat (*Fagopyrum tataricum*) (He et al., 2022).

In this study, a series of novel buckwheat were created by crossing Tartary buckwheat and Golden buckwheat. Their agronomic traits, such as length of crop growth period, plant height, leaf number, yield, etc., showed that these novel buckwheat had good potential to become new commercial buckwheat lines. Thus, a further study on their starch physicochemical properties (including molecular structure, amylose content, crystalline structure, granular structure, thermal properties, pasting properties, and digestibility) would give better understandings of good traits about these novel buckwheat.

2. Material and method

2.1. Material

The novel buckwheat materials (*F.tatari-cymosum* Q.F. Chen), were created by crossing annual auto-tetraploid Tartary buckwheat(*Fagopyrum tataricum*) and perennial tetraploid *F.cymosum*, using the main agronomic traits (plant height, yield, grain color, etc.) and key molecular markers as screening criteria. After multiple generations of systematic selection, a series of stable lines were screened and identified, named Duoku series (Dks). The field performance of Dks was good, the maturity period was consistent, and the lodging resistance was good. Six Dks (Dk6, Dk9, Dk12, Dk13, Dk37 and Dk38) were analyzed in this study. The strain named Cimiqiao (CM) was a cultivated Tartary buckwheat line, and Yeku (YK) was a wild Tartary buckwheat line, and both of them were used as controls.

Dimethyl sulfoxide was purchased from Merck & Co, Inc. (Kenilworth, USA); lithium bromide (Reagent Plus) and protease from Streptomyces griseus (type XIV) were purchased from Sigma – Aldrich Chemical Co. (St. Louis, USA); Total Starch (AA/AMG) assay kit (K-TSTA) and isoamylase from *Pseudomonas* sp. (E-ISAMY) were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland).

2.2. Appearance of buckwheat grain

The thousand kernel weight of randomly picked 1000 buckwheat were measured by a balance (Wanshen, Hangzhou, China). The kernel area, perimeter, diameter, length and width were measured by SC-A type kernel analyzer (Wanshen, Hangzhou, China). The pictures of buckwheat kernels were taken by a Canon EDS800D camera (Tokyo, Japan).

2.3. Preparation of buckwheat flour and starch

The buckwheat flour was prepared by cryo-milling. The kernels were pre-cooled in liquid nitrogen for 5 min, then milled using a cryo-grounder (MM400, Retsch, Haan, Germany) at rate 10 s^{-1} for 5 min. The buckwheat starch was prepared by wet-milling following the method described in other study (Li, Wu, Yu, Gilbert, & Li, 2019). The kernels were soaked in 0.45 % sodium bisulfite (w/v) in 4℃ refrigerator for 2 days. Then the kernels were blended with a homogenizer (XHF-DY, Scientz Biotechnology, Ningbo, China) at 3000 rpm for 2 min, and the slurry was filtered with a 100 mesh screen. This step will be repeated twice. The filters were centrifuged at 4000g for 10 min, and the precipitate was incubated with protease (final concentration is 0.9U/mL) in tricine buffer (250 mM, pH 7.5) for overnight to remove the protein. The suspension was centrifuged at 4000 × g for 10 min and the precipitate was washed by distilled water twice and absolute ethanol twice. The moisture content of buckwheat flour and starch was measured by the difference in sample weight before and after drying in an oven at 110 °C overnight.

2.4. Starch and amylose content

The starch contents of buckwheat kernel were measured in duplicate following the method described in Total Starch (AA/AMG) assay kit (AACC, 1999). The amylose content of starch was measured in duplicate using the iodine colorimetry method described in other study (Vilaplana, Hasjim, & Gilbert, 2012).

2.5. Molecular size distribution of both whole starch and debranched starch

The wet-milled starch and debranched starch were used for *SEC* analysis of whole starch and debranched starch, respectively (Zhao et al., 2021). The analysis was carried out with a LC20AD system (Shimadzu Corporation, Kyoto, Japan) connected with a differential refractive index (DRI, Shimadzu). A series of pre-column, Gram 3000 and Gram 30 column (Polymer standard service (PSS), Mainz, Germany) were used for whole starch analysis. The resulting distributions were calibrated with pullulan standards (PSS) with peak molecular weight ranging from 342 to 2.35 × 10^6.

