Characterization and comparative study on structural and physicochemical properties of buckwheat starch from 12 varieties

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Abstract: Buckwheat is an important starch source because of its health benefits. In this study, buckwheat starches isolated from 12 varieties were analyzed based on the morphological, structural and physicochemical properties. The results showed that starch samples from different varieties had high purity with the total starch ranging from 91.29 to 95.11%, while showing significant differences in ash content (0.12-0.25%), protein content (0.26-0.34%) and amylose content (29.55-36.13%), respectively. All samples presented spherical and irregular shapes and typical A-type crystalline structure, but obvious differences in granule size distribution and relative crystallinity (26.37-35.21%) were observed among 12 varieties. Starch samples differed in lamellar structures, showing higher values of thickness of the samples with higher amylose content. In addition, buckwheat starches with higher amylose content showed higher values in light transmittance and rheological properties, while starch samples with lower amylose content obtained higher values in terms of water solubility, swelling power, pasting behaviors and thermal parameters. The principal component analysis and cluster analysis based on starch property parameters indicated that there were significant similarities and differences among 12 varieties, which might be related to the genotypes. This study would provide valuable information for the full use of buckwheat starch in food and non-food industries.

Keywords: Buckwheat; starch; structural properties; physicochemical properties

1. Introduction

Buckwheat is an annual dicotyledonous crop belonging to the genus *Fagopyrum* of the Polygonaceae family. It is widely recognized that buckwheat grain is rich in starch, protein, lipid, minerals, vitamins and dietary fiber (Ahmed, et al., 2014; Gao, et al., 2016). Buckwheat has also been considered as a source of herbal medicine for preventing and controlling the cardiovascular disease, obesity and cancer due to the high proportion of beneficial health components (phenolic compounds and phytosterols) (Kaur, Jha, Sabikhi, & Singh, 2014; Liu, Wang, Cao, Fan, & Wang, 2016). Recently, there has been an increasing emphasis on natural and healthy foods, making buckwheat an ingredient in functional food based on the low glycemic index. It has also been reported that buckwheat is a suitable food ingredient for different types of food such as noodles, pasta, biscuits and baking food (Yang, et al., 2019), showing good market potential in the functional and healthy food industry. Therefore, more research needs to be done on the food aspects of buckwheat.

Starch is mainly composed of amylose and amylopectin (Perez-Pacheco, et al., 2014), which can be used as a raw material or a food additive in developing food products or be applied as a delivery vehicle for substances of interest in the food and pharmaceutical industries (Ovando-Martinez, Bello-Perez, Whitney, Osorio-Diaz, & Simsek, 2011). Amylose is largely linear with a smaller molecular weight, while amylopectin is highly branched with relatively large molecular weight (Zhu, 2015). The amylose content and fine structure of amylopectin are critical for the physicochemical and functional properties of starch, thereby determining its application. Buckwheat starch is the major component of the grain and appropriately accounts for 60-80% of the whole grain with about 25% amylose and 75% amylopectin (Qin, Wang, Shan, Hou, & Ren, 2010). Previous studies have shown that variations in amylose content and amylopectin chain length distribution of buckwheat starch result in differences in the light transmittance, swelling power, thermal and textural properties (Gao, et al., 2020; Hu, et al., 2022; Liu, et al., 2016). It has also been reported that the structure and properties of starch are critical for the quality of the buckwheat-based products (Zhu, 2015). For example, amylose content is positively correlated with the elasticity of heated buckwheat dough due to the gelling capacity of amylose (Ikeda, Kishida, Kreft, & Yasumoto, 1997), and short chains of amylopectin is negatively correlated to the water solubility of buckwheat starch. Compared with maize and potato starch, buckwheat starch has the smallest granule size (3-14 μm) with lower water solubility and gelatinization enthalpy but higher gelatinization temperatures (Gao, et al., 2016). Kreft and Skrabanja (2002) have found that buckwheat starch had a slow glucose release rate and a large amount of resistant starch compared with other cereal starches, making it suitable for diabetic diets. The relationship between structure and properties of starch is a research hotspot. However, there have just few reports on the physicochemical properties of buckwheat starches, and these reports are on relatively fewer varieties. Therefore, understanding the relationships between the structural and physicochemical properties of buckwheat starches isolated from different varieties is essential for the development of starch-based products of buckwheat in food industry.

In this study, buckwheat starches with different amylose contents were isolated from 12 varieties collected from 8 countries. The chemical composition, structural (morphological, crystalline structure, lamellar structure and short-range ordered structure) and physicochemical (water solubility, swelling power, light transmittance, pasting, thermal and rheological) properties were determined and compared, and the correlation between structural and physicochemical properties was investigated. The main aim of study was to reveal the relationship between structural and properties of buckwheat starch and provide useful information for starch production and utilization of buckwheat in food industry.

2. Materials and methods

2.1 Materials

A total of 12 buckwheat varieties collected from 8 countries were used in this study. All buckwheat varieties were planted in the Bottelare field (50°59'N, 3°49'E) of Belgium and harvested at maturity. An overview of the samples was listed in Table 1.