2.6. Chain length distribution of amylopectin and model fitting

The debranched starch was labeled by APTS (8-aminopyrene-1,3,6,-trisulfonaten), and then analyzed by a fluorescent-assisted capillary electrophoresis system (MDQ+, AB Sciex, Framingham, USA) for obtaining chain length distributions (CLD) of amylopectin, using the method described elsewhere (J. Zhu et al., 2021). The amylopectin CLDs were fitted by a mathematical model established in former studies (Wu, Morell, & Gilbert, 2013). In the model, amylopectin CLD is divided into three regions: short (DP (degree of polymerization) < 35), medium (35 < DP < 65), and long (DP > 65). Three parameters are obtained for each region: two β_{Ap} values which are the ratios of the relative activity of SBE (starch branching enzyme) to SS (starch synthase) in the first and second part of the region; and h_{Ap} value which is the relative activity of SS in the region.

2.7. Crystalline structure

The crystalline structure of buckwheat starch was measured in duplicate using an X-ray powder diffractometer (D8, Bruker, Germany). The analysis was operated at a voltage of 40 kV and a current of 40 mA, and starch crystalline profiles were obtained over an angular range of 2θ from 3° to 40° with a step of 0.05°. The relative crystallinity of starch was calculated using OriginPro 2021 software.

2.8. Granular structure and size of starch

The morphology of the starch granules was observed using a scanning electron microscope (SEM, JSM-6610, JEOL, Tokyo, Japan). The size of starch granules was determined in eight times using a Malvern Mastersizer Hydro 2000MU (Malvern Instruments Ltd., Malvern WR14 1XZ, UK) according to the method described elsewhere (Li, Zhang, Tan, Li, Fu, & Huang, 2019). The granule sizes were evaluated using d (0.1), d (0.5) and d (0.9) values.

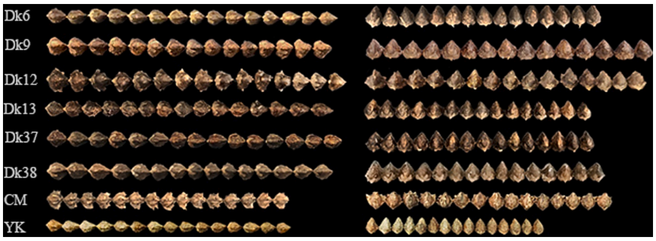

Fig. 1. The appearance of buckwheat grains, the left side is shown in horizontal orientation, and the right side is shown in longitudinal orientation.

2.9. Starch digestibility

The *in vitro* starch digestibility of buckwheat flour were analyzed using the method which simulated digestion in the small intestine (Li et al., 2019), and pancreatin & amyloglycosidase were two enzymes used in the experiment. The test timepoints were set at 0, 15, 30, 60, 120, 180 and 240 min after the addition of pancreatin and amyloglucosidase.

2.10. Thermal properties

Thermal properties of buckwheat starch were analyzed in duplicate using a Netzsch DSC 214 Polyma (Netzsch- Gerätebau GmbH, Selb, Germany) following the method described elsewhere (Li, Cao, Cao, & Li, 2021). Thermal properties of cake batter were characterized as the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH).

2.11. Statistic analysis

IBM SPSS Statistics 26 software was used to do statistical analysis on most of the obtained results. Mean ± standard deviation (SD) and statistically significant differences were reported using analysis of variance (ANOVA) with Turkey pairwise comparisons at $p < 0.05$.

3. Results and discussions

3.1. Grain appearance

All eight buckwheat kernels had different appearance properties (Fig. 1). The six Dk samples had significantly larger kernel weight, kernel area and kernel diameter (perimeter) compared with Cimiqiao and Yeku (Table 1), which showed Dk buckwheat had very good appearance properties. Among six Dk samples, Dk9 had the largest kernel weight and size, Dk12 had the second largest kernel size while Dk37 had the smallest kernel weight. The eight buckwheat kernels also had different shapes, with different length, width and length / width ratio. The length / width ratio of Dk9, Dk12 and Cimiqiao were smaller than the other samples, showing that they had approximately round shape. All the appearance results showed that Dk samples, especially Dk9 and Dk12, had better appearance properties than two control samples. Dk samples also showed larger size and weight compared with commercial buckwheat described in former studies (Unal, Izli, Izli, & Asik, 2016). This suggested that Dk buckwheat had good potential to become high yield buckwheat lines.