2.2 Starch isolation

Buckwheat starch was isolated from the hulled seeds following the method described by Hu, et al. (2022) with slight modifications. Buckwheat flour (500 g) was soaked in sodium hydroxide solution (0.2%, w/v) at a ratio of 1:3 and left at room temperature (25°C) for 16 h. Then, the samples were passed through a 150-mesh sieve and centrifuged at 4000 g for 10 min (the above step was repeated 3 times). Next, the supernatant was poured off and the white sediment from the bottom was washed with distilled water until it became clean. Finally, the samples were dried at 40°C, ground into powders and passed through a 100-mesh sieve.

2.3 Analysis of chemical composition

The moisture content was measured using the rapid moisture determination instrument (Sartorius, MA37-1). Ash content was determined according to standard method ICC no. 104/1. Protein content was measured using a Kjeldahl nitrogen analyzer and a factor of 6.25 was used to calculate the protein content. Amylose content and total starch content were quantified by the Amylose Assay Kit and Total Starch Assay Kit (Megazyme Ltd., Wicklow, Ireland), respectively.

2.4 Cryo-scanning electron microscopy

The starch samples were visualized using a JSM-7100F TTLS LV TFEG-SEM (Jeol Europe BV, Zaventem, Belgium). The starch powder was placed on a carbon double sided sticky tape that was fixed on an aluminum stub, vitrified in a nitrogen slush and transferred under vacuum conditions into a PP3010T cryo-preparation system (Quorum Technologies, East-Sussex, UK) conditioned at -140°C. Subsequently, the sample was sublimated for 10 min at -70°C to remove frost artefacts, sputter-coated with platinum using argon gas, transferred to the SEM stage at -140°C and electron beam targeted at 3 keV.

The images of buckwheat starch granules were further analyzed using ImageJ (National Institutes of Health, USA). The starch granules (50) with complete morphology were selected for labelling in each cryo-scanning electron microscopic image, and the obtained results of particle size were then made into the frequency distribution histograms.

2.5 Granule size analysis

The granule size of buckwheat starch was measured using a laser diffraction particle size analyzer (Malvern Instruments Ltd., Malvern, UK) equipped with a 300 F lens. Data analysis was conducted using the Mastersizer software, and the refractive index of real and imaginary particles was 1.45 and 0.1, respectively (Hellemans, et al., 2017). The granule size distribution was reported in terms of the volume distribution.

2.6 Wide angle X-ray scattering (WAXS)

The WAXS pattern of buckwheat starch was determined using an X-ray scattering instrument (GeniX ^{3D} Cu HFL, Xenocs, France) following the method of Zhang, et al. (2018). The XRD patterns were recorded from 5° to 50° (2θ) with a scanning speed of 1.2°/s. XSACT software (Xenocs, France) was used to normalize the results. The relative crystallinity (RC) was the ratio of the crystallinity area to the total diffraction area.

2.7 Small angle X-ray scattering (SAXS)

2.7.1 SAXS measurement

The SAXS test was conducted by a small-angle X-ray scattering instrument (GeniX ^{3D} Cu HFL, Xenocs, France). The optics and sample chamber were under vacuum to reduce air scattering. The 1D scattering curves were in the range of $0 < q < 0.3$ Å^{-1} from the 2D scattering patterns.

2.7.2 SAXS analysis

The obtained data was calibrated from the background scattering using the XSACT software (Xenocs, Sassenage, France). The data was further analyzed to calculate the lamellar parameters, through the normalized 1D correlation function as described by Kuang, et al. (2017) based on the following equation (1):

$$L(r) = \frac{\int_0^{\infty} I(q)q^2 \cos(qr)\, dq}{\int_0^{\infty} I(q)q^2\, dq} \quad (1)$$

Where I(q), q and r were scattering intensity, scattering vector and the direction along the lamellar stack, respectively.

2.8 Water solubility and swelling power

The water solubility (WS) and swelling power (SP) of buckwheat starch were determined following our previous method (Gao et al., 2020). The WS (%) and SP (g/g, dry basis) was calculated as follows:

WS = mass of dried supernatant/mass of dry starch x 100% (2)

SP = sediment weight/mass of dry starch x (100-WS) (3)

2.9 Light transmittance

The starch sample (0.2 g) and distilled water (20 mL) were mixed and heated in boiling water for 30 min. After the samples were cooled to 25°C, the light transmittance (LT) was measured at 620 nm using a spectrophotometer with distilled water as a control (Gao, et al., 2020).

2.10 Pasting properties

Pasting profiles of buckwheat starch were measured using a Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria) through the method of Hellemans et al. (2017) with slight modifications. A 6% (w/v) starch-water suspension corrected for its moisture content was prepared for the measurements. After the pre-shearing, the suspension was held at 50°C for 1 min and then heated to 95°C at a rate of 5°C/min, held at 95°C for 5 min, cooled to 50°C at the same rate and finally held at 50°C for 2 min. The pasting parameters, including peak viscosity (PV), holding strength (HS), final viscosity (FV), breakdown (BD), setback from peak (SBP), setback from trough (SBT) and pasting temperature (PT), were automatically obtained through the RheoCompass software.