3.2. Starch and amylose content

The starch and amylose content were listed in Table 2. Buckwheat had similar starch content with maize and sorghum, but much lower than that of rice and wheat (El Halal, Kringel, Zavareze, & Dias, 2019). Cimiqiao had the highest starch content, which indicated that domestic buckwheat had been adjusted to high starch content to meet human's consumption desire. Dk37 and Dk38 had relatively higher starch contents; but the other 4 Dk samples showed lower starch content compared with Yeku. However, as all Dk samples had much larger kernel weight than two control samples (Table 1), Dk buckwheat kernel still had larger starch amount than Cimiqiao and Yeku. Buckwheat with more starch could produce more ingredients for producing foods such as noodles, bean jelly, bread and beers (Takahama & Hirota, 2010).

Amylose contents of the buckwheat (based on total dry starch content) were ranged from 26 to 29 % (Table 1). These values were higher compared with other common consumed cereal such as rice, wheat and maize (Changfeng Li, Ji, Zhou, & Li, 2021; Zhang, Fan, Yang, Li, Li, &

Table 1
The kernel weight and size information of 8 buckwheat samples.

Samples	Thousand kernel weight (g)	Kernel area (mm^2)	Kernel perimeter (mm)	Kernel diameter (mm)	Kernel length (mm)	Kernel width (mm)	Length / width ratio
Dk6	43.25	17.318	16.428	4.683	5.603	4.301	1.304
Dk9	56.72	20.646	18.151	5.113	5.505	4.993	1.108
Dk12	46.214	19.654	18.389	4.991	5.772	4.795	1.201
Dk13	43.188	16.327	16.045	4.549	5.557	4.197	1.329
Dk37	37.732	16.621	16.321	4.589	5.679	4.223	1.34
Dk38	43.913	17.481	16.823	4.711	5.547	4.357	1.273
CM	25.957	14.274	15.479	4.224	4.684	4.526	1.034
YK	23.362	11.227	13.228	3.775	4.715	3.352	1.408

Table 2
Starch content, amylose content, relative crystallinity, and starch granular size of 8 buckwheat samples.

samples	starch content (%)	Amylose content (%)	relative crystallinity (%)	d (0.1) (μm)	d (0.5) (μm)	d (0.9) (μm)
Dk6	65.4 ± 0.21ab	26.0 ± 0.96a	19.4 ± 0.16ab	5.3 ± 0.03a	61.4 ± 0.91a	149.1 ± 0.97a
Dk9	64.1 ± 0.41a	26.1 ± 0.48a	20.2 ± 0.06bc	6.7 ± 0.05c	61.7 ± 0.9c	152.8 ± 1.12c
Dk12	66.5 ± 0.53bc	26.1 ± 0.16a	20.5 ± 0.43c	6.9 ± 0.06d	73.1 ± 0.83e	160.3 ± 1.39d
Dk13	66.2 ± 1.12bc	25.8 ± 0.32a	19.3 ± 0.00a	7.2 ± 0.06e	74.1 ± 0.36e	158.4 ± 0.32d
Dk37	70.3 ± 0.82e	26.8 ± 0.48a	19.5 ± 0.34ab	6.7 ± 0.05 cd	72.3 ± 0.60e	160.6 ± 0.73d
Dk38	68.3 ± 0.04d	27.5 ± 0.48ab	20.8 ± 0.15c	8.1 ± 0.07f	80.9 ± 0.44f	163.9 ± 0.37e
CM	72.1 ± 0.77f	29.4 ± 0.32c	21.8 ± 0.10d	7.1 ± 0.07f	65.9 ± 0.65d	147.1 ± 0.55b
YK	67.1 ± 1.00 cd	28.8 ± 0.16bc	19.4 ± 0.05ab	5.5 ± 0.01b	56.0 ± 0.34b	140.2 ± 1.15a

Note: values shown are mean ± SD. Values with different letters in the same column are significantly different at $p < 0.05$.

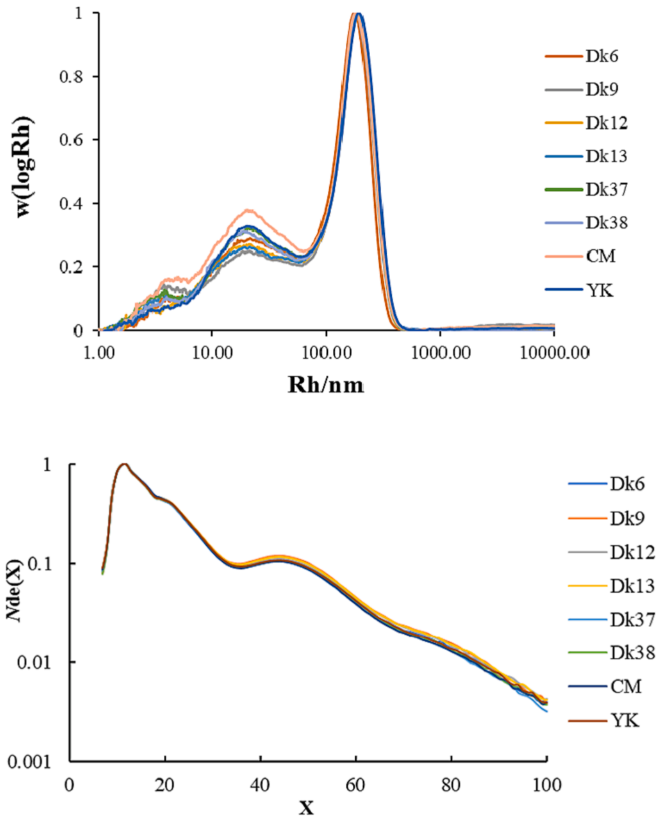

Fig. 2. (A) whole starch molecular size distributions of 8 buckwheat samples, (B) chain length distributions of 8 buckwheat samples.

Gilbert, 2021). Six Dks showed significantly lower amylose content compared with Cimiqiao and Yeku, but no significant differences in amylose content were observed between these six samples. Starchy food made from low amylose starch generally had good textural properties (H. Li, Prakesh, Nicholson, Fitzgerald, & Gilbert, 2016; Tao, Yu, & Gilbert, 2019). Both starch and amylose content results suggested that Dk buckwheat were good food producing materials.

3.3. Whole molecular size distributions

The whole molecular size distributions of starch were shown in Fig. 2A. The peaks (R_h ranged from 10 to 75 nm with peak at $R_h \sim 20$ nm) were the amylose peaks, and the peaks (R_h ranged from 100 to 5000 nm with peak at $R_h \sim 200$ nm) were amylopectin peaks. There were also small peaks or shoulders ranged from R_h 1 to 8 nm, which were from the remaining protein molecules. Although shear scission effect, the size of amylopectin molecular still could be used as for comparison if the samples were analyzed in the same run (Cave, Seabrook, Gidley, & Gilbert, 2009). No large differences were observed in the whole amylopectin size distributions, suggesting eight samples have similar amylopectin molecular structure. While Cimiqiao and Yeku have a larger proportion of amylose compared with six Dks, which are consistent with amylose content results.

3.4. Chain length distributions of amylopectin

The amylopectin chain length distribution (CLD) of eight buckwheat samples were very similar (Fig. 2B). Based on the amylopectin model fitting results (Table 3), amylopectin chain-length distributions (CLD) can be divided into three regions: short chains ($10 < DP < 32$, can be interpreted into $\beta_{Ap,i}$, $\beta_{Ap,ii}$), medium chains ($36 < DP < 66$, can be interpreted into $\beta_{Ap,iii}$, $\beta_{Ap,iv}$ and $h_{Ap,iii}$) and long chains ($DP > 70$), can be interpreted into $\beta_{Ap,v}$, $\beta_{Ap,vi}$ and $h_{Ap,v}$). The β values are negatively related to the average chain length of the region, and the h values are positively related to the total number of chains of the region (Yu, Li, Zou, Tao, Zhu, & Gilbert, 2019). This model could interpret amylopectin CLD by logarithmic scale, and it could give more information on amylopectin molecular structure (Yu et al., 2019). No significant differences were overserved for all six β values of all eight buckwheat samples, suggesting their amylopectin had similar chain lengths. For h values, Dk37, Dk38, Cimiqiao and Yeku have smaller $h_{AP,iii}$ & $h_{AP,v}$ values compared with the other four samples. This shows that these four samples have relatively less long and medium chains of amylopectin molecules.

3.5. Starch crystalline structure

The X-ray diffraction patterns of starch of all 8 buckwheat samples showed typical A type crystalline structure (Gao et al., 2020a, b), which had four major peaks on at 2θ ~ 5.3°, 17.1°, 18.2° and 23.5° (Fig. 3). A-type crystalline structure was commonly found in cereal starch, which contains more short amylopectin chains (A and B1 chains). Crystalline structure plays important roles in determining starch functional properties such as thermal properties, pasting properties and digestibility (Li