2.11 Thermal properties

The thermal properties of buckwheat starch were performed by differential scanning calorimetry (DSC) (Q1000, TA instruments, New Castle, DE, USA) following the method of Guo, et al. (2019) with slight modifications. Briefly, starch and distilled water were mixed into suspension at a ratio of 1:3, and the samples were sealed in an aluminum pan at 4°C overnight. Then, the samples were heated from 30 to 100°C at a rate of 10°C/min with an empty pan as a reference. The transition temperatures (onset, To; peak, Tp and conclusion, Tc) and gelatinization enthalpy (ΔH) were obtained from the DSC curve.

2.12 Rheological properties

The rheological analysis was performed using a Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria) following the method of Jiang, et al. (2020) with some modifications. The starch suspension (8%, w/v) was cooked in boiling water for 15 min. After the samples were cooled to room temperature (25°C), the starch gel was loaded onto the bottom plate at 25°C combined with a thin layer of silicone oil to reduce evaporation loss. The strain sweep test was carried out to determine the linear viscoelastic range (LVR).

2.12.1 Frequency sweep

The frequency sweep of buckwheat starch was conducted from 0.1 to 100 rad/s at

1% strain that was within LVR. The storage modulus (G′), loss modulus (G″), complex viscosity (η*) and loss angle (tan δ = G″/ G′) were recorded.

The obtained data could be analyzed by a Power law model with the following formula (Li, et al., 2021):

$G' = K' \times \omega^{n'}$ (4)

$G'' = K'' \times \omega^{n''}$ (5)

Where K' and K'' represented model constants (Pa/s^n), n' and n'' were the frequency modulus exponents (dimensionless), and ω was the frequency (rad/s).

2.12.2 Creep-recovery test

The creep-recovery test was studied with the constant stress of 1 Pa for 300 s. Then, the applied stress was removed and the performance was recorded for another 600 s. The obtained data was fitted using the four-parameter Burger's model (Zhao, Li, Wang, & Wang, 2022):

$J(t) = 1/G_0 + 1/G_1(1 - e^{-t/\lambda}) + t/\mu_0$ (6)

Where J, G_0, G_1, λ and μ_0 was the creep compliance (1/Pa) at t time, the instantaneous elastic modulus (Pa), the retarded elastic modulus (Pa), the retardation time (s) and the viscous modulus (Pa s), respectively.

2.13 Statistical analysis

The results were expressed as means ± standard deviations. One-way analysis of variance (ANOVA) and Duncan's multiple-range test ($p < 0.05$) were conducted using SPSS software (v. 22.0, IBM, USA) for analyzing the significant difference among the data. Principal component analysis (PCA) and cluster dendrogram analysis based on single-linkage were performed using the OriginPro software (v. 2021, Originlab, USA) to determine the similarities and differences among 12 buckwheat starches.

3. Results and discussion

3.1 Main chemical composition

The main chemical compositions of buckwheat starch collected from different countries are summarized in Table 1. The yield of 12 buckwheat starches ranged from 22.48 to 31.58%. There was significant difference in the total starch content ranging from 91.29 (BU9) to 95.11% (BU5), indicating that the purity of the sample was reasonably high (> 90%). The moisture content was between 8.43 and 13.65% with the lowest value in BU3 and the highest value in BU5, which was within the moisture level recommended for commercial starches (Soni, Sharma, & Gharia, 1993). Differences in moisture content among 12 buckwheat varieties may be due to the degree of starch drying. Significant differences were observed in ash content ranging from 0.12 (BU1) to 0.25% (BU5), which was similar to the results of quinoa starch (Jiang, et al., 2020) but slightly lower than that in sweet potato starch (Abegunde, Mu, Chen, & Deng, 2013). The protein content of 12 starch samples was significantly different ranging from 0.26 (BU6) to 0.34% (BU5). Normally, the proteins in starch granules are mainly surface proteins and internal proteins. The former can be easily removed, while the removal of the latter requires the destruction of the starch granule structure (Swinkels, 1985). It has been reported that the isolation process can influence the protein content and that surface proteins can be removed from starch granules with NaOH solutions (Guo, et al., 2019), which can be used to explain the low protein content of buckwheat starch of this study. In addition, the samples were low in ash and protein content, which met the experimental requirements for the absence of non-starch lipids and hydrated fine fibers (Jan, Panesar, Rana, & Singh, 2017). Significant differences were also observed in amylose content ranging from 29.55 to 36.13%, with the lowest value in BU3 and the highest value in BU8. The result of amylose content in this study was lower than that of previous results (Gao, et al., 2020), indicating that buckwheat variety can influence the amylose content of starch. It has been reported that amylose content has a crucial effect on the functional characteristics of starch, and the differences are mainly related to genotype background, growing environment, and measuring method (Zhang, et al., 2018).

3.2 Morphological properties and particle size distribution

The morphology of buckwheat starch granule was observed using cryo-scanning electron microscope at two different magnifications of 1000 and 5000 (Fig. 1). Most starch granules were irregular polygons with obvious edges and a few granules appeared spherical shape, which was consistent with previous studies on buckwheat starch of different varieties (Gao, et al., 2016; Gao, et al., 2020; Hu, et al., 2022). At high magnification, some hollows were observed on the surface of the starch granule, which could be explained by the fingerprints of the native protein bodies (Dura, Blaszczak, & Rosell, 2014). The image analysis showed that the granule sizes of 12 buckwheat starches followed a normal distribution (Fig. 1), and there were significant differences in the granule size among different varieties with the maximum value in BU3 (7.29 μm) and the minimum value in BU8 (6.02 μm).

The volume distribution and standard average diameter can be obtained by using the laser diffraction particle size measuring instrument, assuming that the particles are spherical. As shown in Fig. 2 A, a smooth curve with two peaks was observed for the volume distribution of 12 starch samples with the weak peaks showing at 1 μm and the strong peaks occurring at about 10 μm. There were significant variations in the volume distribution among different varieties, showing the largest volume distribution in BU10 and the smallest distribution in BU11. The size of most starch granules ranged from 3 to 20 μm, smaller than that of maize starch and sweet potato starch (Lin, et al., 2016; Zhang, et al., 2018). The D [4,3] ranged from 7.158 (BU8) to 8.576 (BU3) μm and the D [3,2] was in the range of 4.052 (BU12) to 4.583 μm (BU3), slightly lower than the results of the previous study (Gao, et al., 2020). The d (0.1), d (0.5) and d (0.9) were in the range of 2.590-3.569, 7.251-8.307 and 10.883-16.558 μm, respectively, with the maximum value in BU3 and the minimum value in BU8 (Table 2). It has been reported that starch granule size plays an important role in affecting the pasting behaviors of starch. Abegunde et al. (2013) have found that the granule size of sweet potato starch was positively correlated with the PV, BD and SB, which was consistent with the results of this study as shown in Fig. 6 A. The granule size of starch can be affected by the variety, growing condition and plant physiology (Guo et al., 2019). In this study, the 12 buckwheat starch samples were planted in the same experimental site, indicating that the variations in particle size distribution of 12 starch samples due to the various genotype backgrounds.

3.3 WAXS

Generally, the X-ray scattering is widely used to study the helical structures of starch crystals at longer range scales (Kuang, et al., 2017). The XRD patterns of buckwheat starches and their relative crystallinities are shown in Fig. 2 B. The typical A-type crystalline structure can be observed with strong diffraction peak at around 15° and 23° 2θ and an unresolved peak at 17° and 18° 2θ, which was consistent with the results of normal cereal starches (Cheetham & Tao, 1998). It has been reported that the peak intensity at $2\theta = 5.4°$ represents the B-type polymorphic form and the peak intensity at $2\theta = 20°$ corresponds to the amylose-lipid complex. In this study, slight difference was obtained in the peak positions of starch samples, which might be due to the genotypes and amylose content among different buckwheat varieties. As shown in Fig. 2 B, there were significant variations in the RC of 12 starch samples ranging from 26.37% in BU8 to 35.21% in BU3, indicating that the BU3 presented more crystalline regions in comparison with other buckwheat varieties. The difference in the RC among 12 buckwheat starches might be related to the variations in granule size and chemical compositions (Table 1). Compared with maize starch and bean starch (Lin, et al., 2016; Ovando-Martinez, et al., 2011), buckwheat starch showed the highest value in the RC, which could be related to the genotypes. The crystalline region of starch can be affected by the structure and content of amylopectin molecules, while the amorphous region is related to amylose molecules. In this study, the RC of buckwheat starch was negatively correlated with the amylose content (Fig. 6 A), which was similar to the results of maize starch (Cheetham, et al., 1998).

3.4 SAXS

The variations of the lamellar structure of buckwheat starches were further determined through the small angle X-ray scattering (SAXS). The SAXS one-dimensional (1D) scattering intensity distribution of various starch samples are presented in Fig. 2 C. One "shoulder-like" scattering peak was observed around the q value of 0.56-0.73 nm^{-1} in each SAXS curves, exhibiting difference for the peak position among 12 buckwheat varieties. It has been reported that the scattering peak represented a long period in starch granules, and the position of the SAXS peak correlates with the average total thickness of the crystalline and amorphous regions in lamellar arrangements (Blazek & Gilbert, 2011). The scattering intensity is proportional to the square of electron density at the corresponding scale, that is, the peak intensity is related to the $\Delta\rho$ and $\Delta\rho u$ (Tan, et al., 2015). $\Delta\rho$ indicates the difference in electron density between the amylopectin crystalline lamella ($\rho 1$) and amylose/amylopectin based amorphous region ($\rho 3$), which is helpful to increase the overall intensity (Zhu, 2015). $\Delta\rho u$ represents the difference in electron density between amylose background region ($\rho 2$) and $\rho 3$, which is related to the low-angle intensity (Yu, et al., 2022). These results indicated that the electron density varied between different buckwheat starches.