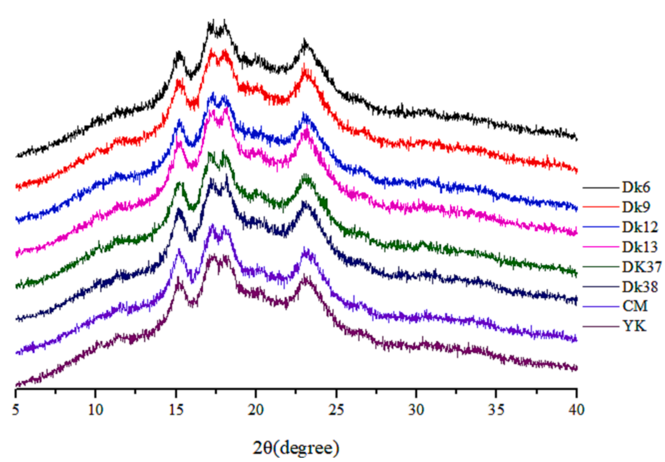

Fig. 3. X-ray diffraction patterns of 8 buckwheat samples.

Table 3
Amylopectin CLD fitting parameters.

Samples	$\beta_{AP,i}(10^{-3})$	$\beta_{AP,ii}(10^{-3})$	$\beta_{AP,iii}(10^{-3})$	$\beta_{AP,iv}(10^{-3})$	$\beta_{AP,v}(10^{-3})$	$\beta_{AP,vi}(10^{-3})$	$h_{AP,iii}(10^{-3})$	$h_{AP,v}(10^{-3})$
Dk6	84.9 ± 2.44a	1.6 ± 0.24a	61.8 ± 0.54a	2.9 ± 0.65abc	46.4 ± 1.94ab	5.9 ± 1.25a	96.2 ± 0.37c	7.2 ± 0.09bc
Dk9	84.5 ± 1.45a	0.9 ± 0.14a	59.7 ± 0.43a	4.2 ± 0.65c	46.2 ± 0.3a	5.6 ± 1.45a	96.8 ± 1.77c	7.7 ± 0.32c
Dk12	85 ± 0.13a	2.1 ± 0.04a	60.4 ± 0.37a	2.9 ± 1.32abc	46.5 ± 0.74a	3.7 ± 0.09a	91.8 ± 0.71b	7.3 ± 0.21bc
Dk13	83.7 ± 0.78a	2.4 ± 0.14a	60.5 ± 0.43a	3.8 ± 0.3bc	42.7 ± 0.48c	4.3 ± 0.8a	91.3 ± 0.5b	7.4 ± 0.06bc
Dk37	86.6 ± 1.25a	1.2 ± 0.2a	59.8 ± 0.31a	1.3 ± 0.06ab	48.9 ± 1.45ab	3.6 ± 0.37a	83.8 ± 2.37a	5.7 ± 0.15a
Dk38	84.5 ± 0.95a	2.3 ± 0.32a	61.5 ± 1.4a	1 ± 0.24ab	51 ± 1.25a	3.3 ± 0.39a	86.5 ± 0.57a	6 ± 0.23b
CM	87.1 ± 1.34a	2.7 ± 0.77a	62.3 ± 0.82a	3.4 ± 0.08bc	43.4 ± 1.31c	3.9 ± 0.85a	87.8 ± 1.47a	6.4 ± 0.26b
YK	85.8 ± 1.55a	1.6 ± 0.15a	62.1 ± 1.06a	1.1 ± 0.02a	47.4 ± 0.2ab	3 ± 0.53a	88.6 ± 1.02a	6.3 ± 0.29b

Note: values shown are mean ± SD. Values with different letters in the same column are significantly different at $p < 0.05$.