Lorentz correction was used to clearly analyze the peak position, and the corrected SAXS curves were shown in Fig. 2 D. The peak intensity presented a "shoulder-like" position at around 0.6-0.7 nm^{-1} with the maximum value in BU1 and the minimum value in BU7. The increased peak intensity indicated that there was also increase for the contrast of electron density, and the differences in 12 samples might be related to the variations in water absorption and swelling of the amorphous fraction and/or leaching of amylose from the amorphous parts (Kuang, et al., 2017).

The correlation function can be used to analyze the starch aggregation structure and can provide the structure parameters of lamellar structures, including the crystalline layer thickness (d_c), amorphous layer thickness (d_a) and long period distance ($d_{ac} = d_a + d_c$) (Chen, et al., 2016). The normalized 1D correlation function is shown in Fig. 3 and the lamellar structure parameters are summarized in Table 3. According to the Bragg's formula ($d = 2\pi/q$), significant difference was observed in the thickness of the semi-crystalline layers (d_{Bragg}) among 12 starch samples, ranging from 8.465 (BU9) to 11.310 nm (BU8). There were also obvious variations in the d_{ac}, with BU8 having the largest value of 11.70 nm and BU9 having the lowest value of 8.56 nm, indicated that the d_{ac} from the correlation function had a proper fitting with d_{Bragg} from the Bragg's equation. The d_a was in the range of 3.20-4.85 nm with the largest amorphous thickness in BU8 and the lowest amorphous thickness in BU9. For the d_c, it was between 5.29 and 6.85 nm with the order of BU8 > BU1 > BU5 > BU10 > BU3 > BU4 > BU11 > BU2 > BU12 > BU6> BU9 > BU7. These results showed that there were significant variations in lamellar structure of buckwheat starch. Lan et al. (2017) have found that there was positive correlation between thickness layer and light transmittance but negative correlation between thickness layer and resilience of canna starch. Ma et al. (2022) have reported that the starch gelatinization of wheat starch can be affected by the lamellar structure. During cooking, the granular and lamellar structures of starch are fully gelatinized, thereby influencing the digestibility. Therefore, the suitable varieties of buckwheat should be selected based on the specific needs in food processing and production. For example, buckwheat starches with low thickness are more suitable for the production of food additives, while starch samples with high thickness can be used to make adhesives.

3.5 Water solubility and swelling power

Water solubility can reflect the dissolution degree of starch during swelling and swelling power is used to measure the water holding capacity (Carcea & Acquistucci, 1997). The WS and SP of buckwheat starches at different temperatures are summarized in Table S1. The results showed that the WS and SP values of 12 starch samples varied at different temperatures, and the values of WS and SP significantly increased with the increase of temperature. At 75℃, the WS and SP were both low (the average WS was 6.04% and the average SP was 12.85 g/g). After 75℃, the WS and SP sharply increased with increasing temperature, with the average value of 10.76% and 19.41 g/g, respectively. When the temperature reached 95℃, both the WS and SP showed the maximum value, with an average WS of 12.52% and an average SP of 22.05 g/g, respectively. Similar change trend was observed in quinoa starch and sweet potato starch (Jiang, et al., 2020; Zhang, et al., 2018). The relationships between WS and SP of buckwheat starch showed a linear relationship, and the SP significantly increased with the increase of the WS at different temperatures (Fig. 4 A). In addition, the linear relationship was more significant at high temperatures, indicating that increasing temperature was helpful to promote the absorption and expansion of buckwheat starch granules. Generally, the water solubility and swelling power of cereal starches are used to study the interaction between water molecules and starch chains in crystalline and amorphous regions during heating (Abegunde, et al., 2013). The extent of this interaction can be affected by the amylose content, amylose to amylopectin ratio and fine structure of amylopectin, resulting in variations in water solubility and swelling power (Kaur, Singh, McCarthy, & Singh, 2007). It has been concluded that amylose could inhibit the starch swelling, hinder the breakage of amylopectin double helix, and maintain the integrity of swollen granules (Lai, et al., 2016). In this study, the amylose content of buckwheat starch was negatively correlated with the WS (P = -0.4256) and SP (P = -0.5027) (Fig. 6 A), for example, the variety of BU3 with the lowest amylose content (Table 1) had the highest values of WS and SP (Table S1), which can be used to explain the variations in the WS and SP among 12 starch samples. In addition, differences in genetics and growing areas of buckwheat starch also contributed to the changes in the WS and SP.