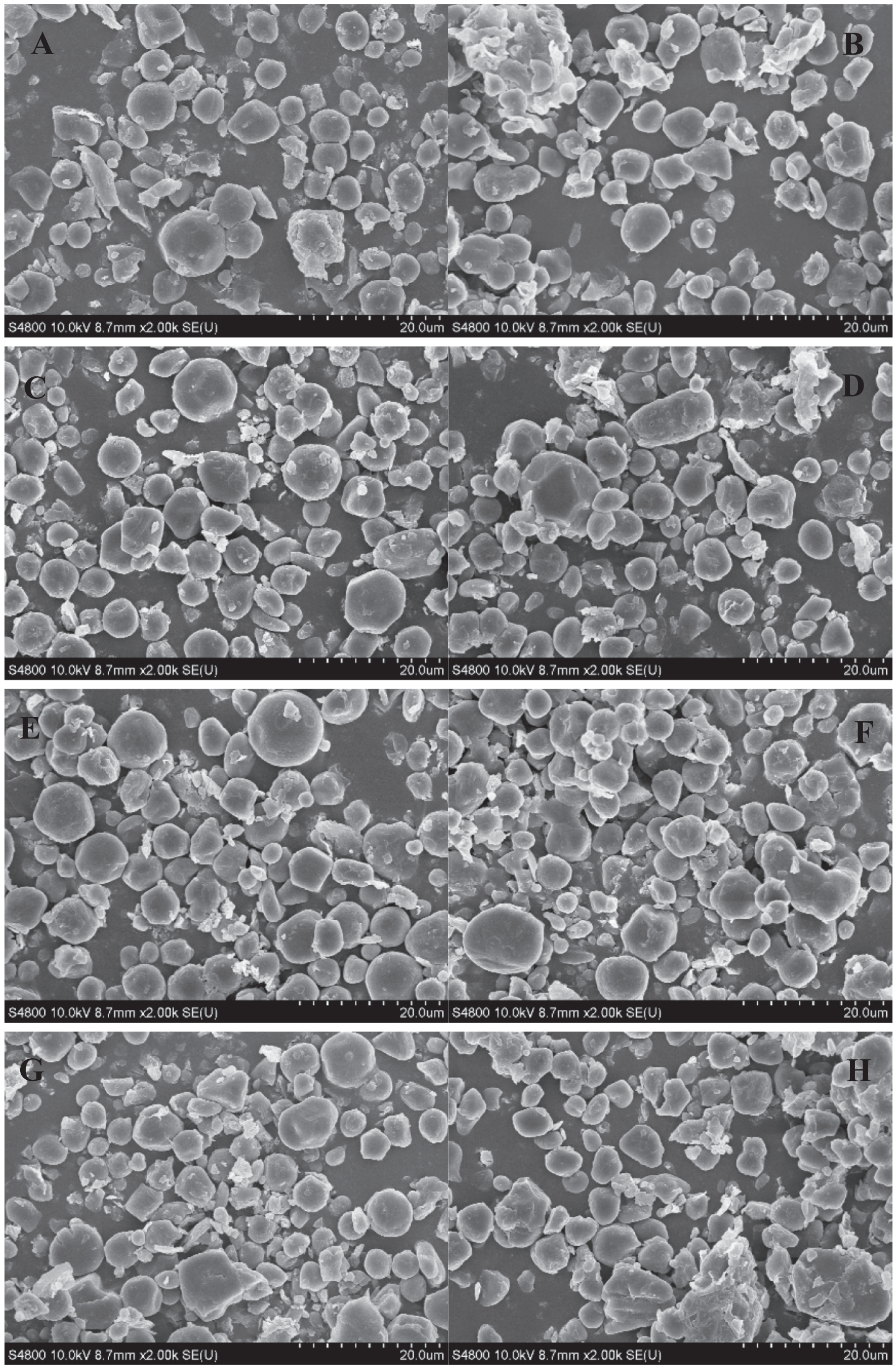

Fig. 4. The SEM image of 8 buckwheat samples, A to H represent Dk6, Dk9, Dk12, Dk13, Dk37, Dk38, Cimiqiao and Yeku, respectively.

et al., 2021; Zhang et al., 2021). Cimiqiao, the only domestic buckwheat, had the highest degree of crystallinity among these eight samples (Table 2), indicating domestic selection trends to cultivate buckwheat with higher crystallinity. The crystallinity of Dks could be divided into two groups: Dk9, Dk12, and Dk38 had higher crystallinity compared with Dk6, Dk13, and Dk37. The latter group had similar crystallinity with Yeku. However, the differences of crystallinity among eight samples were not very large (~2.5 %), which was consistent with amylopectin CLD results (Fig. 2B & Table 3). Starch crystallinity was mainly contributed by crystalline structure of amylopectin, which was determined by CLD of amylopectin (Zhong et al., 2021).

3.6. *Starch granular structure*

SEM micrographs of buckwheat flour were shown in Fig. 4. No significant differences were observed from the morphology of starch granules for all eight buckwheat samples. Though buckwheat had similar starch content compared with maize and sorghu, no indentations were observed on the surface of granules, which showed that buckwheat starch granule and protein bodies were not packed as close as maize or sorghum (Hasjim, Srichuwong, Scott, & Jane, 2009; Li, Hasjim, Dhital, Godwin, & Gilbert, 2011), This result might indicate that protein components in buckwheat should not have big influences on buckwheat starch physicochemical properties.