3.6 Light transmittance

Light transmission can be used to indicate the clarity of the starch paste, reflecting the retrogradation process (Huang, et al., 2021). The results of the light transmittance (LT, %) of buckwheat starches are displayed in Fig. 4 B. It was clearly shown that there were significant differences in the LT among 12 starch samples, ranging from 18.48 to 26.98%, with the largest value in BU3 and the lowest values in BU8, indicating that the starch granules of BU3 had the largest dispersion in water, leading to the highest light transmittance. In addition, the LT of all buckwheat starches was above 18%, which was significantly higher than the results of the previous study (Gao, et al., 2020), and this difference might be contributed to the varieties and growing conditions. It has been reported that increasing the amorphous area could make it easier for water molecules to enter the starch and make starch granules expand and disperse better in water, thus reducing the light refraction and dispersion and increasing the light transmittance of the starch paste (Hu, et al., 2016). In this study, buckwheat starch with higher amorphous area showed lower value of the LT, and there was a significant negative correlation between the LT and amylose content (Fig. 6 A), which was consistent with the above conclusion. The LT of cereal starch can be influenced by the swelling power ability, arrangement of molecular structure and the ratio of amylose/amylopectin (Jacobson, Obanni, & Bemiller, 1997), which can be used to explain the variations in the LT of 12 different buckwheat starches.

3.7 Pasting properties

Pasting behavior is helpful to determine the quality and utilization of starch (Abegunde, et al., 2013; Sun, et al., 2021). The viscosity profiles of buckwheat starches are presented in Fig. 4 C, and the pasting parameters are summarized in Table 4. It was shown that all starch samples exhibited a smooth curve with significant variations in their pasting behaviors among different buckwheat varieties (Fig. 4 C). PV ranged from 601 to 862 mPa·s, showing the largest value in BU3 and the lowest value in BU8. Holding strength (HS) is the difference between peak viscosity and breakdown viscosity. The HS of 12 starch samples was between 535.70 and 753.47 mPa·s, with the maximum in BU1 and the minimum in BU8. Final viscosity (FV) is due to the reduced movement of water molecules surrounded by amylose and amylopectin as the temperature decreases and the viscosity increases again, reflecting the stability to swollen granule structure. In this study, the FV was in the range of 1004.37-1537.33 mPa·s, corresponding to BU8 and BU3, respectively, which was slightly lower than the results of buckwheat starches reported by (Gao, et al., 2016). The difference might be related to the genotypes, starch purity and the interaction among starch components. Breakdown viscosity (BD) can reflect the heat resistance and shear resistance of starch paste, and starch with higher value means lower resistance to heat (Guo, et al., 2019). The BD ranged from 29.07 to 112.60 mPa·s with the lowest in BU6 and the largest in BU3, which indicated that BU3 contained lower resistance to heat and shear and was much easier to gelatinize during the heating process. Pasting temperature (PT) refers to the temperature where starch viscosity begins to rise. The PT of buckwheat starch significantly varied from 66.42 (BU3) to 71.13℃ (BU8) with a mean value of 68.55℃. It has been reported that pasting temperature of starch is positively correlated with amylose content (Zhou, Shi, Meng, & Liu, 2013), which was similar to the results of this study as presented in Fig. 6 A. Differences in the PT of buckwheat starch might be related to the variations in granule size distribution as shown in Table 2. The results of the PT in this study were higher than the PT range (59.12-63.9℃) reported in previous studies (Gao, et al., 2016; Gao, et al., 2020; Liu, et al., 2016) but lower than that of quinoa starch (72.60℃) (Jiang, et al., 2020). In this study, the pasting properties of buckwheat starches were significantly different among various varieties, which might be related to the granule size, amylose content and chain length distribution of amylopectin. Therefore, the suitable buckwheat variety should be selected to achieve the desired properties in food industry.

3.8 Thermal properties

The thermal properties of buckwheat starches were analyzed using DSC, the thermograms are presented in Fig. 4 D, and the thermal parameters are summarized in Table 4. All starch samples showed smooth thermogram curves, while the positions of peaks and the degrees of peak openings were significantly different. Similar results have been reported in sweet potato starch (Guo, et al., 2019). Significant differences were also observed in the thermal parameters of this study (Table 4). Both the To and Tc showed the lowest value in BU8 and the highest value in BU3, ranging from 55.70 to 62.53℃ and from 73.60 to 80.32℃, respectively. The Tp was in the range of 66.85-70.45℃, with the minimum value in BU12 and the maximum value in BU3. Starch with higher gelatinization transition temperatures would require higher heat of solubilization (Vasanthan, Bergthaller, Driedger, Yeung, & Sporns, 1999). The results observed in this study indicated that the BU12 was the easiest of the 12 buckwheat varieties to heat from the crystalline state to the gel state. Gelatinization enthalpy (ΔH) reflects the melting of starch crystals, and a relatively high value means that much energy is needed to melt starch granules (Gao, et al., 2016). Higher crystallinity degree can lead to higher transition temperatures, making the starch granules more resistant to gelatinization (Uarrota, et al., 2013). In this study, the ΔH varied from 6.44 (BU8) to 8.92 J/g (BU3) with mean value of 7.43 J/g, suggesting that BU3 possessed more stable crystal structure and was more difficult to melt, which was consistent with the results of the RC and granule size distribution as shown above. The ΔH values previously reported for buckwheat starches by Gao, et al. (2020) were similar to the values reported in this study. Differences in the gelatinization parameters of starch are thought to be influenced by the main chemical compositions, granule size and the molecular structure of the crystalline region (Kaur, et al., 2007).