The granular size results were shown in Table 2. The value of d (0.1),

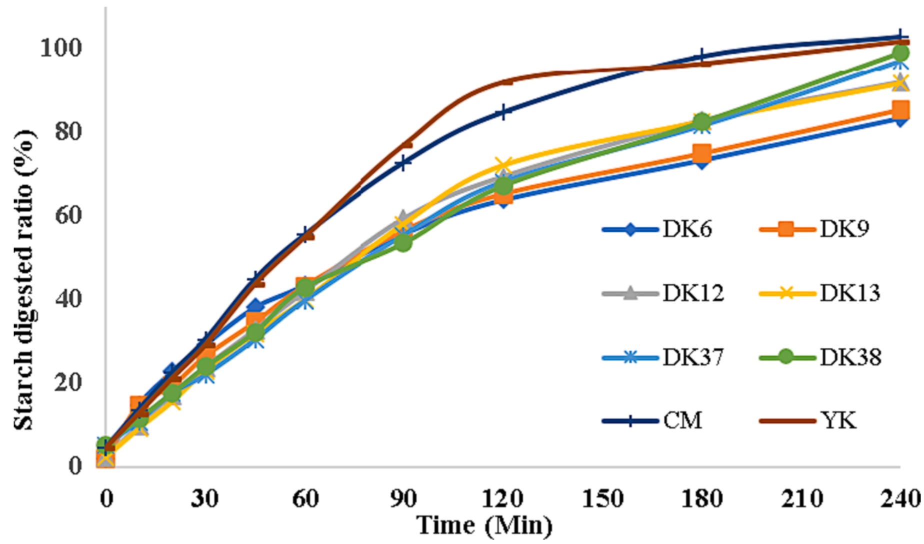

Fig. 5. Digestion profiles of 8 buckwheat samples.

d (0.5) and d (0.9) represents 10 %, 50 % and 90 % of the starch granules had smaller size than that value, respectively. Eight buckwheat samples had similar d (0.1) values, ranged from 5 to 8 μm. However, Dk6, Dk9, Cimiqiao and Yeku showed significantly lower d (0.5) and d (0.9) values, suggesting that these eight samples can be categorized into two groups: larger granular size group (Dk12, Dk13, Dk37 & Dk38) and smaller granular size group (Dk6, Dk9, Cimiqiao and Yeku). Starch granular size could affect several starch functional properties including pasting properties, digestibility, rheology (Qi & Tester, 2016; Zhang, et al., 2020), which would be discussed later.

3.7. Starch digestibility

The digestion profiles were shown in Fig. 5. Former studies had shown that starch digestion rate in first 30 min was too fast (Du, Pan, Obadi, Li, & Xu, 2021), thus the amount of digestion enzyme was decreased in this study. In the early stage of digestion process (the first 60 min), Cimiqiao and Yeku showed the fastest digestion rate, and Dk6 and Dk9 showed faster digestion rate compared with other 4 Dk samples. In the later stage of digestion, Cimiqiao and Yeku still had the fastest digestion rate, while the digestion rate of Dk12, Dk13, Dk37 and Dk38 became faster than Dk6 and Dk9 samples. By the end of digestion (at 240 min), CM and YK had the highest starch digestion ratio; followed by Dk37, Dk38, Dk12, and Dk13; Dk6 and Dk9 had the lowest digestion ratio of starch. The *in vivo* digestion in human small intestine generally took 3 to 4 h before food reaches large intestine (C. Li et al., 2023), which suggested that Dk6 and Dk9 had highest resistant starch content.

The digestibility of starch could be affected by several factors. In the early digestion stage, starch granules with smaller size had better chance to interact with enzymes because they have larger surface area. Cimiqiao, Yeku, Dk6 and Dk9 had smaller starch granular size than the other four buckwheat (Table 2), thus they had faster starch digestion rate in the first 60 min. In the later stage of starch digestion, starch granule collapsed into small parts, (Dhital, Butardo Jr, Jobling, & Gidley, 2015), and starch molecular structure and amylose content became the major determining factors affecting starch digestion rate (Li et al., 2021). Cimiqiao, Yeku, Dk37 and Dk38 had less amylopectin long and medium chains, indicating they were more vulnerable for enzyme hydrolyzation (Table 3). All the Dk samples had low amylose content (Table 2), and starch digestion rate were found to have negative correlation with amylose content (Copeland, Blazek, Salman, & Tang, 2009; Saito et al., 2001). So Dk12, Dk13, Dk37 and DK 38 had faster digestion rate in the later digestion rate. Furthermore, Dk37 and Dk38 had higher final starch digestion ratio than the other four DK samples. The digestion results suggested some Dk samples had very special digestion properties. Dk6 & Dk9 had high resistant starch content, and they could be used to produce low GI (glycemic index) food. While Dk37 & Dk38 had relative slow digestion rate in the early stage and relative fast digestion rate and high digestion ratio in the later stage, indicating that they could release enough glucose in a steady way and not cause rapid increase of blood glucose level. Dk37 & Dk38 could be used to produce healthy hunger-resistant food.

Table 4
Thermal properties of 8 buckwheat flour.

Samples	ΔH (J/g)	T_o(℃)	T_p(℃)	T_c(℃)
Dk6	11.0 ± 0.39a	64.6 ± 0.14e	72.1 ± 0.00d	82.7 ± 0.21c
Dk9	12.7 ± 0.47d	64.0 ± 0.35de	72.1 ± 0.21d	81.5 ± 0.35bc
Dk12	11.4 ± 0.12a	63.5 ± 0.42 cd	71.4 ± 0.14c	82.0 ± 0.28bc
Dk13	11.7 ± 0.06abc	64.1 ± 0.07de	71.8 ± 0.28 cd	81.9 ± 0.35bc
Dk37	11.9 ± 0.20bc	64.1 ± 0.07b	71.4 ± 0.00b	81.2 ± 0.28b
Dk38	12.5 ± 0.28bc	62.8 ± 0.07bc	71.7 ± 0.07 cd	82.4 ± 0.00c
CM	11.6 ± 0.00ab	62.2 ± 0.00b	69.4 ± 0.07a	77.8 ± 0.21a
YK	11.5 ± 0.18ab	60.5 ± 0.28a	68.8 ± 0.21a	78.4 ± 0.21a

Note: values shown are mean ± SD. Values with different letters in the same column are significantly different at $p < 0.05$.

3.8. Thermal properties of starch

Starch thermal properties are important functional properties of starch, as they can reflect the cooking performance and eating qualities of cereal food (Benmoussa, Moldenhauer, & Hamaker, 2007). The thermal properties of buckwheat flour were summarized in Table 4. The enthalpy changes (ΔH) of starch were similar between all eight samples, ranged from 11 to 12.7. This was consistent with crystalline results, as ΔH was positively correlated with starch crystalline structure (Zhang, Li, Fan, Yang, Ma, & Gilbert, 2020).

Unlike ΔH, the gelatinization temperatures of Dk samples, including T_o, T_p and T_c, were all higher than those of Cimiqiao and Yeku. The gelatinization temperatures were mainly affected by the arrangement of amylopectin double helices (C. Li & Hu, 2021). The packing of double helices structure was mainly determined by the CLD of amylopectin, but it could also be affected by the interaction between amylose chains and amylopectin chains (Tao, Li, Yu, Gilbert, & Li, 2019). Dk samples had similar amylopectin molecular structure (Fig. 2A) and CLDs (Fig. 2B), but they had significantly lower amylose content (Table 2). This suggested the amylopectin double helices of Dk samples were less disturbed by the amorphous amylose chains, and they had more rigid structures than those of Cimiqiao and Yeku. The thermal properties results suggested that Dk samples could be very good material to produce buckwheat glass noodles, whose production process preferred to use raw starchy materials with good toughness and not easy to be fractured.

4. Conclusions

Compared with Cimiqiao and Yeku, the novel buckwheat lines Dk showed very good appearance properties and starch physicochemical properties. All Dk buckwheat had better appearance properties than Cimiqiao and Yeku, and Dk9 & Dk12 had the best appearance properties among six Dk sample. Six Dk samples had very different digestion properties: Dk6 & Dk9 contained high resistant starch content, while Dk37 & Dk38 showed steady digestion curves. This was attributed to their different amylose content, amylopectin chain length distributions and starch granular size. Dk samples also showed higher gelatinization temperatures than two control samples, due to their low amylose content. The thermal properties result suggested that Dk samples were good starchy materials for producing glass noodles.

Ethics declarations

Ethics approval and consent to participate

The samples used in this study were grown in China and collected by our lab (College of Biological Sciences and Technology, Taiyuan Normal University, Taiyuan, Shanxi, China). Special thanks to Professor Chen Qingfu of Guizhou Normal University for his outstanding contribution in the early creation of perennial germplasm. All authors stated that this study comply with the Chinese legislation and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

CRediT authorship contribution statement

Dongao Huo: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Project administration. **Xue Xiao:** Methodology, Formal analysis, Investigation, Writing – original draft. **Xiao Zhang:** Formal analysis, Resources. **Xuefeng Hao:** Formal analysis. **Zhanyang Hao:** Investigation. **Enpeng Li:** Conceptualization, Validation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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