3.9 Rheological properties

3.9.1 Frequency sweep analysis

Frequency sweep test was conducted to determine the viscoelastic properties of buckwheat starch. The storage modulus (G′), loss modulus (G″), complex viscosity (η*) and loss angle (tan δ) as a function of the angular frequency (ω) of buckwheat starch are displayed in Fig. 5 A-C. Generally, G′ and G″ represent the elastic behavior (solid-like system) and the viscous behavior (liquid-like system), respectively (Guo, Tao, Cui, & Janaswamy, 2019). If G″ exceed G′ (large tan δ), the gel behaves more like a liquid because the energy used to deform the gel is viscous dissipated (Zhang, et al., 2022). In this study, the G′ exceeded G″ and without any intersection within the angular frequency range of 0.1-100 rad/s (Fig. 5 A), indicating the solid-like systems for buckwheat starch. In addition, there were significant differences in the G′ and G″ of the 12 starch samples with the largest value in BU8 and the lowest value in BU3, which was probably related to the various sensitivity of buckwheat starch to frequency. As shown in Table S2, the G′ and G″ were well fitted by Power low model. K′ and K″ reflects the viscous and elastic behaviors, respectively, and n is related to the frequency dependence. The fitted results showed that the gel of buckwheat starch exhibited a solid-like system. As can be seen from Fig. 5 B, the η* decreased sharply within low angular frequencies and then gradually stabilized at high angular frequency. Loss angle (tan δ) reflects the relative contribution of the viscous part and elastic part to the viscoelastic response of starch samples (Ma, Zhang, Jin, Xu, & Xu, 2022). As shown in Fig. 5 C, the tan δ firstly increased and then decreased with the increase of angular frequency and ranged from 0.01 to 0.15 (lower than 0.4), indicating that all starch samples behaved as an elastic material. Han plot was used to further analyze the different buckwheat starch pastes (Fig. 5 D), and the Han plot firstly decreased and then significantly increased with the increase of G″ and the relaxation mechanism of buckwheat starch was longer in the high G″ region.

3.9.2 Creep-recovery analysis

To further analyze the variations in the viscoelastic behavior of buckwheat starch, creep-recovery test was conducted to record the strain and compliance over time. As shown in Fig. 5 E, when subjected to instantaneous stress, the strain of all starch samples increased with time (within 300 s), and then significantly decreased after the stress was removed. There were significant differences in the strain among 12 buckwheat starches, showing the maximum value in BU4 and the minimum value in BU8, which indicated that BU4 possessed more viscous components, resulting in lower formability. After removing the stress, all samples exhibited stable strain, suggesting that the strain of buckwheat starch was well recovered.

As can be seen from Fig. 5 F, all samples showed a nonlinear response to stress. The compliance of buckwheat starches ranged from 0.01 to 0.03 J with the largest value in BU4 and the lowest value in BU8. The creep compliance can reflect the freedom extent of molecular movement and the strength of gel, and higher compliance values correspond to a weak gel system with high deformability (Samutsri & Suphantharika, 2012). The finding of this study suggested that the gel system of BU8 was the strongest of the 12 buckwheat varieties. Differences in various buckwheat varieties were probably due to the molecular weight and the shorted chain length of starch granules. In addition, the creep compliance curves were well fitted by Burger's model with the range of R^2 from 0.915 to 0.996, and there were significant differences in the G0, G1, λ and μ_0 among 12 buckwheat starches (Table S2). These results indicated that buckwheat starch showed solid and elastic gel network structure, which was consistent with the frequency sweep tests.

3.10 Principal component analysis (PCA)

The PCA is widely used to investigate the interrelationships between the structural properties of starch and the differences and similarities among starches collected from different sources (Kaur, et al., 2007). The relationship between the structural and physicochemical properties of 12 buckwheat starches were subjected to PCA, the loading and score plots are presented in Fig. 6 and the Pearson's correlation coefficients are shown in Fig. S1. The first principal component (PC1) and second principal component (PC2) are considered to explain the variance of data when multidimensional data is projected as one-dimensional data (Lee, Lee, & Chung, 2017). In this study, the PC1 and PC2 accounted for 65.92% and 12.30%, respectively. The correlations among the relative properties could be observed from the loading plot (Fig. 6 A). The curves that are close to each other on the plot are positively correlated, while those are in opposite directions are negatively correlated (Kaur, et al., 2007; Singh, McCarthy, Singh, & Moughan, 2008). Among the main chemical compositions, Ts and Pr were loaded negatively on PC1 but positively on PC2, while Mo, As and Am were all in the negative direction of PC1 and PC2. Among the structural properties, RC was loaded positively on PC1 but negatively on PC2, while da, dc and dac were loaded negatively on PC1 but positively on PC2. Among the pasting properties, PV and HS were loaded positively on PC1 but negatively on PC2, PT was loaded negatively on PC1 but positively on PC2, while FV, BD, SBP and SBT were all in the positive direction of PC1 and PC2. Among the thermal properties, To, Tp and Tc were loaded positively on PC1 but negatively on PC2, while ΔH were loaded positively on both PC1 and PC2.

The distance between any two starches on the score plot refers to the similarities and differences among the starches. A total of 12 buckwheat starches collected from 6 countries are regularly distributed in the quadrants of the score plot with four groups based on the structural and physicochemical properties (Fig. 6 B). For example, BU1 and BU3 had positive scores on PC1 and PC2, while opposite trend was observed on BU4, BU9 and BU12; BU5, BU8 and BU10 were loaded at the left of the score plot with positive scores on PC2, whereas BU2, BU6, BU7 and BU12 showed positive scores on PC1 but negative scores on PC2. Overall, the 12 buckwheat starches with different amylose content were clearly classified according to their different properties.

3.11 Cluster analysis

In order to compare the relationships of different buckwheat varieties, the hierarchical cluster was performed based on chemical compositions, granule size distribution, relative crystallinity, lamellar parameters, water solubility, swelling power, light transmittance, pasting properties and thermal parameters. As shown in Fig. 6 C, the dendrogram consisted of two major clusters and these two clusters were separated by the distance of 3.35. One cluster just contained one buckwheat variety (BU3), and other 11 varieties were contained in other groups. It was obvious that the remained 11 varieties could be further separated into two groups. One group included BU1, BU2, BU6, BU7, BU9 and BU11, and the other group contained BU4, BU5, BU8, BU10 and BU12. Furthermore, BU1 can be separated from the former group at the distance of 2.10, and BU8 can be separated from the latter group at the distance of 1.85. These results indicated that buckwheat starches isolated from different varieties showed various structural and physicochemical properties, which was consistent with the results of the PCA analysis.

4. Conclusions

Starch characteristics of 12 buckwheat varieties collected from 8 countries were investigated in this study. The results showed that the contents of moisture, ash, protein, amylose and total starch varied from 8.43 to 13.65%, 0.12 to 0.25%, 0.26 to 0.34%, 29.55 to 36.13% and 91.29 to 95.11%, respectively. All starch samples showed irregular polygonal and spherical shapes and typical A-type crystalline structure, while had obvious differences in crystallinity ranging from 26.37% (BU8) to 35.21% (BU3). Among the 12 buckwheat starches, BU3 with the lowest value in amylose content presented higher values of water solubility, swelling power, light transmittance, pasting properties, thermal parameters and loss angle. However, starch with higher amylose content showed higher values of amorphous region, storage modulus, loss modulus and complex viscosity. PCA analysis and cluster analysis showed that there were significant differences in structural and physicochemical properties among various buckwheat varieties. In all, buckwheat starches with high amylose content (such as BU8, BU10 and BU12) were more suitable as a food packaging material or food additive, while the starch samples with low amylose content (such as BU1 and BU3) can be used as an adhesive, which would provide valuable information for the further utilization of buckwheat starch in food and non-food industries based on the specific genotypic sources.

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Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Figure and Table legends

Fig. 1 Cryo-scanning electron microscopy images and relative image analysis of buckwheat starches from 12 varieties (magnification of 1000 and 5000).

Fig. 2 The volume distribution (A), X-ray diffraction pattern (B), 1D SAXS curves (C) and Lorentz-corrected 1D SAXS profiles (D) of buckwheat starches from 12 varieties.

Fig. 3 The normalized 1D correlation function of buckwheat starches from 12 varieties.

Fig. 4 Physicochemical properties of buckwheat starches from 12 varieties. The relationship between water solubility and swelling power (A), light transmittance (B), pasting curve (C) and DSC thermogram (D).

Fig. 5 Rheological properties of buckwheat starch from 12 varieties. Frequency sweep curves (A, B and C), Han plot (D), creep recovery curves (E) and creep compliance (F).

Fig. 6 PCA analysis and cluster analysis based on the structural and physicochemical properties of buckwheat starches from 12 varieties. The loading plot (A), score plot (B) and dendrogram (C) (Ts: total starch; Mo: moisture; As: ash; Pr: protein; Am: amylose; Rc: relative crystallinity; dc: crystalline layer thickness; da: amorphous layer thickness;

dac: long period distance; WS75: water solubility at 75℃; WS85: water solubility at 85℃; WS95: water solubility at 95℃; SP75: swelling power at 75℃; SP85: swelling power at 85℃; SP95: swelling power at 95℃; Lt: light transmittance; PV: peak viscosity; HS: holding strength; FV: final viscosity; BD: breakdown; SBP: setback from peak; SBT: setback from trough; PT: pasting temperature; To: onset temperature; Tp: peak temperature; Tc: conclusion temperature and ΔH: gelatinization enthalpy).

Table 1 The origin, yield and main chemical compositions of buckwheat starches from 12 varieties.

Table 2 The particle size distribution of buckwheat starches from 12 varieties.

Table 3 The lamellar structure parameters of buckwheat starches from 12 varieties.

Table 4 The pasting properties and thermal properties of buckwheat starches from 12 varieties.